PART II. POPULATION AND COMMUNITY RESPONSE TO RADIATION

EFFECTS OF RADIATION ON SURVIVAL OF WILD COTTON RATS (SIGMODON HISPIDUS) IN ENCLOSED AREAS OF NATURAL HABITAT

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Abstract. Each month for 4 successive months 4 groups of wild adult female cotton rats (8 females per group) were irradiated at dose levels of 500, 750, 900, 1050, and 1200 R whole-body. Treatment was accomplished at 1.5 m using a 9200 Ci $^{137}$Cs source at a rate of approximately 20 R per minute. Each group was released into a one acre enclosure with 4 unirradiated (control) females and 4 untreated males. Fifteen days later the animals were removed by live and/or snap trapping. Recapture and survival data on the females were subjected to analysis of variance for the effects of dose level and month, and a field LD$_{50}$ was determined. Results of these field experiments substantiate the high radiosensitivity determined for this species in previous laboratory experiments and suggest quantitative similarity and qualitative differences in stressors existing between the two environments. A direct correlation was established between dose level and survival with recaptures ranging from 91% at the 500 R level to 25% at 1200 R. Survival was essentially 100% among controls. No seasonal effects were noted in these experiments.

Introduction

The effects of whole-body irradiation on domestic and laboratory animals have been investigated by numerous authors measuring many parameters. More recently, research also has been concerned with the lethal effects of radiation on wild animals held under laboratory conditions (Provost 1964, Dunaway 1965, Gambino et al. 1965, Golley et al. 1965, and Kellogg 1965).

The cotton rat (Sigmodon hispidus) is one of the most common small mammals in the southeast and therefore a basic food item for many larger vertebrates. High population densities of this animal have resulted in extensive damage to agricultural crops (Hamilton 1939) and, at times, may be a major limiting factor to bobwhite quail populations (Stoddard 1931).

Provost (1962) suggested possible high radiation resistance in the wild cotton rat. The effects of acute radiation on this animal held in the laboratory were investigated by Kellogg (1965). He reported an LD$_{50(30)}$ of approximately 1120 R for adult females. This is substantially higher than figures available for domestic or laboratory mammals.

The importance of studying radiation effects on animals in their natural surroundings has been stressed by Dunaway and Kaye (1964) and Provost et al. (1965). Population characteristics such as density, natality and mortality rates, age distribution, biotic potential, and dispersion may vary with such environmental stresses as radiation and are important parameters that cannot be measured on individuals in the laboratory. Practically no information is available concerning the effects of acute whole-body irradiation on the survival of wild mammals under natural conditions.

Enclosure experiments on which this paper is based were conducted on the Atomic Energy Commission Savannah River Plant, Aiken, South Carolina, between March and September 1965. This area is located in the Upper Coastal Plain of west-central South Carolina. The enclosures were located in field number 3-412, which was a corn and cotton field when the government project was established in 1952, but which has been abandoned since that time. Vegetation

1This investigation was conducted under Contract AT(38-1)-310 Task III between the U.S. Atomic Energy Commission and the University of Georgia.
within the enclosures consisted of a thick stand of mixed forb and broomedge (Caldwell 1960), typical of natural cotton rat habitat in the area.

Methods

Wild cotton rats were captured on the Savannah River Plant with Sherman spring-door traps using mixed grain as bait. Individuals were marked by toe clipping using the method suggested by Baumgartner (1940). These animals were held in laboratory cages (13.5 x 7.5 x 4.5 in.) for a minimum of 30 days and fed a regular diet of mixed grain (sunflower seed, corn, and oats), commercial rat chow, and lettuce. Animals showing weight loss or apparent abnormalities were discarded. Only adult animals weighing over 100 g were used in this study.

Two hundred and fifty-six cotton rats were utilized in 16 enclosure experiments. Each experiment consisted of eight irradiated females, four unirradiated (control) females, and four untreated males (Table 1). The males were utilized in some concomitant experiments on the effects of irradiation on reproduction in feral populations.

All of the animals were wild-caught with the exception of the 48 females used in the August experiments, which were born and reared in the laboratory. Stocking levels were held to 16 animals per acre since previous experience and data from Schnell (1965) indicated that higher stocking densities may occasion increased predation.

The animals were irradiated in groups of eight with each female contained in an individual cardboard carton. The position assumed by the animals within these irradiation chambers essen-

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Inclusive Date¹</th>
<th>Total Number Animals</th>
<th>Enclosure Number</th>
<th>Dose Level (R)</th>
<th>Number of Irradiated Females</th>
<th>Control Females</th>
<th>Males</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>5-3-65 to 5-31-65</td>
<td>64</td>
<td>1</td>
<td>500</td>
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<td></td>
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<td>2</td>
<td>750</td>
<td>8</td>
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<td>4</td>
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<td>900</td>
<td>8</td>
<td>4</td>
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<td>4</td>
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<td>5-31-65 to 6-26-65</td>
<td>64</td>
<td>1</td>
<td>500</td>
<td>8</td>
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<td></td>
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</tr>
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<td>6-30-65 to 7-28-65</td>
<td>64</td>
<td>1</td>
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<td></td>
<td></td>
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<td>2</td>
<td>750</td>
<td>8</td>
<td>4</td>
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<td>4</td>
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<tr>
<td>4</td>
<td>7-29-65 to 8-25-65</td>
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<td>500</td>
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<td></td>
<td>2</td>
<td>750</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>3</td>
<td>900</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>1050</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

¹For convenience, these periods are referred to as “May,” “June,” “July,” and “August” experiments.
tially resulted in a spherical body exposed to the source. The cartons were supported by a wooden rack 1.5 m from a 9200 Ci $^{137}$Cs source.

Air dose rates were determined with a Victoreen R-meter remote area monitoring system (McCormick and Golley 1964). Dose rate readings at four to six locations in the exposure rack were averaged to determine the length of exposure time required for each dose level. The average dose rate readings of the experiments in May, June, July, and August were 20.9, 19.9, 18.4, and 19.2 R/min, respectively. Differences in average monthly dose rates were probably due to the slight variation in position of the source and radiation rack during the four-month experimental period. The animals were irradiated at levels of 500, 750, 900, 1050, and 1200 R. All animals were irradiated in the late afternoon to avoid the possible stress of high temperatures. Control females received identical treatment but without radiation.

