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THE UNIVERSITY OF ALBERTA

VARIABLE EFFECTS OF X-IRRADIATION ON *TRIBOLIUM CASTANEUM*

AND *T. CONFUSUM* DUE TO STRAIN AND "AGE AT PUPATION"

by

(C) HANI SOLIMAN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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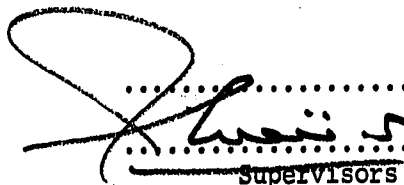OF DOCTOR OF PHILOSOPHY

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The undersigned certify that they have read, and
recommend to the Faculty of Graduate Studies and Research,
for acceptance, a thesis entitled "Variable effects of
x-irradiation on *Tribolium castaneum* and *T. confusum*
due to strain and "Age at Pupation" submitted by Hani
Soliman in partial fulfilment of the requirements for
the degree of Doctor of Philosophy.

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ABSTRACT

Eight geographic populations of *Tribolium castaneum*
and six of *T. confusum* were studied after single exposure
(10KR) to X-rays at 33°C and 70% relative humidity.
Second-day old pupae only were irradiated during the entire
pupation period.

T. castaneum exhibited a marked population difference
in radiosensitivity with a range from 6.8% (Canada) to
47.5% (Kenya) of adult emergence after irradiation.
T. confusum was less variable with a range of 1.7%
(Chicago) to 11.7% (Kansas). In general, *T. confusum* was
more radiosensitive than *T. castaneum* (the overall percent
emergence was 5.4 and 28.5, respectively). However,
within populations increasing pupation time is associated
with decreasing radiosensitivity. Populations originating
from warmer geographic localities were more resistant to
radiation than those from colder localities of *T. castaneum*.

Mean survival time after irradiation was different
among *T. castaneum* populations, within the range of 8.1
days for the population originated from Kenya and 5.5
days for the population from France. *T. confusum* did not
show any significant difference among populations. The
overall difference between the two species means (7.3
days for *T. castaneum* and 5.70 days for *T. confusum*) was
highly significant. The overall survival patterns of the

the two species were similar. However, after 5 days of adult emergence 65% of *T. castaneum* and only 27% of *T. confusum* still survived. Effect of age at pupation was only investigated in *T. castaneum* and found to be different among the four populations studied. A general decrease in the mean adult survival after irradiation with increasing age at pupation was observed for all eight populations (pooled). Intermediate pupae from the total pupation period yielded adults that survived longer than those from early or late pupae. Distribution of death of the emerged adults was also examined.

Inter-population correlation (genetic correlation) between mean adult survival time after irradiation and age at pupation was found to exist among populations of *T. castaneum*.

Developmental wing abnormalities and undifferentia-ed wing and abdomen were found to differ among populations and species. Populations originally from warm localities were more resistant to X-ray induction of developmental abnormalities than populations from cold localities. Also *T. castaneum* was more resistant than *T. confusum* in this respect. The length of pupal stage was also studied in *T. castaneum*. The pupal stage is decreased by increased age at pupation. The emerged adults after irradiation had shorter pupal periods than their controls.

Artificial selection for fast, slow and intermediate time to pupation in *Tribolium castaneum* (Jamaica) was successful in producing lines which differed from the unselected line for this character. After seven generations of selection all four lines were compared with respect to their percent survival and percent split wing after x-irradiation. Although the four lines differed in both traits no correlated response between percent survival or percent split wing and time to pupation was observed. However, there is an indication that rate of development may be negatively associated with rate of repair for the induction of split wing. There was no difference between males and females in their response to either selection or x-irradiation.

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GENERAL INTRODUCTION

Ionizing radiation constitutes a considerable part of
our environment, and consequently, it is important to obtain
information about its effects on different biological sys-
tems. Until recently most of our knowledge in this field

was obtained from bacteria, fungi, *Drosophila* and the mouse.
The use of *Drosophila* as an experimental organism in radia-
tion studies is based on very well known advantages it pre-
sents. However, these advantages are somewhat counter-
balanced by a rather short ($\simeq$ 50 days) adult life span to-
gether with the requirement for a complex artificial rearing
medium.

Two species of *Tribolium*, *viz.*, *T. castaneum* and *T.*
confusum, both of considerable ecological importance, are
attracting more investigators in the fields of evolution
and genetics. The advantages of using these two species in
experimental work are:

1. Size: The beetles are small. Pupal weights for *T.*
castaneum are about 2.5 mg. *T. confusum* is somewhat heavier.
Adults weigh less than the pupae (Soliman and Hardin, 1971a).

2. Life Cycle: The two species have a short life cycle.
At $34^{0}C$ *T. confusum* completes its development in about 26
days whereas *T. castaneum* needs about 23 days (Park and
Frank, 1948 and Soliman and Hardin 1971b).

3. Longevity: Both species are long-lived. The average
life span for *T. confusum* males amounts to 634 days (females:
447 days). *T. castaneum* males on the other hand require

547 days (females: 226 days), (Good, 1936).

4. Productivity: The two species are prolific. *T. confusum* females were found to produce on the average 860 viable eggs in 180 days and *T. castaneum* 614 eggs in 135 days when the medium was renewed at intervals of 30 days (Park, De Bruyn and Bond, 1958). If the medium is renewed every three or four days *T. confusum* is able to produce an average of 500 viable eggs in 60 days and *T. castaneum* 636 eggs in the same period of time (Sokoloff, 1961). Old females were found to lay eggs which are, however, inviable. Old males continue to produce viable sperm throughout their life span (Good, 1936).

5. Sexual Precocity: Erdman (1964) found that at 29°C and 70% relative humidity, males mature sexually on the second day of adult life, and females mature on the fourth day. Between 25° - 32°C, the onset of reproduction is more rapid at higher temperatures. At 29°C females of *T. castaneum* mature sexually two days earlier than females of *T. confusum* (Erdman, 1962a). *T. confusum* males are sexually productive from the first day of emergence, whereas those of *T. castaneum* mature during the second day after emergence (Erdman, 1964).

6. Breeding Medium: Both species can easily be reared at wide ranges of temperature and humidity in whole wheat flour supplemented with brewer's yeast as a source of Vitamin B. A culture including both sexes requires little care and will maintain itself at room temperature for about a year; the population is maintained at a steady level by cannibalism.

If the two sexes are separated, 5 grams of flour may be
sufficient to maintain about 100 adults for about six months.
All stages of the life cycle can be conveniently isolated
from the medium.

7. Resistance to Starvation: Both species can stand starva-
tion for prolonged periods of time. The resistance to star-
vation increases inversely with temperature. High humidity
(60 percent) enables the beetles to survive a longer period
than a low humidity (30 percent). Larvae are found to be
slightly more resistant to starvation than adults at all
temperatures studied (Good, 1936).

8. Resistance to Extreme Physical Conditions: Both species
seem to withstand extreme temperatures (Payne 1926, Sokoloff
and Shrode 1962, and Raros and Chiang 1970), low atmospheric
pressure (Kennington, 1953), and extreme forces of
acceleration (Sullivan and McCauley, 1960).

9. The genetic information on *Tribolium* has been growing
rapidly over the last few years. Until 1958 the description
and the mode of inheritance were limited to three mutations
in *T. castaneum* and two in *T. confusum*. During the six
years that followed, more than one hundred mutations have
been found in *T. castaneum* and fifty in *T. confusum*.(Sokoloff,
1966).

The few disadvantages of using *Tribolium* in radio-
biological research are the following:

1. Their very small chromosomes makes cytological and
cytogenetic studies difficult.

2. *Tribolium* is susceptible to a number of protozoan
parasites and mites.

3. Adults and larvae are cannibalistic. Both stages could
eat any of the different stages including their own stage.

4. Both species secrete quinones with which they condition
the medium. An allergic reaction to these quinones may
develop in people handling *Tribolium*.

There have been few studies on the effect of irradia-
tion on *Tribolium* since 1917 till the beginning of the last
decade. Since then more detailed studies have been conduc-
ted as the suitability of the flour beetles is being appre-
ciated in basic radiobiological studies. In general the
effect of irradiation on *Tribolium* has been found to vary
according to species, developmental stage and kind of
irradiation used. In the radiobiological literature on
Tribolium, there are only a few studies that consider
x-irradiation as an ecological factor (e.g., Park, *et al.*,
1958 and Erdman, 1966, 1970). These studies considered
fecundity, fertility, productivity and mortality separately
or in association with some other environmental factors
such as temperature and DDT concentration.

The present study deals with the effect of
ionizing radiation on various geographic populations of
Tribolium. The main objective is to determine the mode of
variation in resistance to a single exposure to X-ray
within and between the species *T. castaneum* and *T. confusum*.

Percent adult emergence, mean survival of adults, mode of death, and percent abnormalities after irradiation have been chosen as the parameters serving as criteria for the comparison.

A secondary objective is to correlate the difference in sensitivity to radiation with some components of the fitness of the population, e.g., age at pupation.

In addition, the genetic relationship between radio-sensitivity and age at pupation is studied. A selection experiment for the latter (which is known to be under genetic control, e.g., Englert and Bell, 1970) is carried out in one population of *T. castaneum* and the correlated response of the former is tested in a later generation after continuous selection.

It is evident that information about population fitness after exposure to irradiation is essential for the understanding of evolution at the intra- and inter- specific levels. However, the difficulty in studying the genetic-environmental complex in natural populations of *Tribolium* (as in many other species) limits us necessarily to laboratory populations. Left with this choice, the present study is conducted using laboratory populations with different geographical backgrounds.

I. DIFFERENCES WITHIN AND BETWEEN POPULATIONS OF

T. castaneum AND *T. confusum*

INTRODUCTION

Before starting any genetic study, it is of prime
importance to examine the existence of genetic variation in
the organism involved. For instance, various aspects of
variation in radiosensitivity have been studied in *Drosophila*
(Parsons *et al.*,1969) and in the mouse (Spalding *et al.*,
1969a). The existence of such variation has
not as yet been demonstrated in any of the *Tribolium* species.

The present study is an investigation into the compar-
ative x-irradiation resistance of the two closely related
species of *T. castaneum* and *T. confusum*. In order to gain
some information about the intra-specific variations in
radiosensitivity, a number of populations of each species
with different geographical background was employed. Some
of these populations of *T. castaneum* have been used pre-
viously by Soliman (1968) and Soliman and Hardin (1971 a,b)
to demonstrate other variant characters which influence
fitness.

MATERIALS AND METHODS

Populations:

Eight populations of *T. castaneum* and six of
T. confusum with different geographical origins and pre-
vious laboratory history were used in this study. The

populations of *T. castaneum* were originally derived from
samples obtained from Canada (Manitoba), United States
(Chicago and Kansas), Kenya, Jamaica, India, Portugal and
France. Similarly, the *T. confusum* populations originated
from Kansas, Chicago, Germany, Kenya, Scotland and Canada.
Since it is difficult to obtain recently captured samples
of *Tribolium* from their natural habitat, all the stocks
were obtained from various research laboratories in Canada,
United States, Europe and Asia. (See acknowledgements).

