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RESEARCH ARTICLE

# Relative Frequencies of Deleterious Genes in Natural Populations of *Drosophila melanogaster* Originating from the Nucleoelectric Plant of Laguna Verde, Veracruz

**Víctor M. Salceda**

*Departamento de Biología,Instituto Nacional de Investigaciones Nucleares, Carretera México-Toluca S / NLa Marquesa, Ocoyoacac, Edo. de México.*

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Corresponding Author: Víctor M. Salceda, Departamento de Biología,Instituto Nacional de Investigaciones Nucleares, Carretera México-Toluca S / NLa Marquesa, Ocoyoacac, Edo. de México.

## Abstract

In order to obtain information about possible changes and/or damages that could be caused by the operation of the nuclear reactors of the Laguna Verde Nuclear Power Plant, over populations of Drosophila melanogaster that live in the area, a series of semi-annual collections (summer and winter) during the years 1991-1992 and 1996-1998 were done and later subjected to a battery of tests. Flies of this species after been capture were carry to the ININ Biology laboratory where they were subjected individually to a series of crosses that allowed us in the third generation to detect the presence of deleterious genes that, depending on their viability, were classified as normal, lethal or semi-lethal according to Wallace's methodology. In this way a total of 933 second chromosomes were analyze and from them the relative frequencies for each of the categories calculated, as well for each sampling season, this information is shown in tables 1 and 2.Resultsfrom the statistical test applied indicates that there are nonsignificant difference between the populations and that the differences if they existed, are due only to environmental changes, which normally occurs in all populations, due to this we can point out that the presence of the reactors does not seem to negatively influence the behavior of the populations that live in the area.

## 1. Introduction

The current energy needs require the use of new technologies, among which the generation of electricity through the construction and operation of nuclear power plants that partially cover these requirements. The operation of these plants is coupled with the concern of governments to inform the community of the advantages and disadvantages that this type of facility may cause. Mexico has two nuclear power plants located in Laguna Verde, Veracruz; their construction began in 1986 and one started to operate in 1989 and the second in 1994. Consenting to the responsibility of evaluating the possible biological effects that the operation of a nuclear power plant represents, the Mexican authorities, in this case the Instituto Nacional de Investigaciones Nucleares (ININ), approved a long-term project to study the possible consequences of ionizing radiation in populations of organisms that inhabit the area of influence of the two reactors. The organisms selected for the study are two dipterous species widely used in biological research: *Drosophila melanogaster* and its sister species *D. simulans*. The use of these species makes it possible to compare the responses of similar genomes, which will serve as an indicator of possible changes due to exposure to the same factors, as well as an extensive knowledge of their biology.

The study would include the answer to the following biological parameters: relative frequencies of the species, resistance to desiccation, displacement capacity, radioresistance and egg-adult viability and others that could be incorporated during the development of the project, it should be noted that these parameters by themselves represent the natural response of organisms to natural adversities.

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Preliminary studies of these biological parameters, during the construction phase, were presented by Levine *et al*.(1989), de la Rosa *et al. (1989)*, Rockwell *et al*. (1991) and Olvera *et al*.(1993), information that serves as a reference for later studies. Recently,and considering the information regarding the operational phase of the two reactors, there are already responses of both species to the different parameters, thus Pimentel *etal*.(2003) report on radio resistance, Pimentel *et al*. (2004) with reference to adult egg viability and Pimentel *et al*.(2007) with respect to resistance to desiccation; These three studies are comparative between the two species involved and considering the response over a period of ten years and in none of them was found evidence of negative impact.

Now our attention is focused on the genetic load that will give us an idea of its variability because it determines the frequency of recessive lethal genes, with respect to this parameter there is a lot of information and here we present some reports considered as pioneers in their region and therefore of greater relevance.

Thus, the genetic variability in natural populations of *Drosophila melanogaster* has been widely analyzed, highlighting those studied by Ives (1945), in populations of USA; Dubinin (1946) in Russia; Goldschmidt *et al.* (1955) and Dawood (1961) in the Mediterranean; Paik (1960) in Korea and Minamori and Saito (1964) in Japan and in Mexico among others by Salceda a and b (1977), Espinoza-Velázquez and Salceda(1977)and Maganhotto *et al.*(1979). In all of them, reference is also made to the average viability of the carriers of different categories of deleterious genes. This parameter was selected because, among other aspects, it gives us information regarding the hidden variability present in the populations that are studied and although this way of measuring the variability has been surpassed by molecular techniques based on DNA and that are more precise, however, the advantage of using the genetic load is that it allows us to have additional information regarding the genetic health of the population by quantifying the presence of deleterious genes present in the populations and also to obtain values of viability and average fecundity in the populations. Pioneering studies of this type are those of Wallace (1956).