Immediately after treatment irradiated and control females and untreated males were released into each of the four enclosures. With the exception of the 500 and 1200 R groups, the same enclosure was used each month for the same experimental dose level, providing four replications in time for three treatment levels. A 1200 R group was substituted for the 500 R treatment in July (Fig. 1).

The four adjacent one-acre enclosures used in this study were constructed of 20-gauge steel sheeting buried 12 to 15 in. and extending approximately 3 ft above the ground. A 3 ft high, 2 in. mesh chicken wire fence was added on top of the metal fence forming the perimeter of the four enclosures. Trees and large bushes were removed from the inside and for a distance of 70 ft outside the enclosures, and weeds were cut along the enclosure fence. These measures were taken to minimize predation, a factor thought to be important in the loss of experimental animals in earlier enclosure studies. Cotton rats had been introduced into the enclosures prior to these experiments to prepare the habitat (i.e., provide runways and nest sites), and thus possibly reduce loss of experimental animals released into unfamiliar areas. All such cotton rats were removed from the enclosures prior to experimental releases.

Environmental conditions in all four enclosures were ostensibly similar. Initially, five trap lines of nine Sherman live-traps per line were established in each enclosure. Later, four more lines per enclosure were added in an attempt to decrease the time necessary to recapture the experimental animals. Thus, a total of 81 live-traps were maintained in each enclosure. Approximately 40 snap traps were added per enclosure during the last two nights of each trapping period to facilitate removal of all animals.

<table>
<thead>
<tr>
<th>Enclosure 1</th>
<th>Enclosure 4</th>
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<tbody>
<tr>
<td>500 R</td>
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</tr>
<tr>
<td>May</td>
<td>May</td>
</tr>
<tr>
<td>June</td>
<td>June</td>
</tr>
<tr>
<td>August</td>
<td>July</td>
</tr>
<tr>
<td>1200 R</td>
<td>August</td>
</tr>
<tr>
<td>July</td>
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<table>
<thead>
<tr>
<th>Enclosure 2</th>
<th>Enclosure 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>750 R</td>
<td>900 R</td>
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<tr>
<td>May</td>
<td>May</td>
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<tr>
<td>June</td>
<td>June</td>
</tr>
<tr>
<td>July</td>
<td>July</td>
</tr>
<tr>
<td>August</td>
<td>August</td>
</tr>
</tbody>
</table>

*Fig. 1. Design of cotton rat enclosure experiments.*
Animals were left in the enclosures for 15 days. Traps were prebaited and left open during this period. Chitty and Kempson (1949) and Moore (1936) indicated that prebaiting improves capture and recapture efficiency. On the fifteenth day the traps were cleaned and set with fresh baits consisting of mixed grain and/or peanut butter and rolled oats. Traplines were run two to four times daily until it was believed that all animals had been removed.

Recaptured males were weighed and discarded. No data on males were utilized in calculating results. Recapture data (time and location) were recorded on all females. They were then returned to the laboratory for further studies. Recapture data were subjected to analysis of variance for the effects of dose level and month. The means were separated at the 5% level using Duncan’s multiple range and F tests (Duncan 1955). The field LD₅₀ was determined by probit analysis (Finney 1952). The analyses were performed on an IBM 7094 computer at the University of Georgia Computer Center with the special help of J. T. McGinnis.

**Results**

*Recapture Response.* Ninety-nine (77.42%) of the 128 irradiated females were recaptured during the 16 enclosure experiments. Sixty (93.75%) of the 64 controls were recaptured. Except for the May, June, and August 750 R groups, 100% recapture of controls was accomplished in all the experiments (Table 2). The pooled data from the 16 experiments revealed a differential survival of 26.33% between treated and untreated females.

With the exception of the 750 and 900 R groups, highly significant differences in the mean numbers recaptured were obtained between all dose levels (Table 3). Significant differences in the mean numbers recaptured were also obtained between all irradiated groups and the pooled controls. Significant variations in mean recapture times for the treatments occurred only at the 1050 R treatment level (Table 4). The 1050 R group gave a highly significant response when compared to the mean recapture times for all lower dose levels and the pooled control group. With the exception of three individuals, 10 to 12 days were required to recapture released animals from any given enclosure in all of the monthly experiments. No significant differences were found in the mean numbers recaptured or in the mean recapture times between the four experimental periods (Tables 3 and 4).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number Released</th>
<th>Number Recaptured</th>
<th>Percent Recaptured</th>
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<tr>
<td>500 R</td>
<td>24</td>
<td>22</td>
<td>91.66</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>12</td>
<td>100.00</td>
</tr>
<tr>
<td>750 R</td>
<td>32</td>
<td>27</td>
<td>84.37</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>12</td>
<td>75.00</td>
</tr>
<tr>
<td>900 R</td>
<td>32</td>
<td>27</td>
<td>84.37</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
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</tr>
<tr>
<td>1050 R</td>
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<td>21</td>
<td>65.62</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>16</td>
<td>100.00</td>
</tr>
<tr>
<td>1200 R</td>
<td>8</td>
<td>2</td>
<td>25.00</td>
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<tr>
<td>Control</td>
<td>4</td>
<td>4</td>
<td>100.00</td>
</tr>
<tr>
<td><strong>Total irradiated</strong></td>
<td><strong>128</strong></td>
<td><strong>99</strong></td>
<td><strong>77.42</strong></td>
</tr>
<tr>
<td><strong>Total control</strong></td>
<td><strong>64</strong></td>
<td><strong>60</strong></td>
<td><strong>93.75</strong></td>
</tr>
</tbody>
</table>
Mortality Response. It was assumed that all animals not recaptured were dead. All irradiated and control animals that were recaptured alive survived at least 30 days post-treatment. Probit analysis of all recapture data indicated an LD$_{50(15)}$ of 1130 R.