Culturing Technique:

After populations were obtained they were kept as
closed populations reproducing in mass. Each generation
approximately 100 adults about 2 weeks old, were placed in
a plastic box (5 x 2.5 x 3.5 cm high) with 50 grams
standard medium (95% whole wheat flour and 5% brewer's
yeast). Temperature was maintained at $33\pm1^{o}C$ and
relative humidity at $70\pm2\%$.

Experimental Procedures:

From each population, eggs were collected in mass from
about 100 adults for 48 hours. After 14 days, cultures
were checked daily for pupation by sifting the medium through
a copper sieve (8 mesh per lineal cm). When pupae appeared,
they were isolated from the medium and placed in a 15 gram
glass creamer, covered with an identifying cardboard cap,
for x-irradiation next day. From the daily yield of pupae
a maximum of 100 pupae were used whenever possible for

irradiation and the remainder were kept as controls (Table I). This procedure was continued daily till pupation ceased. The controls were treated in exactly the same manner as the irradiated pupae except for the exposure to ionizing radiation. When not used, the cultures and creamers were kept in a temperature and humidity controlled incubator.

Pupae were used in the present study because pupation constitutes a definite and easily observable event in the life cycle of the insect. Pupation time however is variable even for eggs collected over a short time due to differences in larval growth. Since pupation takes place at a time when differentiation is still in progress, pupae can be used advantageously for studies concerning differential organ and tissue sensitivities to environmental conditions.

Irradiation Treatment:

Radiation was delivered by an X-ray machine operated at 300 kv and 20 ma. The target distance was about 15.5 cm. A dose rate of 500 R/min. measured in air with a Philips dosimeter was administered to all creamers. Dose rate adjustment was always carried out at the beginning of each irradiation treatment. All samples from the same day received during one single exposure a total of 10 KR in 20 minutes. The creamers were placed evenly on a circular line at a turntable and rotated throughout the treatment to ensure uniform X-ray exposure.

Treatment after Irradiation:

. After irradiation, the creamers were returned to the temperature and humidity controlled incubator. When the adults emerged, they were removed daily, counted and trans- ferred to a new creamer with fresh medium.

Statistical Analysis:

The number of pupae irradiated daily and the control for each population are presented in Table I. Sensitivity was measured as percent adult emergence from a given number of irradiated pupae. The significance of the difference between any two percentages was tested using the angular transformation t remove the dependence of the variance on the mean. These transformed values are more likely to be normally distributed than percentages. The test takes into account the sample size and is valid when the sample size is large (Sokal and Rohlf, 1969).

This experiment was originally designed to allow an analysis of X-ray sensitivity during the entire period of pupation for all populations. However, after the data were collected, it was only possible to perform a limited analysis of variance on the angular transformation of the daily percent adult emergence on six out of a total of eight populations of T. castaneum. Since few pupae were available after the 22nd day (Table I), they were included in this day and the analysis of variance was carried on only 7 days of pupation. Due to a shortage of data (zero emergence for various days) populations of T. confusum were not analyzed in this manner.

TABLE I

Number of Pupae Irradiated from Each Day of Pupation of Different Populations of *Tribolium*

Population	Age at Pupation (day)									
	16	17	18	19	20	21	22	23	24	25
T. castaneum										
Kenya	79	100 (66)	100 (42)	100 (31)	100 (25)	100 (7)	23	6		
Jamaica	10	29	61	91	100 (9)	100 (100)	100 (58)	53	17	8
India	100 (100)	100 (100)	100 (32)	100	42	36	13			
Chicago	6	14	51	100 (100)	100 (100)	100 (100)	57	18	8	5
Portugal	88	100 (33)	100 (77)	100 (93)	100 (48)	100 (72)	82	24	9	5
Kansas	64	100 (62)	100 (100)	100	100	84	15			
France				11	39	100 (26)	100 (55)	100 (15)	68	8
Canada	100 (100)	100 (64)	69	10						
TOTAL	447	543	581	623	581	620	290	201	102	26

TABLE I cont.

Number of Pupae Irradiated from Each Day of Pupation of Different Populations of *Tribolium*

| | | | | | Age of Pupation (day) | | | | | |
Population	16	17	18	19	20	21	22	23	24	25
T. confusum										
Kansas					11	54	100 (44)	100	39	11
Germany					14	57	61	14	9	
Kenya				75	100	100	100	71	17	7
Scotland					23	90	100 (17)	79	27	7
Canada			13	70	79	100 (62)	100 (100)	100	31	7
Chicago				11	34	100 (57)	100 (92)	83	20	7
TOTAL			13	156	261	501	561	447	143	37

Number between parenthesis indicates number of pupae used in control (only a maximum of one hundred and a minimum of seven was used).

RESULTS

Species and Population Survival:

Table II summarizes total survival in each population after exposure to a single dose of x-irradiation within each species as measured by percent adult emergence after irradiation of pupae. Limited variation of the percentage of adult emergence of the controls can be shown from these data. However, comparing the controls with their respective irradiated populations, an overwhelmingly higher percentage of adult emergence exists for the control samples. From Table II it is clear that *T. castaneum* exhibits a marked variation in radiosensitivity with a range from 6.80 (Canada) to 47.53% (Kenya). *T. confusum* is less variable with a range from 1.69 (Chicago) to 11.74% (Kansas). In general, *T. confusum* is more radiosensitive than *T. castaneum*. This relationship is consistent even when population samples of the two species were from the same locality such as Kenya, Chicago, Kansas and Canada. The percent of emergence for both species amounts to 5.35 and 28.48 respectively. This observed difference in x-irradiation sensitivity between the two species investigated is consistent with data obtained by other investigators (e.g., Erdman, 1968).

In addition, Table II suggests that *T. castaneum* populations sampled from warmer countries (Kenya, Jamaica, and India) are more resistant to radiation than those sampled from colder countries (Canada and France), regardless of the

TABLE II

Percentage Adult Emergence after X-irradiation of Second-day
Old Pupa of Various Geographic Populations of *Tribolium
castaneum* and *T. confusum*

	X-irradiated		Control	
Population	N	% Adult emergence[1]	N	% Adult emergence
T. castaneum:				
Kenya	608	47.53	171	100.00
Jamaica	569	37.79	167	100.00
India	491	31.77 a	232	96.55
Chicago	459	26.43 a b	300	99.67
Portugal	708	23.34 b	323	98.45
Kansas	563	18.83 c	162	98.15
France	426	15.26 c	96	100.00
Canada	279	6.80	161	98.76
TOTAL	4103	28.48	1612	98.82
T. confusum:				
Kansas	329	11.74 a		
Germany	171	8.64 a b		
Kenya	484	5.11 b c		
Scotland	326	4.60 b c		
Canada	493	3.34 c	62	98.39
Chicago	380	1.69 c	57	98.25
TOTAL	2183	5.35	119	98.32

[1] Any two populations having the same letter are not
significantly different at P < 0.05

fact that these populations have been maintained for a con-
siderable time under constant laboratory conditions. This
observation suggests a possible correlation between
radiosensitivity variation and geographic variation.

Effect of Age at Pupation:

The results of the analysis of variance on the angular
transformation of the percent adult emergence after irradia-
tion of the daily yield of pupae of *T. castaneum* with popula-
tions and age at pupation as sources of variation, are pre-
sented in Table III. For both variables there is a highly

TABLE III

Analysis of Variance of Adult Emergence Percent[1]
after Irradiation in *Tribolium castaneum*

Source of variation	d.f.	M.S.	F
Population	5	491.99	9.54***
Age at pupation	6	1479.87	28.71***
Error	30	51.55	

[1]After applying the angular transformation.

***$p < 0.001$

significant effect of x-irradiation on percent adult emergence.
Of particular interest is the significant "age at pupation"
effect. This relationship is illustrated graphically in
Figure 1. This graph indicates that an increase of the age

FIGURE 1 Relationship between age at pupation and percent adult emergence (angular transformation) after exposure of second-day pupae to single dose of X-ray (10KR) of various geographic populations of *Tribolium castaneum*. For number of pupae irradiated at each point see Table I. Data after the 23rd day were grouped to increase sample size.

at pupation is associated with an increase in resistance to
x-irradiation. It is hypothesized that such an increase in
resistance to radiation is due to increased tissue differ-
entiation at the late time of pupation. Although the exis-
tence of bimodal (Howe, 1961, 1965) or of a curvelinear
relationship between pupation age and radiation sensitivity
is feasible, the tests performed only considered a simple
linear relationship (Table IV). As can be observed all the

TABLE IV

Relationship Between Sensitivity to X-irradiation
and Age at Pupation of *Tribolium castaneum*

Population	N	r	b ± SE	
Kenya	7	0.92**	7.78	1.52
Jamaica	7	0.82*	7.12	2.24
India	6	0.88*	8.11	2.17
Chicago	7	0.94**	8.25	1.37
Portugal	7	0.83*	6.01	1.79
Kansas	6	0.94**	8.41	1.48

* $P < 0.05$
** $P < 0.01$

correlation coefficients obtained are significant. The
regression coefficients indicate that, within the limitation
of the available data, an increase in age at pupation by one
day results in an increase in adult emergence by approximately
seven percent.

The same relationship between age at pupation and percent adult emergence for the pooled data of all the populations within the two species is presented in Figure 2. Although the mean time to pupation proved to be longer for *T. confusum* when compared with *T. castaneum* (Figure 2), both species exhibit the same trend (b = 3.96 for *T. castaneum* and 3.22 for *T. confusum*) indicating a similarity between the two otherwise closely related species. Similarly a positive relationship between age at pupation and radiosensitivity seems to exist within the two species (Table V) and these

TABLE V

Relationship Between Sensitivity to X-irradiation and Age at Pupation of *Tribolium castaneum* and *T. confusum*

Species	N	r	b ± SE	
T. castaneum	9	0.87**	3.96	0.89
T. confusum	7	0.91**	3.22	0.66

