first 16 generations at least, and afterwards photonegative behavior increased steadily (fig. 1). Secondly, no similar morphological change has occurred in the control line and in the simultaneously selected negative line, all derived from the same founder population. Our results demonstrate that a single gene mutation has spread out within the population and finally became fixed as a result of selection for photonegative behavior. This conjecture is supported by some results of developmental biologists and vision researchers: the mutation *ora* causes a retardation of the retinula cells R_{1-6} and these cells mediate several visually evoked behavior traits, e.g. the optomotoric responses are strongly reduced[6,7]. Furthermore, the mutation may perhaps put out of action the structural gene for the synthesis of certain photopigments[7,8], and the lack of screening pigments leads to an enhancement of photonegative behavior[4]. On the other hand, the remaining intact rhabdomeres R_7 and R_8 contribute to slow phototaxis[9] and we were able to demonstrate that flies showing photonegative behavior in Y-mazes will reproduce this behavior in a test apparatus for slow phototaxis[10]. However, whether this gene mutation causes the negative phototactic behavior directly or whether it serves as a basis to be modified by other genes acting on phototaxis is not yet known and needs further investigation.

In single gene analysis usually new mutations are induced by a mutagen and these mostly qualitative variants of the behavioral trait in question are isolated by a certain test procedure for further analysis. On the contrary, we detect a single gene mutation with a qualitative morphological effect on the eye structure after selecting for photonegative behavior. Our experiments indicate that in natural populations selection for a behavioral trait leads to an increase of gene frequencies and at least to fixation of the behavioral mutants and their morphological correlates. Thus, our findings demonstrate a link between quantitative genetic analysis and single gene analysis in behavior genetics.

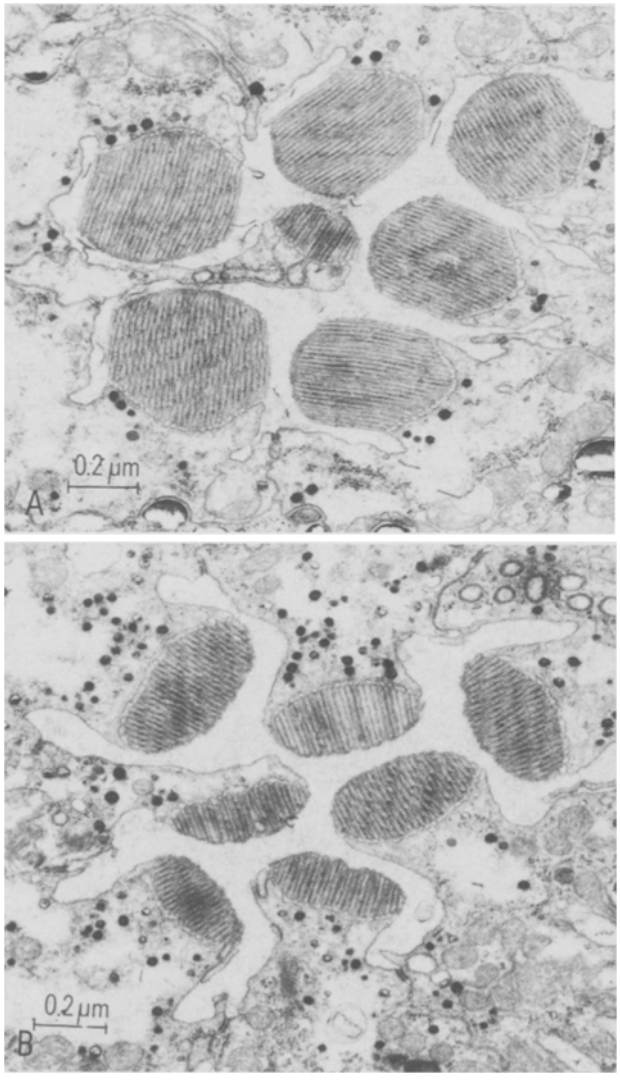
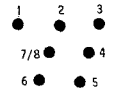


Figure 2. *A* Distal cross section through an ommatidium of the photopositive control line on *D. mauritiana*. *B* Comparable view of an ommatidium of the negative line N316. The rhabdomeres 1 to 6 are of irregular shape. The diagram illustrates the numbering of the rhabdomeres.