Table 3. Individual experiment recapture data on female cotton rats from outdoor enclosures

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Experiment</th>
<th>Mean Number* caught</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td>June</td>
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<tr>
<td>500 R</td>
<td>7</td>
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<tr>
<td>Control</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>750 R</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>900 R</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1050 R</td>
<td>6</td>
<td>7</td>
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<tr>
<td>Control</td>
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<tr>
<td>1200 R</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Mean number caught</td>
<td>5.00</td>
<td>4.57</td>
</tr>
</tbody>
</table>

*Eight irradiated and 4 controls released per experiment.  
*Mean pooled control value is 3.75.

Table 4. Mean recapture time of female cotton rats from outdoor enclosures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment</th>
<th>Mean Number Days*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td>June</td>
</tr>
<tr>
<td>500 R</td>
<td>5.71</td>
<td>1.71</td>
</tr>
<tr>
<td>Control</td>
<td>1.25</td>
<td>2.25</td>
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<tr>
<td>750 R</td>
<td>1.83</td>
<td>1.50</td>
</tr>
<tr>
<td>Control</td>
<td>4.66</td>
<td>1.50</td>
</tr>
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<td>900 R</td>
<td>5.50</td>
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<tr>
<td>1200 R</td>
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</tr>
<tr>
<td>Control</td>
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</tr>
<tr>
<td>Mean number days</td>
<td>4.05</td>
<td>3.37</td>
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</table>

*Mean pooled control value is 2.45.
Discussions and Conclusions

These enclosure experiments represent the second phase in a series of studies designed to quantitate the effects of acute radiation on population parameters in the cotton rat. The initial investigation established a laboratory LD\textsubscript{50} for this species based on a 30-day survival, whereas the LD\textsubscript{50} in the field experiments is based on a 15-day survival, since it was necessary to re-capture pregnant females before parturition occurred. Despite the difference in end points between the laboratory and field studies, we feel that the respective LD\textsubscript{50} figures of 1120 and 1130 R are comparable for the following reasons. Peak mortality in the laboratory experiments occurred between 7 and 10 days, with no animals dying between 17 and 30 days (Kellogg 1965). No live-caught females in the present study died between 15 and 30 days, and all appeared to be in good condition when recaptured. Their subsequent continued survival in the laboratory fortifies this conclusion. Although it was not feasible to locate dead animals in the enclosures and hence a direct measurement of peak mortality was not possible, it seems likely that the peak mortality period was similar in both environments. The similarity between the LD\textsubscript{50} levels in laboratory and field experiments suggests a quantitative similarity of the stress factors under these diverse conditions and encourages the possible application of certain laboratory-established parameters in field experiments. However, the loss of some individuals at all dose levels in these field experiments as compared to 100% survival of laboratory animals through the 1000 R level (Kellogg 1965), indicates a qualitative difference in the stressors of the two environments, and points up the necessity of population studies in relatively natural environments for adequate interpretation of radiation effects. Stressors influencing the response of a wild animal under natural conditions include variable climatic conditions, social interactions, and normal living requirements. Thus, loss of some animals in these experiments at all dose levels may reflect a behavioral response which made them more susceptible to predation or other decimating factors. A great number of avian predators were observed over the enclosure area during the experiments. The excessive loss of control females in the 750 R group as compared to 100 percent survival of all other control groups would indicate extraordinary lethal environmental factors (e.g., predation) acting on this group. This possibility is further substantiated by the equal survival of the 750 and 900 R groups, since one would expect the 750 R group to exhibit greater survival.

Statistical analysis showed no difference in recapture time between monthly experiments.

With the exception of three individuals, the total time required to recapture all the animals from a given enclosure remained between 10 and 12 days throughout the four experimental periods, indicating that the use of additional traps in later experiments did not significantly alter the results. A few trap-shy individuals may account for the lack of change in the 10–12 day period required to recapture all animals alive at the end of each experiment. This trap-shy phenomenon in certain cotton rats is substantiated by data from Wiegert and Mayenschein (1966). The 1050 R level was the only group that showed a significantly longer recapture time than did the controls. Golley (1965) suggested altered behavioral and movement patterns in field populations of irradiated mice. Since 1050 R approximates the field LD\textsubscript{50} in these experiments, the delayed recapture in this group may reflect the debilitating effects of radiation.

The lack of a significant difference between survival of the laboratory-born females of the August experiment, which had never been exposed to field conditions, and the wild-caught females of the May, June, and July experiments, emphasizes the species-specific nature of the radiation-resistance phenomenon, as well as the innate capability of a heterozygous, wild gene pool to cope with "new" environmental conditions.

Although preliminary data (Kellogg 1965, Provost et al. 1965) suggested greater radiosensitivity during the breeding season in the laboratory, data obtained in the present field experiments do not support a lowered resistance to radiation damage associated with breeding activity. This difference may again be explicable on the basis of differential stress factors in the two environments. Similar field experiments conducted during the nonbreeding season are required to evaluate the effects of pregnancy on resistance to radiation.
Further enclosure experiments now in progress with longer exposure of irradiated animals to field conditions should further clarify effects of acute radiation on relatively natural populations.

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MORTALITY RATES IN IRRADIATED RODENT POPULATIONS

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Abstract. Survival curves were computed for the pocket mouse (Perognathus formosus) populations of three fenced 20 acre plots. The animals of one plot were irradiated at a rate of approximately 1 r/day. The data are suggestive of a shorter life span induced by radiation, but are not conclusive. Survival curves for irradiated (1 r/day) and control Peromyscus maniculatus in the laboratory show significant differences between the two groups and between sexes in each group. Extrapolation of comparable shortening of life spans by radiation to survival rates of field populations of the same species indicates the importance of chronic radiation exposure from widespread radioactive contamination in a natural situation. The magnitude of the effect is related to the natural life spans in the field, and varies with the environment. A relatively long-lived species may suffer from such exposure, while a naturally short-lived species will be unaffected. There was no indication of increased survival rates of the wild species under chronic low level exposure as has been indicated in similarly exposed laboratory rats and mice.