**P $\leq$ 0.01

results concur with those obtained by Shipp (1966) who showed that rapidly growing *T. confusum* beetles are more susceptible to killing by gamma radiation than slowly growing beetles.
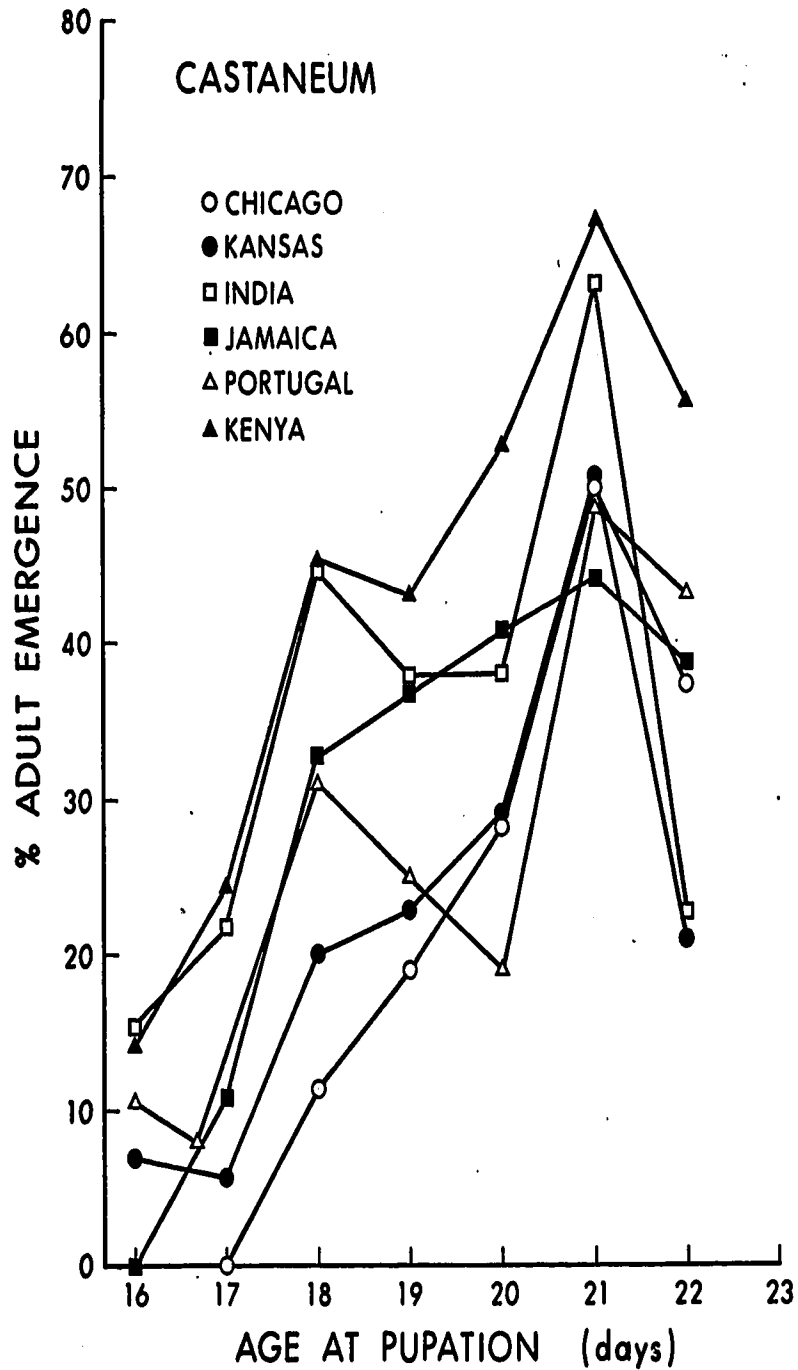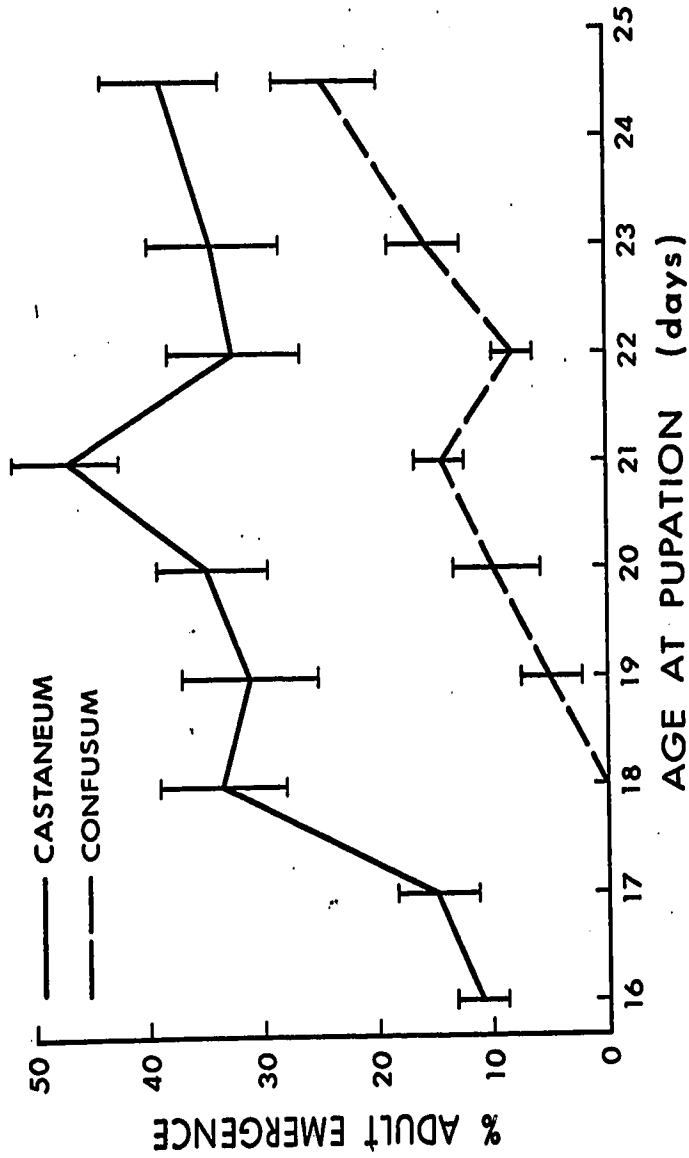
FIGURE 2 Relationship between age at pupation and percent adult emergence (angular transformation) after exposure of second-day pupae to single dose of X-ray (10KR) of the pooled data of *Tribolium castaneum* and *T. confusum*. Each point represents the mean of all populations ± standard error. For number of pupae irradiated at each point see Table I.

<u>DISCUSSION</u>

In *Tribolium* and specifically in *T. castaneum* and *T. confusum*, the spectrum of radiosensitivities among their populations has remained unknown, although extensive experimental use of these two species has been made in radiobiological studies. The present investigation indicates that variation in radiosensitivity exists among populations of the same species, It is postulated here that these differences among populations of the same species or between species might have arisen by divergent evolution due to natural selection resulting in adaptation of each population's gene pool to its local environment. As in *Drosophila*, the significance of the gene-environment complex has been recognized in *Tribolium* for various fitness traits (Park *et al.*, 1964; Karten, 1965 and Soliman and Hardin, 1971 a,b) and in relation to ionizing radiation (Erdman, 1966, 1970).

In the present study it was found that *T. castaneum* showed increased resistance to x-irradiation, as measured by percent adult emergence, compared with that of *T. confusum*. Erdman (1962b, 1963, 1966, 1968, 1970) compared the radiosensitivity of the wild type "Brazil" of *T. castaneum* with the wild type "Chicago" of *T. confusum*. In all his studies, *T. confusum* was consistently found to be far more sensitive, at all dose ranges for all developmental stages of the life cycle, whether the criterion was reproduction, lethality or sterility. Moreover, the low levels of lethality of *T.*

castaneum were found to persist through the adult stage

(Erdman, 1962) or even to decrease with the advancing age of

the insect (Erdman, 1968). In *T. confusum* on the other hand,

young pupae were found to be less radiosensitive when com-

pared with old pupae or adults (Erdman, 1962, 1968).

Only one population of *T. castaneum* (*i.e.,*Canada) is

more sensitive than two populations of *T. confusum* (*i.e.,*

Kansas and Germany, Table II). The reason for this distinc-

tive difference in radiosensitivity between those two closely

related species of *Tribolium* is not as yet completely clear.

The additional pair of autosomal chromosomes in *T. castaneum*

can hardly be considered to cause the difference in radio-

sensitivity. From measurements it appears that the total

chromosomal size in both species is the same (Smith, 1952 a,

b). *T.confusum* possesses a much larger pair of X- and Y-

chromosomes, and eight pairs of autosomes, whereas *T.*

castaneum has nine pairs of autosomes, and a chromosome

approximately the size of the autosomes and a small Y

chromosome. It has been suggested that *T. confusum* descended

from a primitive form with a chromosome complement similar

to that of *T. castaneum* by means of a fusion of the X chromo-

some with one pair of the autosomes, with the other homolo-

gous chromosome taking over the mechanical functions of the

minute Y chromosome (See Sokoloff, 1966). However, it

has been stressed by Smith (1952 a) that with the translocated

autosome forming the neo-X chromosome in *T. confusum,* the

number of fully sex-linked genes in this species must be
relatively higher than those of *T. castaneum*.

When differential radioresistance between the two species
was determined as the percent of dominant lethals induced by
x-irradiation, *T. confusum* contained more lethals than *T.
castaneum* (Erdman, 1963) indicating that specific sites (genes)
of the chromosomes are perhaps more sensitive to radiation in
T. confusum than in *T. castaneum*. Alternatively, dominant
lethals induced by radiation may be associated with the
sex chromosome.

The difference in lethality among populations could
be due to differences in sensitivity of various tissues.
Erdman (1968) found, for instance, an increase in radia-
tion sensitivity of the *Tribolium* prepupal stage when
compared to later stages, which he considered to be a
result of tissue reorganization and cell division in pre-
paration for the pupal stage. In *Drosophila* the somatic
resistance to X-ray radiation was studied in larvae from
isogenic stocks (Eiche, 1970). Somatic resistance developed
in larvae irradiated with 1120R in earlier generations in
comparison with the control line. Significant genetic
differences were found to exist between irradiated and non-
irradiated lines (Valentin, *op. cit.*). Hogan (1968) argues
that the lethal effects in irradiated *Tribolium* are the
result of damage to somatic tissue which is synthesizing

DNA. DNA synthesis was found to occur in pupae and newly
emerged adults of *Tribolium* (Chaudhary *et al.*, 1964, Devi
et al., 1963 and Gall, 1970), contrary to the general obser-
vation in *Diptera* and *Hymenoptera* where no cell division
occurs after larval stage (Grosch, 1962).

The observed variation in radiosensitivity among pop-
ulations is expected to have a genetic basis, since all pop-
ulations were reared under the same environmental conditions.
Parsons *et al.* (1969) found that eighteen strains derived
from a wild population of *D. melanogaster* differed in their
resistance to gamma-rays as measured by percentage of mor-
talities. Genetic analysis of the most resistant strains
and most sensitive ones showed that the differences were
controlled by genes having mainly additive effects. By
using chromosome assay techniques they were able to locate
these genes on the second and the third chromosome. Again
in *Drosophila*, Ogaki and Nakashima-Tanaka (1966) found a
gene in the third chromosome which affects radioresistance
where resistance was dominant over sensitivity.

An association between radioresistance in *Tribolium*
and heat resistance is indicated from studies by Ogaki and
Nakashima-Tanaka (1966) and Eiche (1970). In this respect
it is of particular interest to note that in the present
study the most resistant populations of *T. castaneum*
originated from warmer regions, e.g., Kenya, Jamaica and
India, whereas the most sensitive populations were originally

collected from colder localities, e.g., Canada and France
(Table II), (the world distribution of *T. castaneum* is
mainly in the tropical region, while *T. confusum* inhabits
colder localities). In the present study *T. castaneum* was
shown to be significantly more resistant to x-irradiation
than *T. confusum*. The effect of low temperature on survival
in *T. castaneum* and *T. confusum* was studied by Raros and
Chiang (1970) and Takoshiwa and Nikkuni (1969). *T. confusum*
was found to be more tolerant to lower temperatures than
T. castaneum whereas wild populations were more resistant
than inbred populations. Since all populations used in the
present study were not previously exposed to x-irradiation,
and were reared under the same temperature in the laboratory
it is possible to suggest that population differences in
X-ray sensitivity are a reflection of differences in
temperatures that might have influenced the populations in
their original habitats.