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Disruption of circadian rhythm of tissue respiration in *Channa striatus* by Metasystox®

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Summary. Oxygen consumption of gill, brain and muscle tissues of *Channa striatus* exhibits a clearcut circadian rhythm with a maximum uptake at 18.00 h. Metasystox exposure decreased the oxygen consumption of all tissues. Maximum reduction is noted in gill followed by brain and muscle. The peak period of oxygen consumption is also eliminated in the pesticide-treated fish.
Key words. Channa striatus; circadian rhythm; oxygen consumption; metasystox; pesticide.

Tissue oxygen (O_2) consumption (as an indicator of cellular metabolism) is a valuable parameter for assessing the toxic effects of pesticides[2]. Hiltibran[3] studied the effect of 16 insecticides on the in vivo O_2 uptake and phosphate metabolism of blue-gill liver mitochondria. In *Tilapia mossambica*[4], age-related changes in O_2 consumption of gill, brain and muscle

Effect of metasystox on the circadian rhythm of tissue O_2 consumption in *C. striatus*

Time of day (h)	Gill			O_2 Consumption µl of O_2/g/h brain			Muscle		
	Normal	Experimental	% change	Normal	Experimental	% change	Normal	Experimental	% change
06.00	628 ± 21	212 ± 19	− 66	432 ± 16	212 ± 30	− 51	202 ± 18	110 ± 26	− 46
09.00	712 ± 18	216 ± 26	− 70	516 ± 36	300 ± 10	− 42	226 ± 23	128 ± 30	− 43
12.00	833 ± 31	203 ± 17	− 76	638 ± 46	312 ± 17	− 51	239 ± 19	130 ± 31	− 41
15.00	998 ± 40	298 ± 24	− 70	722 ± 50	298 ± 24	− 59	268 ± 29	157 ± 42	− 41
18.00	1230 ± 38	232 ± 26	− 81	822 ± 49	396 ± 31	− 52*	316 ± 16	188 ± 27	− 40
21.00	1164 ± 49	182 ± 22	− 84	665 ± 32	246 ± 18	− 63	276 ± 31	208 ± 41	− 21*
24.00	912 ± 18	193 ± 20	− 79	448 ± 28	200 ± 16	− 53	241 ± 41	149 ± 26	− 38
03.00	761 ± 26	151 ± 19	− 80	394 ± 21	174 ± 20	− 55	200 ± 28	108 ± 31	− 46

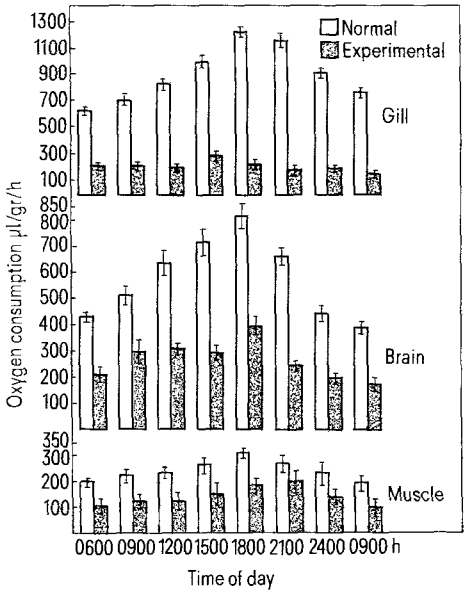
All values are mean ± SD of six individual determinations. The sign − indicates percent decrease compared with control. All experimental values are highly significant $p < 0.05$ except $*p < 0.02$.

tissues were shown after dichlorvos treatment. There are practically no other reports on the effects of pesticides on the circadian rhythm of tissue respiration in any freshwater fish. Hence, in the present work, an attempt has been made to study whether pesticide exposure affects the circadian rhythm of tissue respiration in a freshwater fish. Metasystox was chosen for the studies because it is probably the most widely-used organophosphate pesticide in India against various plant pests[5]. The test fish, *Channa striatus*, shows strong rhythmicities[6] in wholebody O_2 consumption and is frequently exposed to metasystox during agricultural operations.