Introduction

In January of 1964 a radiation source was raised to the top of a 50-foot (15 meter) tower in the center of a 20 acre (8 hectare) study area in the Mojave Desert of southern Nevada. The study area was surrounded by wire mesh fence constructed four months earlier to confine the rodents residing in the area. All rodents in the area had been trapped, marked and released. The objective of the study was to determine the effects of long-term low-level irradiation of a desert ecosystem. Of the organisms present, mammals were expected to be most susceptible to damage by ionizing radiation. However, studies of other organisms were planned.

At the same time, a laboratory colony of wild mice was similarly exposed to long-term low-level gamma radiation to determine effects on life span and on production of young. Such studies had not been performed previously on wild species, and investigations in the laboratory would help differentiate the relative importance to a population of elevated radiation exposure and the environmental variables that control natural populations.

To understand the impact of potential wide-spread radioactive contamination, it is essential to consider separately the hazard to the population and hazard to the individual. Individuals near the center or source of radioactive contamination may be injured or die, but successful species are well suited to overcome such a momentary disadvantage. Hazard to the population will be from wide-spread, and therefore low-level, contamination. In this paper we present our data on decreased longevity from chronic exposure to gamma radiation, and attempt to put them in proper perspective by comparison with the natural longevity.

Previously reported studies of the effect of chronic exposure on life span have been performed on laboratory strains of animals, mainly rats and mice. These have been reviewed by Mole (1959) and by Sacher and Grahn (1964). Mole emphasized the similarity of results from different investigators, and concluded that there was a threshold between 1 and 2 r daily below which no life shortening occurs. Sacher and Grahn reported results of extensive studies in which daily dose
rates ranged as low as 5 r/day. They demonstrated a shorter life span of approximately 23 days per roentgen per day of exposure for laboratory mice, and indicated little difference between strains and between sexes. Carlson and Jackson (1959) irradiated rats at rates between 0.3 and 4.2 r/day, at two different ambient temperatures, and concluded that the effects of radiation interact with the effects of environment in influencing longevity. Grän (1958) found a difference in survival of control animals that lived in different types of cages and suggested this indicated their sensitivity to different environmental conditions.

Methods

Field studies were conducted in the desert shrub community of the northern Mojave Desert at the U.S. Atomic Energy Commission’s Nevada Test Site. Four circular 20-acre (8 hectare) study areas, with a radius of 550 feet (170 meters) were studied by live-trapping three successive nights once a month. Traps were spaced 50 feet (10 meters) apart, making a total of 400 traps in each study area. We attempted to set at least twice as many traps as there were animals in the study area. At times of high population density the number of traps was increased to 600, without altering the spacing, by setting two traps at alternate stations.

The small mammal population of the areas under study was composed almost entirely of four species of heteromyid rodents: pocket mice Perognathus formosus and P. longimembris, and kangaroo rats Dipodomys merriami and D. microps. The first of these was the most abundant. All were easily trapped. This characteristic greatly facilitated our study, because in three nights of trapping we could very nearly census the entire population of a grid. Animals seldom failed to appear in our monthly trapping, except during winter months when the pocket mice hibernated for temporary periods, and may not have appeared above ground.

Three of the study areas were fenced to prevent the entrance or exit of rodents. This enabled us to build up actuarial records of the resident animals. Young animals born in the plots were marked with a numbered metal ear tag (kangaroo rats) or by toe clipping (pocket mice). When the records for an individual animal in one of the fenced plots terminated, the animal was presumed dead.

One of the fenced study areas was irradiated at a low level. Radiation was from a $^{137}$Cs source suspended 50 feet above the ground in the center of the area. It was partially shielded to reduce radiation intensity near the center of the plot. The source was lowered into a shield for approximately 5 days each month to permit biological studies in the area. The remainder of the time, day and night, the source remained in the raised position. Plants of the study area were exposed to dose rates of 2 to 10 r/day, depending upon location within the plot, and rodents were exposed to an average of 1 r/day. Rodents were shielded when in their burrows (French, Maza and Aschwanden 1966).

All records of animals were compiled for each area by a computer program after each trapping period. In the field, an animal was removed from a trap, its number, trap location, physical characteristics and physiological condition recorded, and then released. After three nights of trapping, the field records were punched on IBM cards. A sort-merge program was used to add the data to a magnetic tape containing all the previously accumulated records. A list of all records was printed in duplicate, and one of these was sent to the field for use during the next trapping period. It was considered essential to have the complete record in the hands of the personnel conducting trapping in the field for comparison with new observations of animals. Each new record was checked for consistency and accuracy before it was added to the total. Occasionally it was necessary to re-examine an animal if there was a question about the new record. This system minimized errors in our data. A total of over 45,000 records of capture has been accumulated on the master tape.

The laboratory colony was composed of approximately 200 laboratory-born descendants of wild Peromyscus maniculatus. This species was used because it is easily maintained in the laboratory and it breeds readily, whereas the heteromyid rodents do not. Representatives of
three wild populations were used, one from the pine-fir zone at 8000 feet elevation in the mountains, one from the desert at the Nevada Test Site, and one from the desert in California near Palmdale. These strains were kept separate in the laboratory colony, to test for differences between them and to strengthen the analysis for radiation effects. Inbreeding was avoided. Litters from the wild pairs were divided between irradiated and control groups. Animals were paired in glass cages with litter in the bottom. A shelter and an exercise wheel were provided in each. The shelter was made from an old-style coffee can 5 inches in diameter and 3 inches high with four 2-inch holes in the sides. In the irradiated colony a lead hemispherical shield, of slightly smaller diameter than the can, was placed on top of the shelter. One mCi of $^{137}$Cs sealed in a stainless steel container was placed in a recess in the under side of the lead shield. This subjected the inside of the shelter to a dose rate of 45 to 50 mr/hr. Exposure of the mice was determined by the light and dark cycle of the windowless room in which both control and irradiated animals were maintained. The animals spent most of the light cycle (12 hours daily) inside the shelters, and most of the dark cycle outside. Their exposure averaged slightly over 1 r/day, the same as the irradiated field population.

Dosimetry was performed with microthermoluminescent dosimeters. To determine the exposure of animals, the dosimeter was placed in small plastic tubing (electrical spaghetti) and attached by a single suture to the skin of the back of the neck. Dosimeters could be removed and replaced with fresh units after a period of exposure.