The relation between radiosensitivity and temperature
in *Tribolium* is of interest and deserves closer attention.
Hogan (1968) observed a spectacular increase of mortality
when irradiated *T. confusum* was fed on an artificial diet
and transferred to a low temperature environment. Ducoff
and Bosma (1967) found that *T. confusum* adults at 22° were
distinctly more X-ray sensitive than when kept at $30^\circ C$.
Yang and Ducoff (1969) showed that exposure of *T. castaneum*
larvae to $15^\circ C$ before X-irradiation increased their radio-

sensitivity. The same workers have recently (1971) found
that the rate of recovery from radiation induced damage in
T. castaneum larvae is dependent on each of the three tem-
peratures studied (30, 22 and 15°C). Rate of recovery showed
a maximum at 30°C and at 15°C proceeded at a very slow rate.
Moreover, Crenshaw (1965) reported that at sub-optimal
temperatures, progeny of irradiated *T. confusum* males ex-
hibited a higher productivity than progeny from control
(unirradiated) males. The author infers that "induced
polygenic overdominance" may have a greater fitness value
to the carriers under conditions of stress, than under normal
conditions.

Although comparison of wild populations has not pre-
viously been carried out in any radiological studies con-
cerning *Tribolium*, a few studies have included comparison
of mutants and wild types. For example Erdman (1966) found
that although the black autosomal mutant "sooty" of *T.
castaneum* showed an increase lethality post-irradiation when
compared with the wild type "Brazil", the productivity of
the two populations remained the same for a given X-ray
exposure. However, radiation sensitivity during the differ-
ent developmental stages of the two populations appeared to
be the same (Erdman, 1968). In addition, the mortality of
the two populations was found to be equal at 4KR and below
this dose the mortality of the mutant increased steadily
(Erdman, 1970). More recently, Ducoff *et al.* (1971a) found

that the survival of the mutant "McGill Black" males is significantly lower than that of wild type "Oklahoma" males of *T. confusum* irradiated with equal doses.

Restriction of food intake of *T. castaneum* has been demonstrated to increase radiation-induced mortality (Rogers and Hilchey, 1960). Also, Hogan (1968) argued that the increased X-ray induced mortality in *T. confusum* is possibly due to starvation. Jafri (1965) reported injuries to the midgut wall of *T. castaneum* adults after exposure to x-irradiation. The injured midgut wall showed an invasion by the intestinal flora and consequently an increased susceptibility to bacterial infection pathogenic to *Tribolium*. On the other hand, Ducoff and Howell (1966) were not able to attribute the death of irradiated *T. confusum* adults to infection. Ducoff *et al.* (1971b) reported that irradiation of the midgut, which possesses a renewable lining in the Coleoptera, is sufficient to cause death.

In *Tribolium* as in so many other insects, the difference between the radiosensitivity of well defined developmental stages is striking. However, little is known about differences within a particular stage. In general, radiosensitivity increases with the stage of development, e.g., eggs are more sensitive and adults more resistant (e.g., Erdman, 1962b, 1968, and Henderson, 1964). Yet a few studies throw some light on the effect of increasing age of a particular stage on

radiosensitivity of that stage. For example, it has been
found that the sensitivity of the egg stage is an inverse
function of its age (Ducoff and Bosma, 1963). Older larvae
of *T. castaneum* were found to be more sensitive than
younger larvae (Erdman, 1962b, 1968, Yang and Ducoff, 1969).
Larval age of *T. confusum,* on the other hand, appears to
have a constant effect on lethality (Erdman, 1962b, 1968).
An increase in pupal age is usually associated with an
increase in radioresistance (Erdman, 1962b, 1968 and Ducoff
and Bosma, 1963). Although the same radiosensitivity has
been reported for one day old as well as for ten day old
adults of *T. castaneum* (Erdman, 1962b, 1968), adults of *T.
confusum* showed an increase in radiosensitivity with an
increase in adult life span (Erdman, 1962b, 1968, Ducoff,
1967, and Ducoff and Bosma, 1967). It is then evident that
the effect of age on radiosensitivity in *Tribolium* is
different at different developmental stages, and with few
exceptions the two species behave similarly in this respect.
Nevertheless, Yang and Ducoff (1969) have suggested that
differences in recovery rate and in time to pupation may be
responsible for the age effect on X-ray sensitivity of *T.
castaneum* larvae. The present study provides evidence for
the validity of the second suggestion. For all the pop-
ulations of *T. castaneum* as well as for the pooled population
data of both species, radioresistance increases with
increasing age at pupation.

Despite the fact that in the present study a linear relationship between age at pupation and radiosensitivity could be established, non-linearity can also be expected. McDonald (1961) showed a departure from linearity for the dose response effect in *T. confusum* spermatogenesis, determined by the meiotic stage at the time of x-irradiation. Non-linearity reflects the differential sensitivities between dividing - and nondividing - cells (Erdman, 1968). For example, the radiation sensitivities of the entire life cycle for a mutant and the wild type of *T. castaneum* were compared with the wild type of *T. confusum* (Erdman 1962b, 1968). As expected the two populations from the same species showed similar non-linear patterns for lethality, while the two wild types of the different species diverged in later stages of their developmental differentiation.

SUMMARY

Eight geographic populations of *Tribolium castaneum* and six of *T. confusum* were examined in regard to their radiosensitivity as measured by percent of adult emergence after single exposure (10 KR) of X-ray at 33° C and 70% relative humidity. Second-day old pupae only were irradiated during the entire pupation period.

T. castaneum exhibited a marked population difference in radiosensitivity with a range from 6.8% (Canada) to 47.5% (Kenya) of adult emergence after irradiation. *T. confusum* was less variable with a range of 1.7% (Chicago) to 11.7% (Kansas). In general, *T. confusum* was more radiosensitive than *T. castaneum* (the overall % of emergence was 5.4 and 28.5, respectively). Within populations there were some indications that increasing pupation time is associated with decreasing radiosensitivity. It is also suggested that populations originating from warmer geographic localities might be more resistant to radiation than those from colder localities.

Age at pupation appears to be positively associated with radioresistance and non-linearity of the relationship is also apparent in some populations.

Physiological and genetical factors that might have been the reason for population and species differences are discussed.

II. SURVIVAL OF ADULTS EMERGING AFTER X-IRRADIATION OF PUPAE

INTRODUCTION

A characteristic effect of ionizing radiation is the reduction of the natural life span; early death of the irradiated organism can result from a sufficient dose. In *Tribolium confusum* changes in life span after exposure to ionizing radiation was first observed by Davey (1919) who used X-rays and later by Cork (1957) employing gamma-radiation. At low dose levels the effect consisted of prolongation of life span, whereas it was shortened after exposure to high or moderate doses. Recently, the factors and mechanisms that influence the survival-time after irradiation have been investigated in mice (Spalding *et al.*, 1969b), *Drosophila* (Blair and Baxter, 1970) and *Tribolium* (Ducoff and Bosma, 1967 and Ducoff *et al.*, 1971b). Ducoff *et al.* (1971b) using *T. castaneum* and *T. confusum*, reported that survival-time after moderate doses of ionizing radiation is dependent on strain and independent of dose; this suggests a single mode of death.

The present study is a continuation of the previous investigation (Section I) on X-ray sensitivity of *Tribolium* pupae after exposure to a moderate single dose of X-ray (10KR). The emerged adults from the previously irradiated pupae were used. Mean survival-time, survival pattern and effect of age at pupation on survival after irradiation served as parameters.

MATERIALS AND METHODS

The analysis of the data is based on the numbers of adults which emerged from x-irradiated pupae derived from the same eight geographic populations of *T. castaneum* and six populations of *T. confusum* which were used in the previous study. (For more details see p. 6).

Whenever the data allow different comparisons were made. All populations were used for comparison of mean survival time. However, only the pooled data from all populations of *T. confusum* were used for comparison of survival patterns in the two species (since the numbers of adults emerging after irradiation of *T. confusum* were small). For the same reason, only four populations of *T. castaneum* (Kenya, Jamaica, India and Portugal) were used when "age at pupation" was studied. Also, data for this parameter were pooled for all populations of this species. (*T. confusum* was not used in "age at pupation" effect).

The survival-time of adults emerging after a single exposure to 10KR of X-rays of two-day old pupae was determined at two-day intervals starting from the fifth day of emergence. The Student-Newman-Keuls test (Sokal and Rohlf, 1969) was used to detect statistical differences between any two populations of *T. castaneum* in respect of average time of survival of adults after emergence. Student t-test was applied to compare the weighted means of all populations of the two species.

On examining the age at pupation effect of the four
populations of *T. castaneum* the data were grouped in four
categories according to age: (A) adult emergences
originated from early pupated individuals during the first,
second and third day of the pupation period, (B) adult
emergences originating from intermediate pupae, pupated
during the fourth and fifth day of the pupation period,
(C) adult emergences originated from intermediate pupae,
pupated at the sixth day of the pupation period and (D)
adult emergences pupated during the seventh, eighth and
ninth day of pupation.

RESULTS AND DISCUSSION

Mean Survival Time:

Mean life span values of the eight populations of
T. castaneum and six populations of *T. confusum* are shown
in Table I. Population means ranged from 5.48 days (France)
to 8.05 days (Kenya). Among *T. confusum* populations there
was no detectable difference. However, the population from
Kenya showed the longest and the population from Germany
the shortest mean survival time post-irradiation. The
overall mean survival time for *T. castaneum* (7.25 days) was
significantly longer than that   observed for *T. confusum*
(5.70 days). These results are in agreement with those
reported by Watters and MacQueen (1967) for populations
exposed to gamma radiation. Although not confirmed, it
appears that the same two Canadian populations as used in the

TABLE I

Adult Survival Time of Geographic Populations of
Tribolium castaneum and *T. confusum* after Single
Exposure to x-irradiation by 10KR

Population	N[1]	Mean Survival time[2] (days)	SE
T. castaneum:			
Kenya	294	8.05 d	0.13
Jamaica	221	7.08 a b	0.13
India	259	7.41 b c	0.12
Chicago	129	6.58 a	0.15
Portugal	232	6.95 a	0.13
Kansas	101	7.73 c d	0.27
France	83	5.48	0.09
Canada	39	8.03 c d	0.36
TOTAL	1358	7.25	0.06
T. confusum:			
Kansas	39	5.46 e	0.20
Germany	15	5.40 e	0.21
Kenya	29	6.38 e	0.30
Scotland	19	6.26 e	0.43
Canada	37	5.54 e	0.20
Chicago	7	5.86 e	0.59
TOTAL	146	5.70	0.11

[1] emerged after irradiation

[2] any two populations having the same letter are not significantly different at $P<0.05$ by Student-Newman-Keuls test.

present communication were employed by these authors.