Material and methods. A total of 120 *C. striatus* (10–15 g) of either sex were acclimated to 29 ± 1 °C for at least 15 days under laboratory conditions, as described by Natarajan[5]. During acclimation, the fish were kept under a 12-h light–12-h dark cycle (LD 12:12). This is a reasonable approximation of the summer photoperiod. Illumination was provided by overhead fluorescent tubes. Technical grade metasystox (Oxydemeton-methyl) (0,0-dimethyl S-(2-ethylsulfinyl)ethyl)phosphothioate) of 95% purity was obtained from Bayer Ltd, Bombay. LC 50/48 h as calculated by the probit method[7] was 5 mg/l for metasystox. Hence, ⅓ of the LC 50/48 h concentration (1.7 mg/l) was selected for sublethal treatment. At this concentration, the fish survived even after prolonged periods of exposure. Fishes in batches of six were exposed each time to 100 l of 1.7 ppm (1.7 mg/l) metasystox solution for 48 h. Simultaneously, controls were maintained under the same conditions with the exception that no metasystox was introduced. After exposure, the control and experimental fish were killed by a blow on the head between 09.00 and 10.00 h to reduce diurnal variations in the parameters measured. Homogenates of gill, brain and muscle were prepared in ice-cold 0.25 M sucrose solution using a Potter-Elvehjem type Teflon glass homogenizer. Tissue O_2 consumption was measured for at least 3 consecutive days with one tissue sample from one fish and the data was pooled and mean values were obtained for the respective time periods.

Data collection began at dawn (06.00 h) on the first day and ended 72 h later. O_2 consumption was measured in a Warburg constant volume respirometer (Gallenkemp, England), with fish Ringer fluid at pH 7.5 as liquid phase and air as gas phase. The time required to prepare a sample was 7 min and each test was replicated for a minimum of six readings. The experiment was repeated for over a week to observe whether the disruption of rhythmic activities recorded in the present experiment was persistent. The test described by Fisher[8] was employed to calculate the statistical significance of differences between control and experimental values.

Results and discussion. A clear-cut circadian rhythm (table, fig.) of tissue respiration is recorded in the control fish. Surprisingly, all the three tissues showed the maximum O_2 consumption at 18 h. This unimodal rhythm of tissue respiration corroborates the findings of earlier authors who have reported a maximum whole body O_2 uptake during the early part of the night in *C. striatus*. Metasystox exposure inhibited the respiratory rates of all tissues. Maximum inhibition is noted in the gill tissue, followed by brain and muscle. Since the gill is the main organ through which pesticide enters[9], it is greatly affected. The greater inhibition of O_2 consumption in the gill tissue may be due to 'Coagulation film anoxia'[10] in which the mucus is lost from the gill, as a result of which absorption of O_2 from the surroundings is adversely affected. Histological observations[5] also indicate that the secondary lamellae are most vulnerable to metasystox intoxication.



Histogram showing tissue respiratory rhythm of normal and metasystox exposed fish head.

Metasystox exposure also disrupted respiratory rhythm. The disruption does not indicate any specific trend. However, the peak period of O_2 consumption is completely eliminated in the pesticide-treated fish. Similar disruption of circadian rhythms has been reported for heavy metals in fish[11]. Since pesticides ultimately act at cellular and molecular levels, disturbing cellular functions by physicochemical reactions, the present findings indicate that at sublethal concentration, the normal physiological rhythm at the cellular level is completely destroyed. This disruption may have a significant effect on the survival ability of the animal in the physical environment. From a chronotoxicological point of view, a change in rhythmic activities of this kind must be regarded as an alarming reaction.

1 Acknowledgments. Prof. G. Sundara Rajulu is thanked for suggestions and UGC for financial assistance.

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Wound-induced alterations in survival of ^{60}Co irradiated mice: importance of wound timing[1,2]

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Summary. Wounding mice shortly before or shortly after lethal ^{60}Co irradiation enhances survival. Survival of wounded-irradiated mice may be due to enhanced hematopoietic recovery as measured by endogenous spleen colony (E-CFU-s) formation.
Key words. Trauma; radiation; combined injury; endogenous spleen colonies.

In previous publications[3-5] we established that skin-wound trauma 24 h prior to graded doses of ^{60}Co radiation resulted in survival of mice that ordinarily would die from radiation-induced hematopoietic failure. We also examined the repopulation of hematopoietic centers with early proliferative cells [colony forming unit-spleen-(CFU-s)] and committed progenitor cells [granulocyte-monocyte colony forming cell (GM-CFC) and monocyte-macrophage colony forming cell (M-CFC)] of sublethally irradiated-wounded mice.