Analysis of the data was performed by computer utilizing programs written by one or all of us for specific problems, or existing BMD programs (Biomedical Computer Programs of the Health Sciences Computing Facility, UCLA School of Medicine). Of special value in results presented here was the program designated as BMDX76, Life Table Analysis.

Results

One species of the field population will be considered, the pocket mouse, Perognathus formosus. It is the only species which was abundant in all plots, and the only one for which sample size was considered adequate. Density of the populations fluctuated greatly (Fig. 1), apparently due to a close correlation between primary production of the desert community and production of young by the small mammal population in the different years of this study. The population of Plot A showed there were 268 (138 ♂) P. formosus present in the fenced area. This population declined rapidly during the next year, and continued to decline until the spring of 1965. There was no recruitment in the spring of 1963 and very little in 1964. This was followed by two very good years, and the P. formosus population attained a maximum of 83 individuals (34 ♂) in 1965 and 169 (71 ♂) in 1966. Study of Plot B was begun in October 1963, with a population of very low density. Irradiation of this area began in January 1964. From an original population of 37 (19 ♂), the P. formosus increased to 130 (72 ♂) in 1964, to 493 (248 ♂) in 1965, and to 400 (209 ♂) in 1966. Work in Plot C was initiated in November of 1963 with a population of 25 (12 ♂) P. formosus, which increased to 54 (30 ♂) in 1964, to 135 (72 ♂) in 1965, and 245 (128 ♂) in 1966. The pattern of population increase and decline was generally similar in the three fenced plots. The main differences were in the very high density population of Plot B in 1965, and the decrease in numbers of this plot in the next year, when other plots continued to increase. The unfenced Plot D was a special problem because it permitted dispersal of the rodents and some additional predation, the implications of which have been discussed elsewhere (French Maza, and Aschwanden 1967).

The most important difference between the survival curves for P. formosus (Fig. 2) was the high initial loss in the population of the irradiated plot during the first 2-month time period represented. The rate of survival during this period was 0.80 in Plot C, 0.69 in Plot A, and 0.58 in Plot B. The difference at this point is highly significant ($P < 0.01$). After this, the difference between the curves became less. There is another 50% loss in the populations by 10 months in A, by 12 months in C, but not until 16 months in B. In none of these periods was the difference
between the curves statistically significant. At 20 months, survival in Plot B again dropped sufficiently so that it was significantly different from Plot A \((P < 0.01)\). At this point so few animals \((10)\) remained of the original total in Plot C that a comparison was of questionable value. It should be noted, however, that the survival curve for the irradiated plot (Plot B) was almost consistently lower than those for the other two plots. The total numbers of animals and the animals of known age (marked as juveniles) used in this analysis were: Plot A, 507 (222 juveniles); Plot B, 945 (865 juveniles); Plot C, 411 (365 juveniles).

Analysis of survival in the laboratory Peromyscus population was based on 50 control females, 49 control males, 47 irradiated females, and 49 irradiated males. Survival curves (Fig. 3) showed the control groups of both sexes to have higher survival rates than their corresponding irradiated groups. There was a difference between the sexes, survival being better in the males than in the females in both the irradiated and the control groups. In each case the difference was statistically significant \((P < 0.01)\). Radiation decreased survival in both sexes, the decrease being statistically significant in both females \((P < 0.05)\) and males \((P < 0.05)\). The sex difference was sufficiently great that survival of the irradiated males was generally better than that of control females. When the data for the two sexes were combined, and the control group compared with the irradiated group, survival was significantly decreased in the irradiated group \((P < 0.05)\).

Discussion

Results of analysis for comparison of survival rates of *P. formosus* populations in field enclosures are provisional at this time. True survival rates and life table data can be computed only from data on animals of known age. In our studies, only animals captured and marked as juveniles were of known age. Because there was very little reproduction by the rodent population during the first two years of our study, those animals born in the fenced plots and therefore of known age have not yet been followed long enough to permit construction of life tables from these data. We have therefore utilized data on all animals in results presented here, including the original population of adult animals that were present in the plots when trapping was begun.
For these reasons our analysis of survival in the field populations is considered preliminary, and we have intentionally avoided presenting the data in the form of a life table lest there be the implication of greater accuracy than is warranted. After one more year of study we will probably be able to make reasonably accurate estimates of life expectancy and survival rates based on animals of known age.

In a previous analysis (French, Maza and Aschwanden 1967) of life spans of the field populations, we conclude that longevity was shorter in the irradiated plot and that this was very likely due to irradiation. This conclusion was based upon computed mean life spans of the different species and comparison of these means. The present analysis is believed to be more precise because it includes an additional season of data and utilizes an improved method of analysis.

Although not conclusive, the field data are suggestive of a reduced life expectancy in the irradiated population. However, other factors besides radiation may have influenced this. The sample from Plot B (irradiated) was composed of a very large proportion of animals that entered our study as juveniles (92%). Hence, the survival curve for Plot B includes the mortality of the very young, which could be the explanation for the sudden and significant drop during the first time period (0 to 2 months) when compared to Plot A which was based on a sample composed of only 44% juveniles. However, Plot C had 89% juveniles, almost as high a proportion as Plot B, and survival over the first time period was significantly higher.

Other differences of unknown importance are the population densities and species composition of the plots. The greatest density of *P. formosus* occurred in the irradiated area, Plot B. The population of Plot B was almost entirely of this species (>90%), while in the other plots the proportion of *P. formosus* in the total population was less (60–70%). The degree of interspecific competition is unknown.

Results from *Peromyscus* reared and irradiated in the laboratory clearly indicate that longevity of a wild species is reduced by 1 r/day of chronic exposure to gamma radiation. This becomes important in ecology, however, only if it affects the ability of a population living in a natural situation to maintain its numbers. Survival rates for the same species and subspecies under field conditions can be approximately computed from the data published by Dunmier (1960), who studied field populations at four different elevations (4600 to 12,400 feet) in the White Mountains of California. Life expectancy was greatest in the population at the highest elevation.