The different factors that may influence the reduction in normal life span after irradiation have been studied by different investigators in *Tribolium* and in other organisms (e.g., Ross and Cochran, 1963; Ducoff and Bosma, 1967). The latter authors found that beetles incubated at 22°C after x-irradiation showed that the period during which mortality occurs is twice as long when compared with beetles kept at 30°C. In the present study it is apparent that the experimental temperature (33°C) favours populations derived from warmer localities. Consequently, the population from Kenya of *T. castaneum* showed the longest mean survival time and the population from France the shortest. The Canadian population, which presumably came from a cold region, exhibited a mean survival time as long as the population from Kenya (Table I). In addition relative humidity was found to affect mean survival time after irradiation (Farkas, 1966). The mean survival time observed under 70% relative humidity was significantly longer than that at either 30 or 90% relative humidity.

Other factors that may interfere with the life span in *Tribolium*, concern pathogenic infections and food uptake. Rogers and Hilchey (1960) found that ingestion of food was reduced after irradiation of *T. castaneum*. The nutritional state of the beetles after irradiation, affected their life expectancy, although starvation was not of primary importance in causing death of the irradiated individuals. The life

span of *T. confusum* after exposure to x-irradiation and infection of the midgut was shortened by approximately 7 days (Jafri, 1965). A breakdown of natural barriers between midgut lumen and body cavity was considered as the cause of death after moderate exposure suggesting an intestinal syndrome as in mammals (Ducoff *et al.*, 1971b).

Beside the physical and biological conditions that might influence the life span of an irradiated beetle, the genetic make up of the individual or of the population appears to be a contributing factor. In *Drosophila*, it has been demonstrated that differences in damage after exposure to irradiation which resulted in shortening of the life span of the fruit flies can be attributed to induction of chromosomal aberrations. A genetic basis for such shortening of the life span has been demonstrated by Oster (1959).

Some of *T. castaneum* populations which showed a long mean survival time (Table I) also showed a high survival rate (e.g., Kenya, Table II, p. 13). In other populations the reverse occurred: short mean survival time was associated with low percent survival (e.g., France, Table II, p. 13). The above observations suggest a possible correlation between percent survival and mean survival time after irradiation. The statistical test indicated that such inter-population correlation does not exist for *T. castaneum* $(r=0.16, P<0.1)$ and for *T. confusum* $(r=0.0)$, at least within the limitation of the present study. This point is of

interest, since it illustrates an interaction between populations and the criteria used to measure sensitivity to radiation among these populations. At the present there are two possible explanations for such a lack of correlation, which are not necessarily mutually exclusive.

Firstly, some or all of the populations consist of two sub-populations, one of them is more resistant than the other and these populations differ in the proportion of the two sub-populations. Irradiation will eliminate the susceptible sub-population and leave the resistant one which may or may not have the same mean of adult survival time. It is possible, according to this explanation, that mean survival time after irradiation is influenced by genes different from those responsible for lethality. This hypothesis is supported by two observations in *Tribolium*: (i) in various experiments it had been found that after irradiation the last group of individuals to die, far outlived the rest of the population (see for example, Watters and MacQueen, 1967), and (ii) two populations with overlapping distributions for pupal weight and developmental periods have been observed in both laboratory (Howe, 1961) and field (Howe, 1965) populations of *T. castaneum* strains from Kenya. In addition, the observed bimodality in the relationship between lethality and age at pupation, in the present study (Figure 1, p. 15) may be a reflection of such population polymorphism. The evolutionary significance of such polymorphic phenomena may be that they provide a

mechanism by which the population could be adjusted to diverse environmental conditions such as exposure to irradiation.

Secondly, there may be different genes controlling resistance for different metamorphic stages in the life cycle. Since all populations have been reared under the same environmental conditions, it is possible to assume that lack of inter-populations correlation is a reflection of lack of genetic correlation (linkage or pleiotropy).

In *Drosophila* Lamb (1964) and Lamb and Maynard Smith (1964) rejected the hypothesis that somatic mutations constitute the cause of life shortening, since diploid and triploid females of *D. melongaster* showed the same fractional life shortening per unit dose. Recently, Baxter and Blair (1967, 1969) and Blair and Baxter (1970) suggested that the two types of injury, i.e., lethality and life shortening are mutually independent. The lethal injury is mainly cytoplasmic and the damage is of a chemical nature and reparable. On the other hand, life shortening is due to structural injury to the nuclear components (chromosomal breaks, Muller, 1963) which in these cases are irreparable.

Survival Pattern:

The general pattern of the time course survival of adults after irradiation of pupae of the eight populations of *T. castaneum* and of the overall populations of both species are plotted in Figures 1 and 2. Although the adult survival
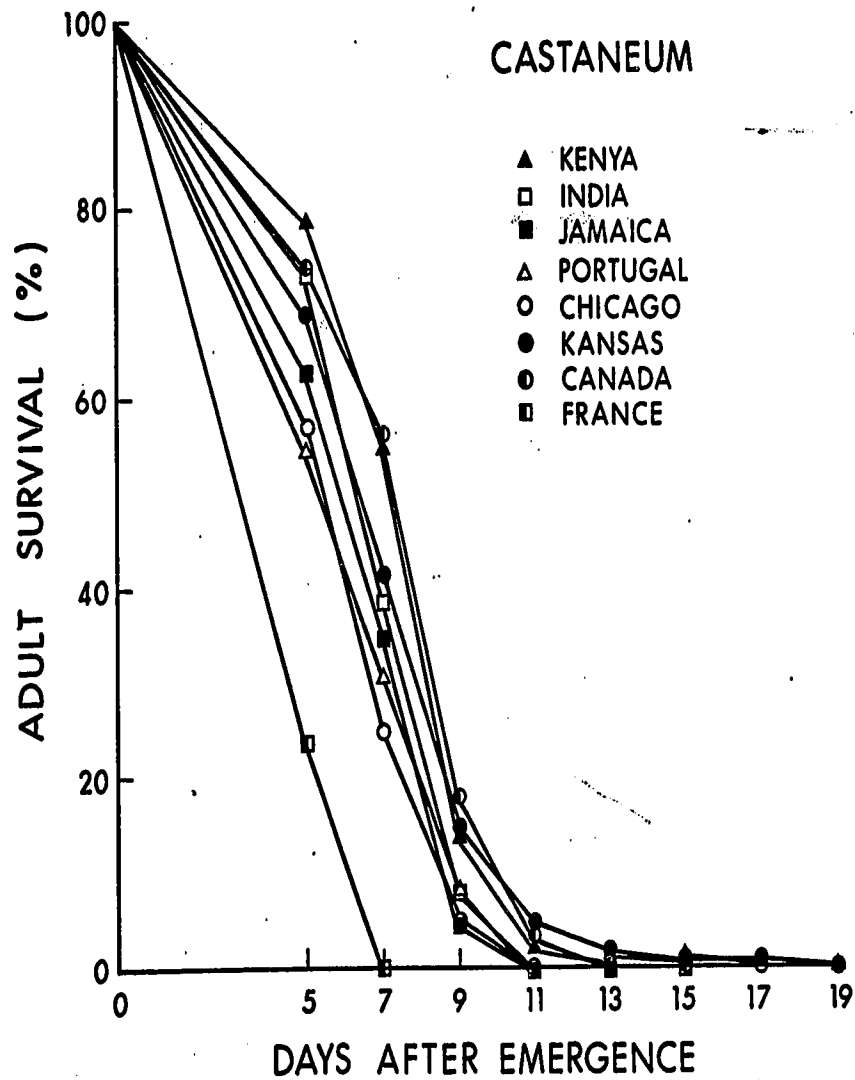
FIGURE 1 Effect of x-irradiation of pupae at 10KR on the survival of adults of various geographic populations of *Tribolium castaneum*.
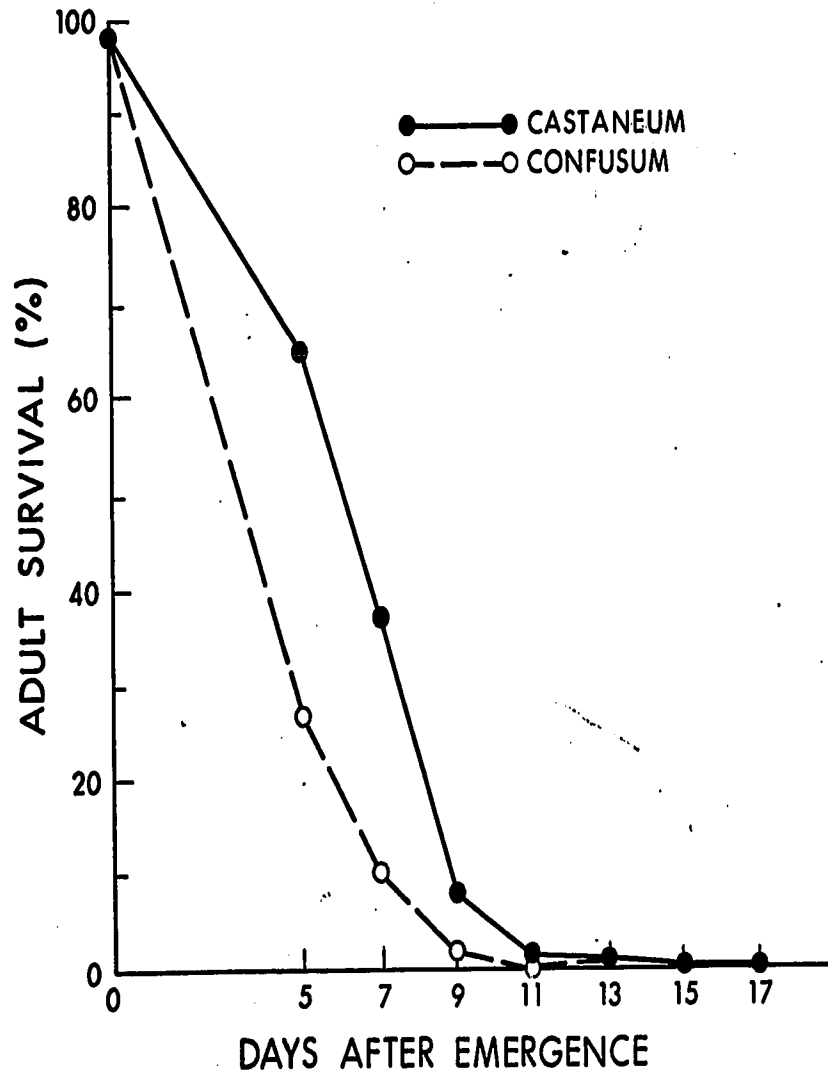
FIGURE 2 Effect of x-irradiation of pupae at 10KR on the survival of adults of *Tribolium castaneum* and *T. confusum*.

patterns are comparable, *T. castaneum* showed 65% survival

after five days of adult emergence, whereas this value

amounted to 27% for *T. confusum*. Watters and MacQueen (1967)

using Canadian populations of the two species found that

complete mortality of adults (less than 8 weeks old) after

gamma irradiation by 12.5KR was achieved for *T. castaneum*

in 52 weeks and in 3 weeks for *T. confusum*.