While radiation has been employed in mice[6,7] and rats[8] in combination with surgical or wound trauma, the timing of wounding prior to or after whole body lethal radiation doses has not been comprehensively studied in both sexes of a single species. Our previous work[9] in combined injury (radiation wound trauma) and that of others dealing with the recovery from radiation injury[10] suggests the use of the endogenous-colony forming unit spleen (E-CFU-s) assay as a potential indicator of survival and recovery from radiation damage in mice. Additionally, wound trauma alone[11] perturbs the clonogenic cell compartments of the hematopoietic system. Since hematopoietic cells are involved in restoration after lethal radiation doses, we posited that wound timing relative to radiation exposure would effect survival from the combined injury. Therefore, in the present study we determined 1) the survival of mice wounded prior to or after lethal irradiation and 2) the number of E-CFU-s in mice wounded prior to or after lethal irradiation.

Materials and methods. Female and male (C57BL/6 X CBA)F1 Cum BR mice were obtained from Cumberland View Farms, Clinton, Tennessee. All mice were acclimated to laboratory conditions as previously described[11].

Between the hours of 10.00 and 14.00, groups of mice were given 4% skin surface wounds under light methoxyflurane anesthesia. The technique for wounding was previously described[11].

Whole-body irradiations of 40 rad/min (midline tissue) from bilaterally-positioned ^{60}Co elements were performed on mice placed in plexiglass restrainers. An ionization chamber calibrated against a National Bureau of Standards ionization chamber was used for dose determinations. Radiation exposures were performed between 10.00 and 14.00 h. Irradiations of traumatized animals were appropriately timed in relation to skin wounding (see figs 1 and 2).

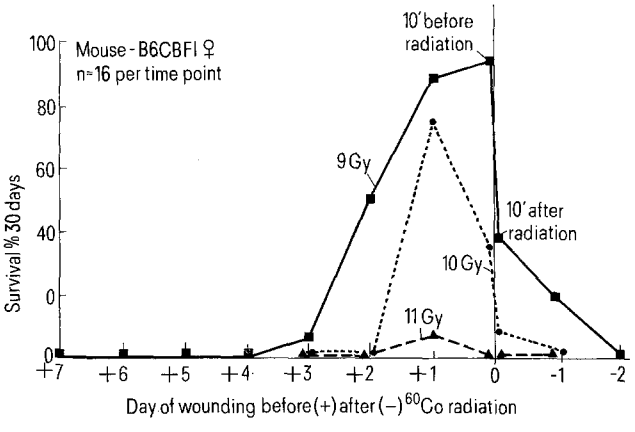

Figure 1. The 30-day survival percentages of wounded irradiated B6CBF1 female mice. At each time point indicated wounding was performed on 16 mice with the exception that 47 mice were injured 1 day after irradiation. All control-irradiated mice died (data not shown) with the exception that one mouse lived after 9.0 Gy. All control-wounded mice survived. 9.0 Gy ■—■; 10.0 Gy ●-----●; 11.0 Gy ▲– – –▲.

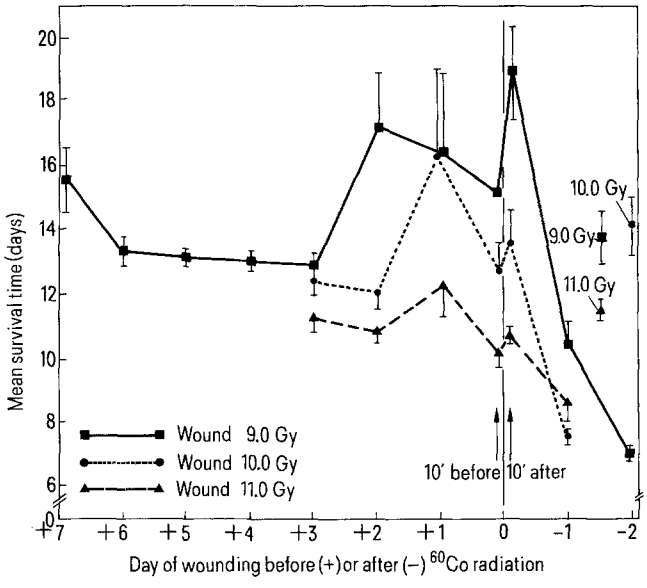

Figure 2. The mean survival times (days) of control irradiated and wounded-irradiated mice. These data reflect the survival of mice only within the 30-day observation period. See figure 1 legend for symbols.