Fig. 3. Survival curves for *Peromyscus* in the laboratory.
Fig. 4. Approximate survival curves for wild populations of *Peromyscus maniculatus sonoriensis* at 12,400 feet elevation (upper curve) and 4600 feet elevation (lower curve) in the White Mountains of California (from data of Dunnier 1960).

studied, and least in the population at the lowest elevation (Fig. 4). It is evident that life expectancy is very much less under field conditions than under laboratory conditions.

To examine the effect of radiation on survival of the field population, the fractional reduction of life expectancy in the irradiated laboratory population (sexes combined) was applied to the appropriate age category in the field population (Table 1). Reduction of life expectancy because of radiation is important only to the field population with the greatest life expectancy. Hence, the shorter the natural life expectancy of a population, the less important is further reduction by radiation exposure. Consequently, populations of animals living in different environmental conditions will respond differently to radiation exposure. This resembles the conclusions of Grahn (1960), based on chronic exposure of various strains of laboratory mice, that “mean survival time and the mean accumulated dose vary directly with the control survival.” In other words, the mean accumulated dose (for chronic exposure) and the LD$_{50}$ (for acute exposure) are related to each other through their common relationship to the normal life expectancy, and have been shown to vary directly with the control life expectancy. Extending this to ecology, radiation effects on life span vary directly with the natural life expectancy. This important consideration for estimates of the effects of widespread radioactive contamination has been overlooked in previous theoretical investigations of radiation exposure to populations (French 1965).

It has been reported that laboratory strains of animals receiving very low chronic exposure (5 r/day and below) demonstrate greater survival than controls. Mole (1957) attributes this to experimental error and does not consider the difference significant. Sacher and Grahn (1964) attribute increased life expectancy in groups receiving low level chronic exposure to a protective effect from one or more of the prevalent infectious diseases that are common in laboratory animals. They reason that the loss of life (in animal days) when the controls are lost due to infectious disease can easily exceed the days lost at the end of the life span due to cumulative deleterious actions of irradiation.
Table 1. Survival rates of natural populations of *Peromyscus* (from data of Dunnier 1960) and survival rates after chronic exposure to low level radiation (extrapolated from data on irradiated and control *Peromyscus* in the laboratory)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Radiation Survival Rate</th>
<th>Population</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12,400 feet (natural)</td>
<td>12,400 feet (after rad.)</td>
<td>4600 feet (natural)</td>
<td>4600 feet (after rad.)</td>
<td></td>
</tr>
<tr>
<td>Post-wean.</td>
<td>0.99</td>
<td>0.35</td>
<td>0.12</td>
<td>0.119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 mo.</td>
<td>0.98</td>
<td>0.102</td>
<td>0.011</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mo.–1 yr.</td>
<td>0.99</td>
<td>0.037</td>
<td>0.009</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 yr.</td>
<td>0.88</td>
<td>0.013</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no evidence of increased life span in any of our irradiated species. This suggests either the absence of infectious diseases comparable to those that affect laboratory animals, a greater natural immunity in wild species, or the lack of any protective action of chronic low level radiation exposure. Further study may contribute evidence to help clarify this problem.

**Literature Cited**


ECOLOGY OF TWO POPULATIONS OF AN AQUATIC ISOPOD (LIRCEUS FONTINALIS RAF.), WITH EMPHASIS ON IONIZING RADIATION EFFECTS

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Abstract. Two populations of Lirceus fontinalis Rafinesque in DeKalb County, Georgia, are isolated from each other in separate river drainages and are evolving in response to different environmental stresses. The background radiation count from naturally occurring radionuclides in the granite at Mount Arabia is several times above the average for terrestrial ecosystems. Drought and high temperatures appear to be the main ecological factors limiting the population on Mount Arabia. These factors are of little moment to the population in Lullwater Creek where biotic factors are of primary ecological significance to the Lirceus population. There is a significant increase in tolerance to gamma radiation and drought stress with age in Mount Arabia Lirceus. Mount Arabia Lirceus are 7.88 times more tolerant to ionizing radiation stress and are 5.36 times more tolerant to drought stress than Lullwater Creek Lirceus.

Introduction

Lirceus fontinalis Rafinesque 1820, an asellid isopod, occurs on Mount Arabia which is a granite gneiss rock outcrop 25 km southeast of Emory University, Atlanta, DeKalb County, Georgia, in the Altamaha River Basin (Atlantic Ocean drainage). The isopods are found in temporary weather pools - small depressions containing water - and in an intermittent creek draining the outcrop. Naturally occurring uranothans in the granite provide a background radiation count several times above the average for terrestrial ecosystems. Animals living in the pools on the outcrop are exposed to severe weather fluctuations (Darlington 1959), particularly drought and high temperatures (Styron 1965, Styron and Burbank 1967). An intermittent creek flows through a shaded mesic pine stand where soil has accumulated and affords a much more buffered habitat. As a consequence of strong selection pressures exerted by the rigorous environmental conditions and of a problematically high mutation rate due to radiation, one might expect to find in this population interesting adaptations involving wide tolerance for various environmental factors. Local L. fontinalis repopulate weather pools after the occurrence of intolerable physical phenomena such as drought and high temperatures.

A second population of L. fontinalis occurs in a typical, physiographically young, spring-fed stream adjacent to the Emory University Lullwater Biological Field Laboratory. Lullwater Creek is in the Appalachian River Basin (Gulf of Mexico drainage). Drought and high temperatures which are the main limiting factors on Mount Arabia are of little moment to the population in Lullwater Creek, since the beech-maple stand tempers the environment. The Lullwater Creek area is, however, an integral part of an Atlanta suburb and is strongly influenced by the surrounding human habitation. A heavy application of herbicide to a tennis court that drains into the creek has been implicated in a marked decrease in the population density (Styron 1967), and a large amount of silt from neighboring construction sites passes through the creek. On Mount Arabia Lirceus is the largest animal that completes its life cycle in the weather pools and is preyed upon only by dragon

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fly nymphs. In Lullwater Creek *Lirceus* is preyed upon and is probably in competition with juvenile salamanders, crayfish, and insect larvae and nymphs.