Although the 10KR dose employed allowed survival for

short periods of time, it certainly sterilized all populations

of both species, and no progeny was obtained. Watters and

MacQueen found that the adults of both species that survived

12.5KR gamma radiation failed to reproduce, while at 6.25KR

only *T. castaneum* reproduced intermittently. On the

other hand, Banham and Crook (1966) reported that *T. confusum*

and *T. castaneum* recovered fertility after irradiation with

a sublethal dose.

Age at Pupation:

Table II represents the mean and standard error of the

survival time of adults emerging after x-irradiation of second-

day old pupae of different "age at pupation" groups (see,

p. 31) for the four populations of *T. castaneum*. The mean

of each group of the four populations is presented graphi-

cally in Figure 3. The graphical analysis indicates that the

four populations differ in respect to the effect of increas-

ing age at pupation on mean survival time after x-irradiation.

For the Kenyan and probably for the Jamaican populations,

increasing age at pupation was associated with decreasing
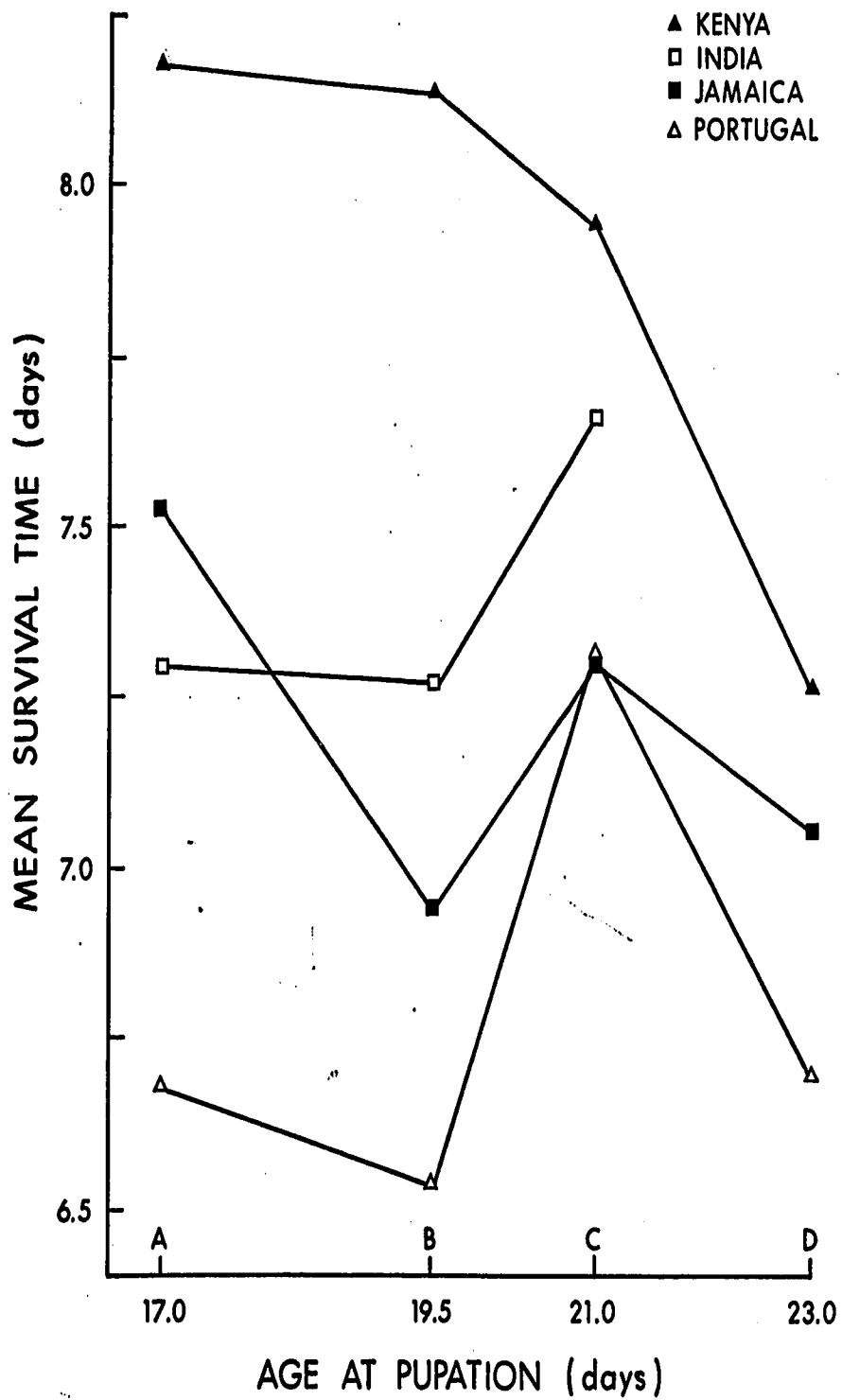
FIGURE 3 Mean survival time after adult emergence of
x-irradiated second-day old pupae of different "age at
pupation" groups (A-D) of four geographic populations
Tribolium castaneum.

TABLE II

Population Mean and Standard Error of Survival Time after
Adult Emergence from x-irradiated Pupae of Different
"Age at Pupation" of *T. castaneum*

	Age at Pupation[1]										
	A			B			C			D		
Popula-tion	N	mean	SE	N	mean	SE	N	mean	SE	N	mean	SE
Kenya	68	8.18	.34	109	8.16	.18	86	7.95	.20	22	7.27	.42
Jamaica	19	7.53	.50	79	6.95	.20	51	7.31	.24	71	7.05	.23
India	71	7.31	.24	59	7.27	.27	39	7.67	.29			
Portugal	32	6.69	.36	38	6.58	.35	62	7.39	.25	52	6.69	.25

[1]See text

mean survival time after irradiation. For the Indian
populations the reverse relation exists. The Portugese
population was completely different: adults emerged from
early and late x-irradiated pupae, survived for shorter
time than those from the intermediate period of pupation.
A similar relationship is presented in Figure 4 for all
populations (pooled) of *T. castaneum*. Time to pupation in
Tribolium is under genetic control and maintained at an
intermediate phenotypic optimum, selection for either fast
or slow pupation leads to a reduction in fitness of the
population (Dawson, 1965; Englert and Bell, 1970). Hence,
the longer survival of the intermediate yield of pupae
(Figure 4) may be due to the genetic superiority of such
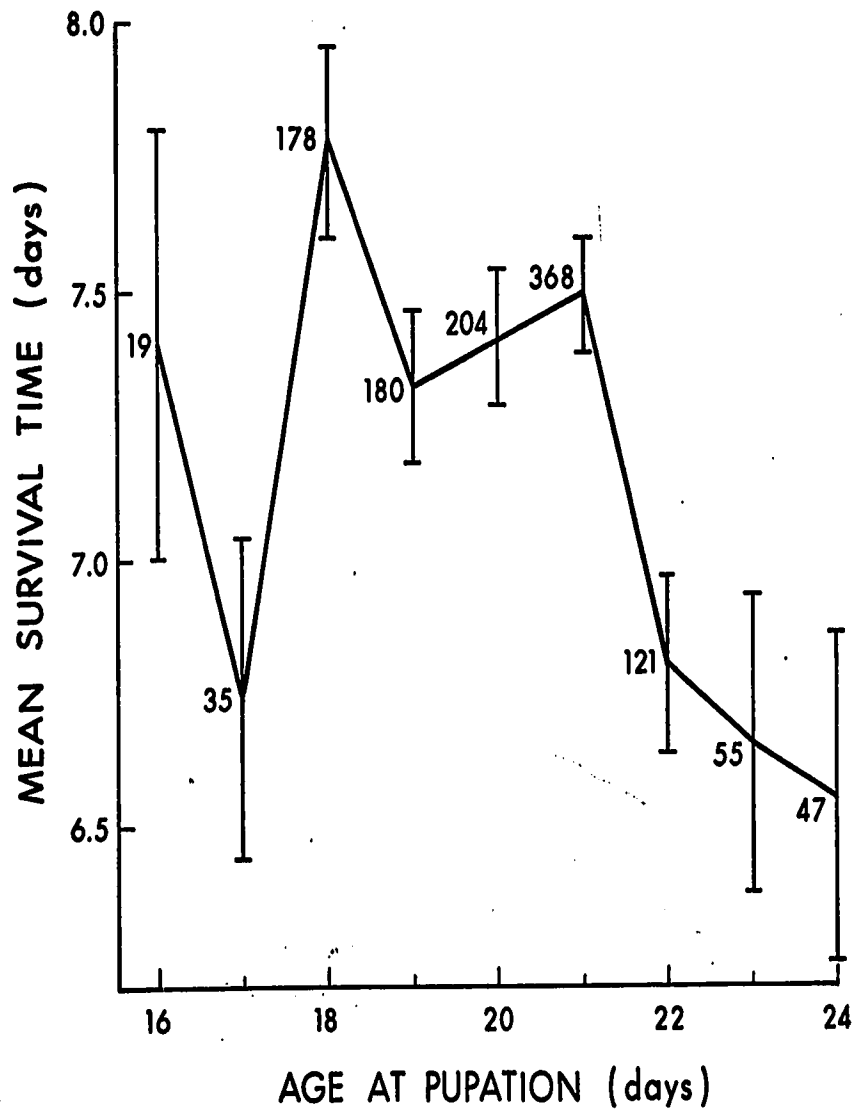
FIGURE 4 Mean survival time and standard error of adult emergence from x-irradiated second-day old pupae of different age at pupation of *Tribolium castaneum*. (Number in body of graph represents sample size).

intermediate pupae. This is an expected result if genetic homeostasis is operating.

Distribution of Time of Death:

The distribution of time of death of the four populations is presented in Figure 5. The means and standard errors of these distributions are presented in Table II. For all populations, except the Indian population, the variance for early pupation (group A) was found to be larger than for late pupation. With increasing age at pupation the distribution became positively skewed. This skewness was more apparent for the Portugese population, in agreement with Lamb (1966) who found that the distribution of time of death was normal for young flies of *Drosophila* but with increasing age at irradiation the distribution became positively skewed.