It is known that a good number of mutations are lethal or semilethal when presented in homozygous condition, but the effects of those lethals in heterozygous flies are scarcely known and conflicting views about these effects have been expressed by various authors, thus Berg (1945) demonstrated that the lethal ones found in natural populations and Stern and Novitski(1948) using experimental populations showed that the lethal genes are deleterious in heterozygous condition, both authors employing*D. melanogaster*.

On the other hand, Wright et al. (1942) using D. pseudoobscura, found that autosomal lethals are less frequent in natural populations than they would be if they were completely recessive. For its part Cordeiro (1952)showed that on average the viability of lethal heterozygotes is significantly lower than in individuals free of lethal and both types of chromosomes, carriers and non-carriers of lethals vary significantly with respect to the effects of viability when combined with other chromosomes.

Also it will be possible to propose this biological system as a test to monitor in similar facilities, the possible existence of leaks or to detect damages caused by extremely low doses due to gaseous emissions, if they occur.

## 2. Material and Methods

The collection of the biological material was done in two sites of Laguna Verde in which the first electric-power plant of the country was established, which is located 72 km north of the city of Veracruz and its coordinates are: 96° 24'30" longitude W and 19° 43 '24" of latitude N.These sites were established by Levine *et al*. (1989), designated Site I the one located in the residential area at 1350m WNW of the reactor and corresponds to the control population, while Site II is located 350 m from the exit of the reactor cooling water in a SSW direction of the first site and corresponds to our experimental population; subsequently, a third site was included outside the facilities approximately 5 km north of them.

The plant started its operation in February of 1989 and although collections of *Drosophila* done in the stage prior to its operation, there is no information regarding the frequency of lethal genes in these populations. The collections of biological material analyzed here were done in three successive seasonal periods: July 1991, January 1992 and July 1992 and correspond to the first years of operation of the plant. The collections were suspended for four years and continued on the following dates, where information on Site III is already included: summers and winters of 1996, 1997 and 1998, the last date on which samples were analyze. Even we have looking for more collections the Authorities of the Plant did not allow the entrance for security regulations.

The flies were capture by means of traps containing fruit in fermentation as an attractant and with the use of an entomological network. Once captured and selected, they were transported to the ININ laboratory where the analyzes were carried out. For this, the flies were subjected to a series of experimental crosses according to the Cy L / Pm technique; H / Sb described by Wallace (1956)and widely used in similar studies that schematically is as follows: P1 male + / + nature X female Cy L / Pm; H / Sb

This initial cross serves to isolate or extract a second chromosome from the male of natureF1 male + / Cy L X female Cy L / Pm; H / Sb

This cross acts as a multiplying agent of the isolated chromosome, since all the descendants will be identical F2 males + / Cy L X females + / Cy L these crosses will originate the test generation F3 CyL / Cy L: Cy L / +: + / +1 (dies): 2 (heterozygote): 1 (wild)

where the CyL / Cyl individuals die because the markers in turn carry in association a recessive lethal gene; CyL / + individuals are heterozygous for the marker chromosome and the one from a male of nature. As noted in the diagram, in the third generation two types of individuals are obtained: the heterozygotes carrying a chromosome with markers and the other a replica of one of the second chromosomes of the male originating from nature and whose phenotype is Curly wings, the other type of individual that appears in this third generation corresponds to homozygous individuals for that second chromosome of the captured male and wild phenotype; the proportion of these two genotypes is 2: 1 or in percentage 66.67 against 33.33. The absence of wild individuals is indicative of the presence of a lethal gene and deviations to the 2: 1 ratio indicate differential viability, when the fraction corresponding to wild types is greater than zero but less than ten percent of the total number of flies counted in the culture, they are individuals carrying semilethal genes. This characterization helps us to determine which category corresponds to each chromosome so extracted from the population and can therefore determine the relative frequencies of these genes in each sample.

With this test we could determine the frequency of recessive genes, lethal, semilethal and normal for each population and with this information suggest the existence or not of genetic damage possibly caused in a natural way and in case of significant differences with the control population suggest the possible effect caused by other agents present in the area, which according to our hypothesis could be due to emanations from the reactors, the absence of changes in the relative frequencies of these genes indicate that there is no damage caused by the presence and operation of the reactors.

The determinations of the type of gene extracted in this way were made by quantifying the number of heterozygous descendants carrying the wild type and the markers (+ / Cy L) *vs* wild (+ / +) that in a 2: 1 ratio indicate normality and deviations of the same degree of viability being the proportion 100% + / Cy L indicative of lethality.