This comparative study of two *Lirceus* populations is based on physiology and behavior (Andrewartha 1961) with correlative field and laboratory techniques (Kinne 1964). The overall objectives of the study are (1) to establish the taxonomic affinities of the two populations; (2) to investigate the biological reactions (Allee et al. 1949) of isopods to various environmental stresses including ionizing radiation; (3) to investigate their behavior patterns; (4) to extend and test previous biometrical conclusions relative to environmental tolerances; and (5) from these to attempt to determine the factors or combinations of factors that control the maintenance, growth, and movement of the populations. This report will be concerned with objectives 2 and 4.

**Materials and Methods**

The biological reactions of isopods of the two populations to acute gamma radiation, high temperature, and drought stresses have been investigated through factorial experiments. These experiments were replicated at five stages in the life cycle of isopods from Mount Arabia and at one stage in the life cycle of isopods from Lullwater Creek. Isopods 2.0 mm long are juveniles 1.6 to 2.1 months old; 4.00 mm long, in a preparatory stage just before maturity and 3.7 to 4.9 months old; 5.00 mm long, sexually mature adult and 5.2 to 6.6 months old; 6.00 mm long, 6.5 to 8.2 months old; 7.00 to 8.00 mm long, 7.9 to 13.2 months old.

Each experiment included all combinations of four levels (Tables 1 and 2) of radiation, temperature, and drought stresses. Four groups of isopods were irradiated in the Emory University $^{137}$Cs gamma radiation field at 16.4 kr/hr (27 ± 2 °C). Isopods from each radiation unit were divided into four groups for temperature treatment. Temperatures were raised at 15 °C/hr and then the units were allowed to cool to room temperature. Five isopods from each temperature unit were placed in each of four 8-cm finger bowls with 100.0 g of sand from the study area and enough

<table>
<thead>
<tr>
<th>Length of Isopods (mm)</th>
<th>Radiation (kr)</th>
<th>Temperature (°C)</th>
<th>Drought (g of water per 100 g of sand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>0.0</td>
<td>24</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td></td>
<td>8.2</td>
<td>24</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>16.4</td>
<td>24</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>32.8</td>
<td>24</td>
<td>1.9</td>
</tr>
<tr>
<td>4.00</td>
<td>0.0</td>
<td>38</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td></td>
<td>32.8</td>
<td>38</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>65.6</td>
<td>39</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>82.0</td>
<td>40</td>
<td>1.7</td>
</tr>
<tr>
<td>5.00 and 6.00</td>
<td>0.0</td>
<td>24</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td></td>
<td>32.8</td>
<td>24</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>65.6</td>
<td>24</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>82.0</td>
<td>24</td>
<td>0.9</td>
</tr>
<tr>
<td>7.00 to 8.00</td>
<td>0.0</td>
<td>24</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td></td>
<td>32.8</td>
<td>24</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>65.6</td>
<td>24</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>98.4</td>
<td>24</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Table 2. Stress levels for $4^3$ factorial experiments

<table>
<thead>
<tr>
<th>Mount Arabia Isopods 5.00–7.00 mm Long</th>
<th>Lullwater Creek Isopods 5.00–7.00 mm Long</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation (kr)</td>
<td>Temperature (C)</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
</tr>
<tr>
<td>0.0</td>
<td>24</td>
</tr>
<tr>
<td>32.8</td>
<td>38</td>
</tr>
<tr>
<td>65.6</td>
<td>39</td>
</tr>
<tr>
<td>131.2</td>
<td>40</td>
</tr>
</tbody>
</table>

water to cover the sand. Drought units were allowed to dry (24 ± 2 C) to the stress levels in 10 days.

Mortality data were subjected to a normit transformation in an attempt to get a linear relationship between treatments and mortality so LD50_{10} (lethal dose for half the animals in 10 days) values could be accurately estimated (Fisher and Yates 1963). Normits and weights following Berkson (1955) were regressed by least squares on treatment. The LD50_{10} values from this analysis were then regressed by least squares on treatment. The constants for a cubic response surface were calculated for the Mount Arabia and Lullwater Creek $4^3$ factorial experiments by equation (1) and for the Mount Arabia $5 \times 4^3$ factorial experiment by equation (2).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_1^3 + \beta_4 X_2 + \beta_5 X_2^2 + \beta_6 X_2^3 + \beta_7 X_1 X_2 + \beta_8 X_1^2 X_2 + \beta_9 X_1 X_2^2,$$

where

- $Y$ = LD in radiation exposure,
- $X_1$ = temperature stress,
- $X_2$ = drought stress,
- $\beta$ = constants;

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_1^3 + \beta_4 X_2 + \beta_5 X_2^2 + \beta_6 X_2^3 + \beta_7 X_1 X_2 + \beta_8 X_1^2 X_2 + \beta_9 X_1 X_2^2 + \beta_10 X_1^3 X_2 + \beta_11 X_1 X_2^3 + \beta_12 X_1^2 X_2^2 + \beta_13 X_1^3 X_2^2 + \beta_14 X_1^2 X_2^3 + \beta_15 X_1 X_2^4 + \beta_16 X_1^2 X_2^4 + \beta_17 X_1^3 X_2^4 + \beta_18 X_1^4 X_2^4,$$

where

- $Y$ = LD in radiation exposure,
- $X_1$ = temperature stress,
- $X_2$ = drought stress,
- $X_3$ = time stress (age at time of irradiation),
- $\beta$ = constants.

The coordinates for the locus of points on these surfaces are temperature ($X_1$ axis), drought ($X_2$ axis), time ($X_3$ axis), and radiation exposure estimated to give the LD50_{10} value ($Y$ axis).
Results

Tolerance of the Mount Arabia *Lirceus* population to acute gamma radiation, high temperature, and drought stresses changed significantly with age (Fig. 1). The LD$_{50}$ for exposure only to radiation ranged from 31.3 kr for juveniles (2.00 mm) to 70.3 kr for adults (6.00 mm). The temperature LD$_{50}$ ranged from 38.6°C for juveniles to 40.5°C for adults (6.00 mm). The LD$_{50}$ for drought conditions ranged from 5.70% moisture for juveniles to 0.70% moisture for adults (7.00 to 8.00 mm). Combinations of these stresses were also more lethal to the younger isopods.