The bimodality of the time of death curves for *T. castaneum* populations (Figure 5) is of interest since a similar phenomenon was observed in *Drosophila* by Lamb (1966) and Nöthel (1968) and explained on the basis that the two distributions may be due to different causes of death. The early distribution reflects damage to the central nervous system, whereas the latter, which has a different appearance even within the same species, seemed to be due to specific metabolic changes not related to those that induce natural aging (Nöthel, 1968).
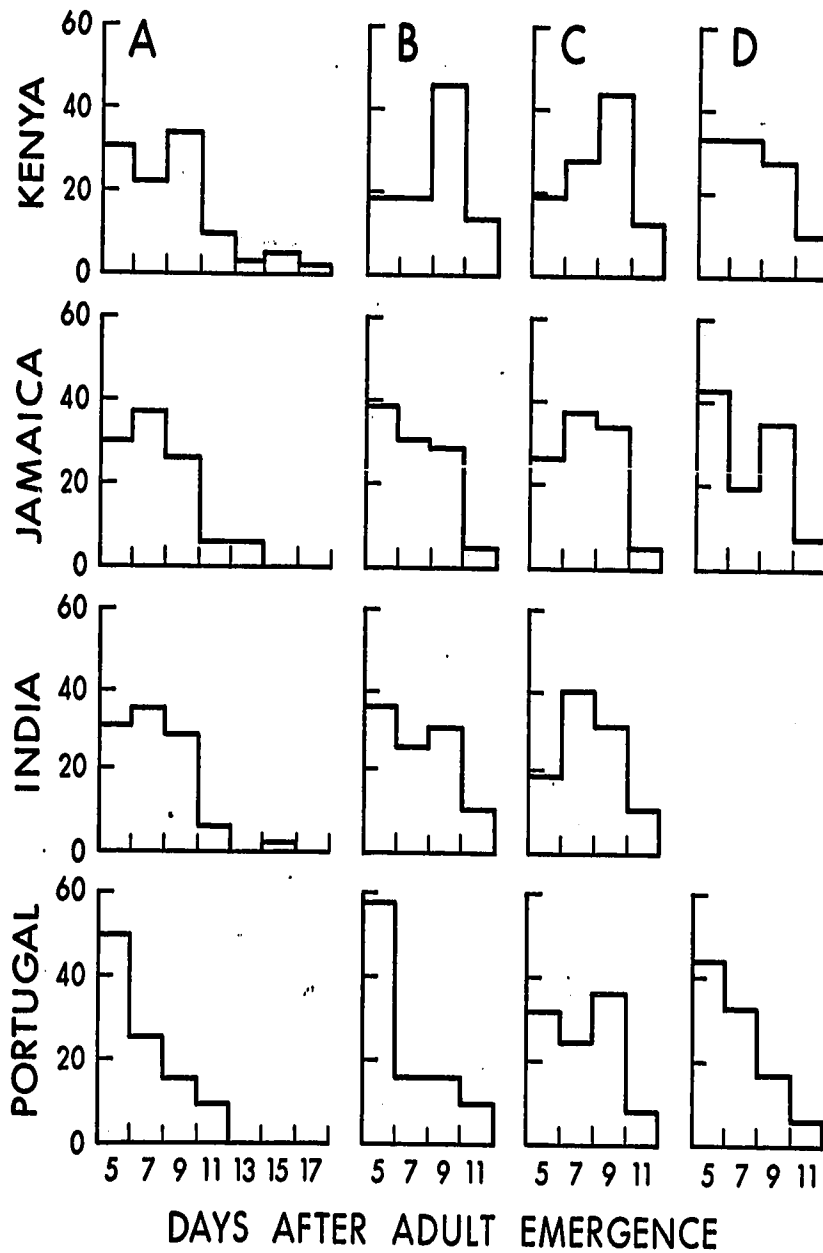
FIGURE 5 Distribution of time of death (in percentage) of different populations of *Tribolium castaneum*. From A to D "age at pupation" increases (see text for further explanation and Table II for sample size).

SUMMARY

The effect of single exposure (10KR of X-rays) of
second-day old pupae on mean adult survival time of eight
populations of *Tribolium castaneum* and six populations of
T. confusum, all with different geographic background was
studied. Mean survival time after irradiation was different
among *T. castaneum* populations within the range of 8.05
days for the population originated from Kenya and 5.48 days
for the population from France. *T. confusum* did not show
any significant difference among populations which ranged
from 6.38 days for the Kenyan population to 5.40 days for
the population from Germany. The overall difference between
the two species means (7.25 days for *T. castaneum* and 5.70
days for *T. confusum*) was highly significant. The overall
survival patterns of the two species were similar. However,
after 5 days of adult emergence 65% of *T. castaneum* and only
27% of *T. confusum* still survived.

Effect of age at pupation was only investigated in
T. castaneum and found to be different among the four popula-
tions studied. A general decrease in the mean adult
survival after irradiation with increasing age at pupation
was observed for all eight populations (pooled). Interme-
diate pupae from the total pupation period yielded adults
that survived longer than those from early or late pupae.
Distribution of death of the emerged adults was also examined.

III. INTER-POPULATION CORRELATION BETWEEN FITNESS TRAITS AND

SENSITIVITY TO X-IRRADIATION IN *Tribolium castaneum*

The relative adaptiveness of a population to its
contemporary environment may be termed its short term fitness
(Thoday, 1970). Developmental rate and productivity are two
intrinsic factors contributing to such fitness. Radiation,
on the other hand, may constitute an environmental hazard
that tends to limit population growth and therefore may affect
its short term fitness. An understanding of the genetic
and phenotypic relationship between the response (lethality
and survival time) of a species or population to an environ-
mental hazard such as x-irradiation and other fitness compon-
ents (developmental rate and productivity) can be gained
by two approaches: 1) A detailed examination of the relation-
ship between the fitness components and the response to the
particular hazard within each population, will indicate the
specific relationship as a consequence of the natural past
selection pressure. This approach has been investigated in
Section I and II. 2) Examination of this relationship by
computing the correlation coefficient between the phenotypic
values of any two fitness components for all the different
populations after a given exposure to irradiation. If there
is a large number of individuals within each population, and
if there is a wide genetic difference between populations then
the computed phenotypic correlation reflects a "genetic
correlation" in a broad sense. This section concerns
itself with this second approach to the problem.

Fitness components of the eight populations of

T. castaneum are presented in Table I and the response in

TABLE I

Population Parameters of Two Fitness Components of Various
Geographic Populations of *Tribolium castaneum*

Population	Number of Parents	Productivity		Age at pupation	
		Total	per/parent	Mean	SE
Kenya	90	737	8.18	17.67	0.06
Jamaica	84	740	8.81	19.63	0.07
India	79	753	9.53	16.70	0.07
Chicago	79	729	9.23	19.07	0.05
Portugal	95	979	10.30	18.23	0.06
Kansas	100	705	7.05	17.47	0.06
France	100	390	3.90	21.14	0.06
Canada	92	560	6.09	15.62	0.04
TOTAL	719	5593	7.78	18.15	0.03

terms of lethality (as percent adult emergence) of each

population to a single exposure of X-ray (10KR) is given

in Table II, Section I (p. 13). In Table I, Section II (p. 32)

the mean adult survival time after x-irradiation is also

given. The inter-population correlation between the popula-

tion parameters and response to x-irradiation is presented

in Table II.

TABLE II

Inter-population Correlation Between Fitness Traits and Radiosensitivity of Eight Geographic Populations of *Tribolium castaneum*

Traits	Survival[1]	Development[2]	Productivity[3]
Lethality[4]	0.16	0.18	0.57
Survival	-	-0.87*	0.31
Development	-	-	0.25

[1] mean adult survival time after x-irradiation of second day pupae

[2] mean age at pupation (in days)

[3] number of pupae produced/individual parent/2 days

[4] percent adult emergence after x-irradiation of a given number of second-day old pupae (after angular transformation)

* significant at $P<0.01$

The non-significant inter-population correlation between most of the present components of fitness and response after x-irradiation indicates that different independent genetic mechanisms may be responsible for change in fitness (Soliman, 1970). The only significant correlation concerns the mean age at pupation and the mean adult survival after x-irradiation. The regression analysis indicates a highly significant negative relationship between both traits (Figure 1), where increasing mean pupation time by one day results in decreasing mean adult survival after x-irradiation by 0.43 ± 0.1 day.
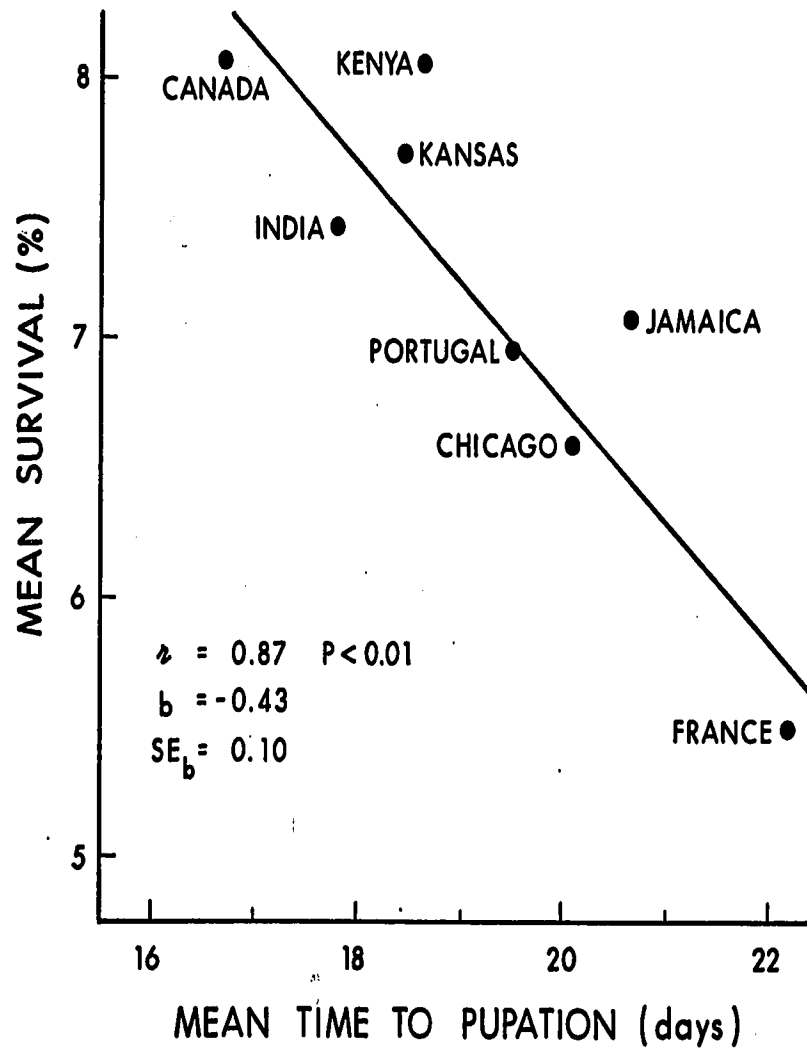
FIGURE 1 Inter-population relationship between mean time to pupation and mean adult survival after x-irradiation of second-day old pupae of eight geographic populations of *Tribolium castaneum*.

If mean adult survival after x-irradiation is proportional to life expectancy in *Tribolium* as in *Drosophila* (Lamb, 1966, and Blair and Baxter, 1970), it could be assumed that the variation between means of adult survival after x-irradiation reflects variation between the life expectancy among populations. This means that increasing pupation time is associated with decreasing life span in *T. castaneum* (Figure 1). These results seem to contradict the conclusion arrived at in *Drosophila* by Lints and Lints (1971) that "a prolongation or a shortening of the duration of development results in an increase or in a decrease in the speed of the ageing process". However, these results substantiate Lints (1971) conclusion and Sokal (1970) finding in *T. castaneum* that longevity of a species is under genetic control. The genetic factors controlling longevity must have evolved as a compromise between the forces tending to prolong life and environmental hazard tending to curtail it (e.g., Edney and Gill, 1968). Other investigators have also observed the effect of particular chromosomes on the variability of longevity in *Drosophila* (e.g., Marinkovic and Wattiaux, 1967).