The comparisons that took place were of two types: comparison of the frequencies of deleterious genes with regard to the different seasons of the year, that is, winter versus summer, since the collections were in January and July and those between the sites. The different comparisons and their differences were analyzed by applying the $X^2$ test, which are observed in the respective tables.

All the cultures were made in ¼ liter jars containing fresh food consisting of a mixture of agar-agar, corn flour, sucrose, dextrose and brewer's yeast to which tegosept and propionic acid were added as a fungicide and preservative; the cultures were maintained at a temperature of 25° C $\pm$ 1 and a relative humidity of 65%.

## 3. Results

Once all the counts have concluded, data were collected, tables prepared and the results analyzed, they are shown in Tables 1, 2 and 3.

As a result of the study, a total of 933 chromosomes were extracted or isolated from the same number of males captured in the sampling sites and seasons; distributed in the three categories of genes as shown in Table 1, in which in order to have a reference data from Salceda and Gallo (2002) from a locality in the vicinity of the City of Veracruz are included as reference.

The most important aspect of the study and from which the subsequent analyzes are derived is to determine the amount of the genetic load, that is, the relative frequencies of normal, lethal and semi-lethal genes in each of the populations, this information is shown in the Table 1. On the other hand, in Tables 2 and 3 we present the result of the application of the statistical test $X^2$, in which to increase the sample size that allows us to make comparisons, the data

of all the collections were added and were obtained the corresponding value per site and per season of the year, which were compared among themselves to be subjected to the aforementioned test. The next step was first to compare the observations between seasons, that is, summers against winters for each site as shown in Table 2; the values between site and site were then compared for each season, as shown in Table 3.

**Table 1.** *Percentage frequency of normal, lethal and semilethal genes in natural populations of Drosophila melanogaster originating from Laguna Verde, Ver*

| Population | Normals | Lethals | Semilethals | n |
|---|---|---|---|---|
| SI, July, 1991 | 100 | --- | --- | 11 |
| SI, January, 1992 | 62.2 | 13.5 | 24.3 | 37 |
| SI, July, 1992 | 65.4 | 7.7 | 26.9 | 26 |
| SI, July, 1996 | 94.4 | 5.6 | --- | 18 |
| SI, January,1997 | 92.9 | 7.1 | --- | 14 |
| SI, July, 1997 | 95.6 | --- | 4.4 | 45 |
| SI, January,1998 | 86.1 | 8.3 | 5.6 | 36 |
| SI, July, 1998 | 95.3 | 1.9 | 2.8 | 108 |
| SII, July, 1991 | 80.0 | 5.7 | 14.3 | 35 |
| SII, January,1992 | 89.1 | 3.9 | 7.0 | 129 |
| SII, July,1992 | 84.6 | 7.7 | 7.7 | 13 |
| SII, July, 1996 | 96.3 | 3.7 | --- | 27 |
| SII, January,1997 | 100 | --- | --- | 12 |
| SII, July, 1997 | 96.9 | 3.1 | --- | 65 |
| SII, January,1998 | 87.0 | 13.0 | -- | 23 |
| SII, July, 1998 | 90.3 | 7.8 | 1.9 | 103 |
| SIII, July, 1996 | 100 | --- | --- | 2 |
| SIII, January, 1997 | 100 | --- | --- | 25 |
| SIII, July,1997 | 92.3 | --- | 7.7 | 13 |
| SIII, January, 1998 | 90.5 | 7.1 | 2.4 | 42 |
| SIII, July, 1998 | 91.3 | 3.4 | 5.4 | 149 |
| Average | 90.01 | 6.3 | 9.2 | 933 |
| SG | 90.01 | 4.44 | 5.92 | 148 |

*SI= Site I; SII= Site II; SIII= Site III; n= population size; SG=Salceda and Gallo, (2002).*

**Table 2.** *Seasonal differences of the relative frequencies of normal, lethal and semilethal genes in natural populations of Drosophila melanogaster originating from Laguna Verde, Ver*

| | Normals | Lethals | Semilethals | n |
|---|---|---|---|---|
| SI Winters | 77 | 10.3* | 12.6 | 87 |
| SI Summers | 91.8 | 2.4 | 5.8 | 208 |
| SII Winters | 89.6 | 4.9 | 5.5 | 164 |
| SII Summers | 90.9 | 5.8 | 3.3 | 243 |
| SIII Winters | 94.0 | 4.5 | 1.5** | 67 |
| SIII Summers | 91.5 | 3.0 | 5.5 | 164 |