![Diagram of response surface analysis](image)

Fig. 1. Cubic response surface of mortality in LD$_{50}$'s from radiation stress on temperature and drought stresses for Mount Arabia *L. fontinalis* 2.00 to 8.00 mm long. Response surface A shows the reactions of *L. fontinalis* 2.00 mm long; B, 4.00 mm long; C, 5.00 mm long; D, 6.00 mm long; and E, 7.00 to 8.00 mm long. The comparison for the Mount Arabia *Lirceus* population of tolerance at age of irradiation to acute ionizing gamma radiation, high temperature, and drought stresses showed a marked shift with age.
The comparison of the reactions of Lullwater Creek and Mount Arabia Lirceus to acute gamma radiation, temperature, and drought stresses showed a significant difference between the two populations (Fig. 2). Mount Arabia Lirceus had approximately 7.88 times (LD50 of 105.2 kr as compared to 13.3 kr) more resistance to radiation stress and 5.36 times (LD50 of 0.70% as compared to 3.75% moisture) more resistance to drought stress. Solutions of roots of the cubic and analyses of variance for these 4^3 experiments and the 5 × 4^3 experiment mentioned above are given in Styron (1967).

Discussion

The biological reactions of Lirceus to acute gamma radiation, high temperature, and drought stresses were investigated through multifactorial experiments to obtain a more detailed analysis than could be achieved by monofactorial experiments and to provide a more substantial basis for development of mathematical models. Factorial experiments give an insight into the effect of each factor in the different conditions furnished by variations in the other factors. This is a particularly valuable approach when considering the ecological factors temperature and drought. Temperature can enlarge, narrow, or shift the moisture range of an individual, and moisture can modify the effect of temperature accordingly (Kinne 1963).

Multifactorial experiments on the reactions of Lirceus from Mount Arabia and Lullwater Creek have served to test and extend previous biometrical conclusions relative to environmental tolerances (Styron 1965, Styron and Burbank 1967). Data on the 4^3 factorial experiment for Mount Arabia Lirceus 5.00 to 7.00 mm long (Styron 1965) were analyzed here by a more general technique. The more general regression of mortality on treatment compared very well with the previous calculations. The earlier analysis indicated an LD50 of 95.6 kr with an r^2 (coefficient of determination) of 0.933, and the second analysis indicated an LD50 of 105.2 kr (r^2 = 0.942) which is not significantly different. The shape of the model (cubic response surface) developed in the first study is also closely approximated in the later experiments. The multifactorial experiment was extended to include the Lullwater Creek population and to include age at time of irradiation as another factor in the Mount Arabia experiment.
There is no generally acceptable quantitative model for the effects of ionizing radiation on animals. The great need for such a model became especially clear during attempts to analyze data for a previous report (Styron 1965, Styron and Burbank 1967). Several qualitative models have been developed and quantitative models are being developed for predicting radiation initiated death and cessation of growth in higher plants by Evans (1965), Evans and Sparrow (1961), Sparrow and Evans (1961), and Sparrow and Woodwell (1963). One type of radiation damage in animals, aging, has received very serious attention by several authors (Alexander and Connell 1960, Cassaret 1963, Cronkite et al. 1955, Curtis 1963, Failla and McClement 1957, Futh et al. 1960, Henshaw 1963, Johnson 1963 and 1964, Jones 1956a and 1956b, Kohn and Guttman 1959, Lindop and Rotblat 1961 and 1962, Mole 1963, Sacher 1956, Upton 1957, Upton et al. 1963). It is true that radiation aging and spontaneous aging share certain basic mechanisms, then in a given species a certain dose of whole-body radiation should be, as far as aging effects are concerned, equivalent to a certain dose of time. Johnson (1964) suggests that the relationship of relative life shortening to age at irradiation varies according to the form of the Gompertz function. Analysis of the $5 \times 4^2$ factorial experiment on the reactions of Mount Arabia Lirceus to radiation, temperature, drought, and age at irradiation revealed a shift in radiation sensitivity with age that approached a Gompertz function. That is, the increase in radioresistance was almost exponential.

An increase in drought resistance with age was also indicated by the analysis. It is suggested, however, that this is more the result of a structural than physiological or genetic change in the isopods with age. Adult Lirceus the exoskeleton that provides a much more effective barrier to water loss than do juveniles. When Lirceus are under drought stress, air pockets form first in the gill region of the abdomen, progress through the thoracic cavity, and finally reach the thoracic appendages before death occurs. These air pockets develop in juveniles under much less drought stress than in adults. No significant difference with age is indicated for resistance to temperature shock in Mount Arabia Lirceus. It is suggested that death from temperature shock results from disruption of enzyme systems that are heat sensitive.

Differences in the reaction of Lullwater Creek and Mount Arabia Lirceus to acute gamma radiation, temperature, and drought stresses may have developed for no particular reason, but it seems likely that they have developed through evolutionary time in response to different selection pressures. It is tempting to speculate that Mount Arabia Lirceus may have been naturally selected for radioresistance, since they have been continuously exposed to a low level of ionizing radiation seven times greater than the level in Lullwater Creek. There is, however, no physiological evidence that these radiation levels are significant in metabolic processes. To say that background radiation levels have been a selective factor in the evolution of radiation sensitivity is highly problematical. The same is not the case though for drought stress. Mount Arabia Lirceus are repeatedly exposed to drought conditions, and occasionally a few individuals survive mild droughts. The summer of 1966 was the first since the observation period began in September 1963 during which the population survived in weather pools on the outcrop. Obviously these drought conditions do not develop in spring-fed Lullwater Creek. The extreme physical-chemical conditions to which Lirceus is exposed on Mount Arabia are the principal ecological factors limiting the distribution and abundance of Lirceus on the outcrop. Since the physical-chemical parameters in Lullwater Creek remained well within the range of tolerance for Lirceus (Styron 1967), the distribution and abundance of Lirceus in the creek must be controlled by biotic factors such as predation and competition.

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