**significant p≤ 0.05; S I = Site I; S II = Site II; SIII= Site III.*

**Table 3.** *Inter-population differences for the relative frequencies of normal, lethal and semilethal genes in three natural populations of Drosophila melanogaster originating in Laguna Verde, Ver*

| | Normals | Lethals | Semilethals |
|---|---|---|---|
| SI January | 77 | 10.3 | 12.6* |
| SII January | 89.6 | 4.9 | 5.5 |
| SI July | 91.8 | 2.4* | 5.8 |
| SII July | 90.9 | 5.8 | 3.3 |
| SI January | 77 | 10.3 | 12.6** |
| SIII January | 94 | 4.5 | 1.3 |
| SI July | 91.8 | 2.4 | 5.8 |

| | | | |
|---|---|---|---|
| SIII July | 91.5 | 3 | 5.5 |
| SII January | 89.6 | 4.9 | 5.5 |
| SIII January | 94 | 4.5 | 1.5 |
| SII July | 90.9 | 5.8 | 3.3 |
| SIII July | 91.5 | 3 | 5.5 |

*Significant * $p \leq 0.05$; ** $p \leq 0.01$ S I = Site I; S II = Site II; SIII= Site III.*

## 4. Discussion

From the enormous amount of existing data in the bibliography regarding the amount of the genetic load or accumulation of lethal genes in natural populations of *D. melanogaster*, it is inferred that this value fluctuates between 5 and 20 percent for the frequency of this type of genes, depending on these values of both the time of year in which the sample was taken and the geographical position of the sample. Regarding the frequency of semi-lethals there is little information and it is only indicative of the genetic health of the population since it does not have more information of an adaptive nature.

The effects caused by irradiation, however, vary according to the type and dose of radiation and in this respect little or nothing is know regarding the effect of emanations of nuclear plants.

The information we detected as shown in Table I, indicates that our populations do not differ from those studied by other authors in studies of natural populations because these are the ones that serve as the basis for any comparison, as the values detected by us are similar to those of other authors we consider that these populations are within the range of normality for the parameter analyzed. Our results clearly show that the frequencies of lethal genes fall within the ranges of normality, in addition, since there are no differences between the values obtained for each population, it is suggested that the absence of damage caused by possible leaks or gaseous emanations proper to the functioning of the reactor do not represent any alteration of the values.

When no significant differences were found or were very small between the two populations, it was decided to do another type of analysis, consisting of comparing the behavior of the populations in relation to the parameter under study but now taking into consideration the temporal differences, that is, seasonal changes.

As the data were collected during several successive seasons two summers, July 1991 and 1992 and a winter January 1992 in a first stage, as well as the Summers of 1996, 1997 and 1998 and the Winters of 1997 and 1998, it is in possibility to detect changes due to the change of seasons. Since the summer samples were small in size and there were no differences in the relative frequency of lethal genes in both populations, we opted to add the data of both years for this season and try to see differences, as shown in the Table 2.

The result of these observations and when doing the statistical tests was the response that the differences between the summer frequencies with respect to the winter ones are so small and probably due to the sample size; these small differences can therefore be due exclusively to seasonal changes fundamentally due to the different temperatures and humidity that prevail in the area.

It is known that in most natural populations in which this type of analysis has been carried out, the behavior is similar and the changes detected are only a reflection of the changes caused by the alternation of the seasons, in our case dry and cold against wet hot.

Having not found differences between the seasons, we will now deal with those present in the different sites. By our design and following the established by Levine et al. (1989), in addition to Site III since no differences were found during the first stage between Sites I and II, we consider it pertinent to include a third sampling site. Site I represents our witness against which to compare the values of Site II or experimental population, the values of these comparisons are seen in the first four rows of Table 3. In the table it is noted that the only two differences found correspond to the semi-lethal frequencies between Site I and Site II in the Winter; for lethal in July between the same sites for Summer and for semilethals in Winter between Site I and Site III.

The difference in the summers between sites I and II may be due to the fact that Site I is located in an urban area and since it frequently suffers bottlenecks in terms of abundance of individuals, our samples probably suffered alterations in which individuals that started the repopulation were those free of lethal, and probably the same happens in the case of the

semilethals in the Winter comparisons between Sites I and II and between Sites II and III.

Therefore, we can conclude that the differences between the three populations are not significant and this suggests the absence of the possible induction of damage due to the possible effect of gas emanations from the reactor and that the differences found are due to seasonal changes. normally occurring in natural populations.

Our information regarding the frequencies of deleterious genes present in natural populations of *D. melanogaster*, with respect to the possible impact due to the presence and operation of the reactors in the Central Nucleoeléctrica de Laguna Verde, Ver., Coincides with the studies reported by Pimentel et al (2003) about radio resistance, Pimentel et al. (2004) with reference to adult egg viability and Pimentel et al. (2007) with respect to drying resistance; These three studies conducted in the same populations presented here indicate that there is no impact whatsoever on the operation of the reactors and we agree with them in their statement that the small differences are only responses to environmental changes.

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