IV. DEVELOPMENTAL ABNORMALITIES

INTRODUCTION

The effect of radiation on the development and
morphogenesis of young pupae have been extensively studied
in *Tribolium*. When pupae aged less than 37 hours are
exposed to a dose of X-ray exceeding 1200R, a wing abnorma-
lity consisting of median elytral split, elytral blistering
and protrusion of the underlying membranous wing will gener-
ally develop in the imago (Buckhold and Slater, 1969; Yang
et al., 1970). These types of developmental abnormalities
appear spontaneously at very low frequencies and are influ-
enced by various genetic mutations (Sokoloff, 1966). In
addition to ionizing radiation, other physical factors such
as temperature, weightless spaceflight, U.V., O_2 tension,
magnetic field, and specific chemicals were found to influence
the frequencies of developmental abnormalities (Buckhold and
Slater, 1969; Slater et al., 1961; 1963, 1964, 1969, Ducoff
and Bosma, 1966; Amer, 1963; Amer et al., 1962, Roth, 1944
and Roth and Howland 1941).

In addition Beck (1963) and Buckhold and Slater (1969)
found that the duration of the pupal period in *T. castaneum*
and *T. confusum* is affected by exposure to x-irradiation.
The present section deals with data pertaining to the
frequencies of developmental abnormalities in various wild
populations of *T. castaneum* and *T. confusum*, and the duration

of the pupal stage of the same populations of *T. castaneum*
after exposure to a single dose of x-irradiation.

RESULTS AND DISCUSSION

Wing Deformities:

The frequency (in percent) of wing abnormalities that
developed after x-irradiation of pupae of various populations
of *T. castaneum* and *T. confusum* is presented in Table I.
The frequency of dead pupae is presented in Table II together
with the percentage of beetles with undifferentiated wings
and abdomen but with adult legs and heads. These kinds of
monstrosities are usually moribund. In all populations of
both species studied, x-irradiation induced an increase in
percent of abnormalities over the control. Canadian popula-
tions for both species were the most sensitive in this res-
pect. For this population *T. castaneum* showed 21.1 percent
wing deformities, whereas *T. confusum* produced 37.5 percent.
For undifferentiated wings and abdomen of the same Canadian
population *T. castaneum* yielded 90.3 percent and *T. confusum*
92.9 percent. Kenyan Populations appear to be the most
resistant to the production of developmental abnormalities
after x-irradiation. For this population the percent of
split wing was 1.0 percent for *T. castaneum* and 16.7 percent
for *T. confusum*. Undifferentiated wing and abdomen appeared
in 50.7 percent of Kenyan *T. castaneum*; the corresponding
figure for *T. confusum* was 93.8 percent.

The number observed for most populations of *T. confusum*

TABLE I

Adult Split Wing Deformities of Various Populations of
Tribolium castaneum and *T. confusum* after Second-day
Exposure to 10KR of X-rays

Population	No. of Pupae		Normal Adults		% Split Wing[1]	
	Exposed	Control	Exposed	Control	Exposed	Control
T. castaneum:						
Kansas	563	162	109	158	4.5	0.6
Kenya	608	171	289	170	1.0	0.0
Chicago	459	300	128	267	7.0	0.0
Canada	279	164	19	159	21.1	0.0
Jamaica	569	167	215	165	1.9	1.2
India	491	232	154	220	5.2	0.0
Portugal	708	323	165	301	15.8	0.7
France	426	96	65	96	6.2	0.0
TOTAL	4103	1615	1144	1536	5.5	0.3
T. confusum						
Kansas	329	44	36	44	13.9	0.0
Kenya	484		24		16.7	
Chicago	380	149	6	148	00.0	0.0
Canada	493	162	24	158	37.5	0.0
Germany	171		13		00.0	
Scotland	326	17	15	17	26.7	0.0
TOTAL	2183	372	118		18.6	0.0

[1]relative to number of normal adult.

TABLE II

Developmental Deformities in the Pupal Stage of Various
Populations of *Tribolium castaneum* and *T. confusum*
after Second-day Pupal Exposure to 10KR of X-rays

Population	% Developmental Abnormalities[1]		% Dead Pupae[1]	
	Exposed	Control	Exposed	Control
T. castaneum:				
Kansas	74.6	0.0	5.2	1.9
Kenya	50.7	0.6	1.3	0.0
Chicago	69.5	0.7	0.7	0.3
Canada	90.3	1.8	15.8	1.2
Jamaica	61.0	0.0	0.5	0.0
India	61.5	2.2	5.5	3.0
Portugal	69.5	0.0	3.5	6.2
France	83.8	0.0	0.0	0.0
TOTAL	66.5	0.7	3.4	2.0
T. confusum:				
Kansas	87.5	0.0		
Kenya	93.8	0.0	0.4	
Chicago	98.4	0.0		0.7
Canada	92.9	0.0	0.4	2.5
Germany	92.4	0.0		
Scotland	94.2	0.0		
TOTAL	93.4	0.0	0.2	1.3

[1]relative to number of pupae Table I

was too small to allow for a meaningful conclusion regarding

differences between populations in this species. However,

by pooling all populations for comparison of species it can

be concluded that *T. confusum* (common in cold regions) is

more sensitive with regard to the production of developmental

abnormalities induced by ionizing radiation than *T. castaneum*

(common in the tropical regions). The frequency of split

wing induced by x-irradiation amounted to 5.5 percent for

T. castaneum and 18.6 percent for *T. confusum;* whereas

undifferentiated wing and abdominal abnormalities amounted

to 66.5 percent and 93.4 percent for both species respectively.

Comparisons between population and between species suggest

that the induction of developmental abnormalities may be

associated with the geographic distribution of the species

and the adaptation of isolated populations to temperature

which seems to be the chief environmental factor. Induction

of developmental wing abnormalities by x-irradiation was

found to be temperature dependent in *T. confusum,* reaching

a maximum at 28°C within the temperature range from 10 to

35°C. The induction of abnormalities was found to be higher

at 10°C than at 35°C (Buckhold and Slater, 1969). Interaction

of radiation induced wing abnormalities with temperature, O_2

tension, weightlessness, magnetic field and dose fractionation

have been reported in literature. For example, Amer (1963b)

found that magnetic fields have a protective effect
against irradiation injury. Also by using dose fractionation
Yang *et al.* (1970) observed a rapid phase of recovery, fol-
lowed by a slow phase when wing abnormalities were used to
determine the kinetics of recovery in the pupae of *T. confusum*.

.. Wing abnormalities were also found when pre-imaginal
stages of *T. confusum* were exposed to natural gaseous
quinones derived from the adults (Roth and Howland, 1941)
and to acetic and hydrochloric acid (Roth, 1944).

The genetic basis of different genes that can modify
the normal wing expression in *Tribolium* has been discussed
by Sokoloff (1966). For instance, in *T. castaneum* 5 sex
linked mutants and 4 autosomal genes were identified and were
assigned to different linkage groups. All these genes
exhibit recessive mode of inheritance with some incomplete
recessive or semilethal effect. Thirteen other autosomal
recessive genes that affect the elytra in this species have
also been isolated, although their linkage groups have not
been identified yet. Similar genes have also been identi-
fied in *T. confusum* (Sokoloff, 1966).

The frequency of some of these mutants has been increas-
ed by artificial selection. For example, the droopy elytra
"*dre*" was found to increase by selection in *T. castaneum*
(Sokoloff and Lasley, 1961) and *T. confusum* (Sokoloff, 1966).
Similarly the frequencies of the wrapped elytra "*we*" in
T. castaneum (Sokoloff, 1960) and bilateral blisters in

T. confusum "*ro*" (McDonald *et al.*, 1963) can be increased by
selection. On the other hand, selection against thumbed
mutant "*thu*^{8}" was found to be unsuccessful: the frequency
of this gene remained constant between 0.28 and 0.32
(Sokoloff, 1966).

An interesting observation is that the high abnormali-
ties observed for the Canadian population of *T. castaneum*
in Table I and II, were also associated with rapid develop-
ment. For instance, the mean time to pupation for the
Canadian population amounts to 15.6 days, whereas the French
population showed a mean time to pupation of 21.1 days
in the same species. This observation is in agreement with
results reported by Dawson (1968) who found that selection
for short time to pupation in *T. castaneum* was correlated
with a greater increase in the frequency of abnormal beetles
when compared with the line selected for long time to
pupation. For instance, Dawson obtained 14.5 percent abnorma-
lities for the unselected populations, 23.8 percent for the
fast line and 18.4 percent abnormalities for the slow line.
This increase in percent abnormalities was attributed to
fixation of genes as a result of (i) inbreeding, (ii)
increased homozygosity (especially for genes for slow
development due to pleiotropy), and (iii) disruption of
developmental homeostasis (due to artificial selection). In
the present study the lowest percent abnormalities in
T. castaneum was obtained for the Kenyan population (one
percent split wing and 50 percent undifferentiated wing and

abdomen) with a mean time to pupation of 17.7 days. The French population, which has the longest mean time to pupation (21.1 days), yielded 15.1 percent split wing and 66.3 percent undifferentiated wing and abdomen aberrations. These results are comparable in their magnitude with those obtained by Dawson (1968) when comparing the correlated response in the frequency of developmental deformities with selection for time to pupation in *T. castaneum*.

Tables I and II indicate that the production of abnormalities in various geographic populations of *T. confusum* is, in general, greater than those of *T. castaneum* (18.5 and 5.5 percent). These results are at variance with those reported by Dawson (1968) who found that the synthetic stock of *T. confusum* produced less developmental deformities, and hence was better able to maintain normal developmental pattern than the synthetic stock of *T. castaneum*. These differences were attributed to greater developmental homeostasis of the synthetic stock of *T. confusum* when compared to the synthetic stock of *T. castaneum*. Both stocks were synthesized by pooling various geographic populations.

The exact mechanism giving rise to wing damage after irradiation is still not known. However, a mechanism of chromosomal or DNA damage of the proliferating somatic cells of the wing imaginal discs during the rapid period of wing development, has been proposed (Slater, *et al.*, 1969).

<u>Pupal Period</u>:

The effect of age at pupation on the mean duration of the pupal period is presented in Figure 1 and the coefficient of variation in Table III, for various populations of

TABLE III

Number of Pupae and Coefficient of Variation of Pupal Period for Each Population of *Tribolium castaneum* used as Control for Each Age at Pupation

		Age at Pupation (days)						
Population		16	17	18	19	20	21	22
Kansas	N		61	97				
	%C.V.		7.3	9.2				
Kenya	N		65	42	31	25	7	
	%C.V.		8.2	9.2	8.1	7.5	7.3	
Chicago	N					99	100	98
	%C.V.					8.6	7.1	8.2
Canada	N	99	40					
	%C.V.	8.7	8.8					
Jamaica	N					9	88	58
	%C.V.					7.9	8.8	5.0
India	N	93	97	32				
	%C.V.	8.2	7.4	8.8				
Portugal	N		32	73	92	48	71	
	%C.V.		7.9	8.9	7.1	7.1	8.2	

T. castaneum. Although not all populations were presented at each age at pupation, it seems that increasing age at pupation is negatively correlated with decreasing pupal period ($r = -0.93$, $P<0.01$). However, x-irradiation of young
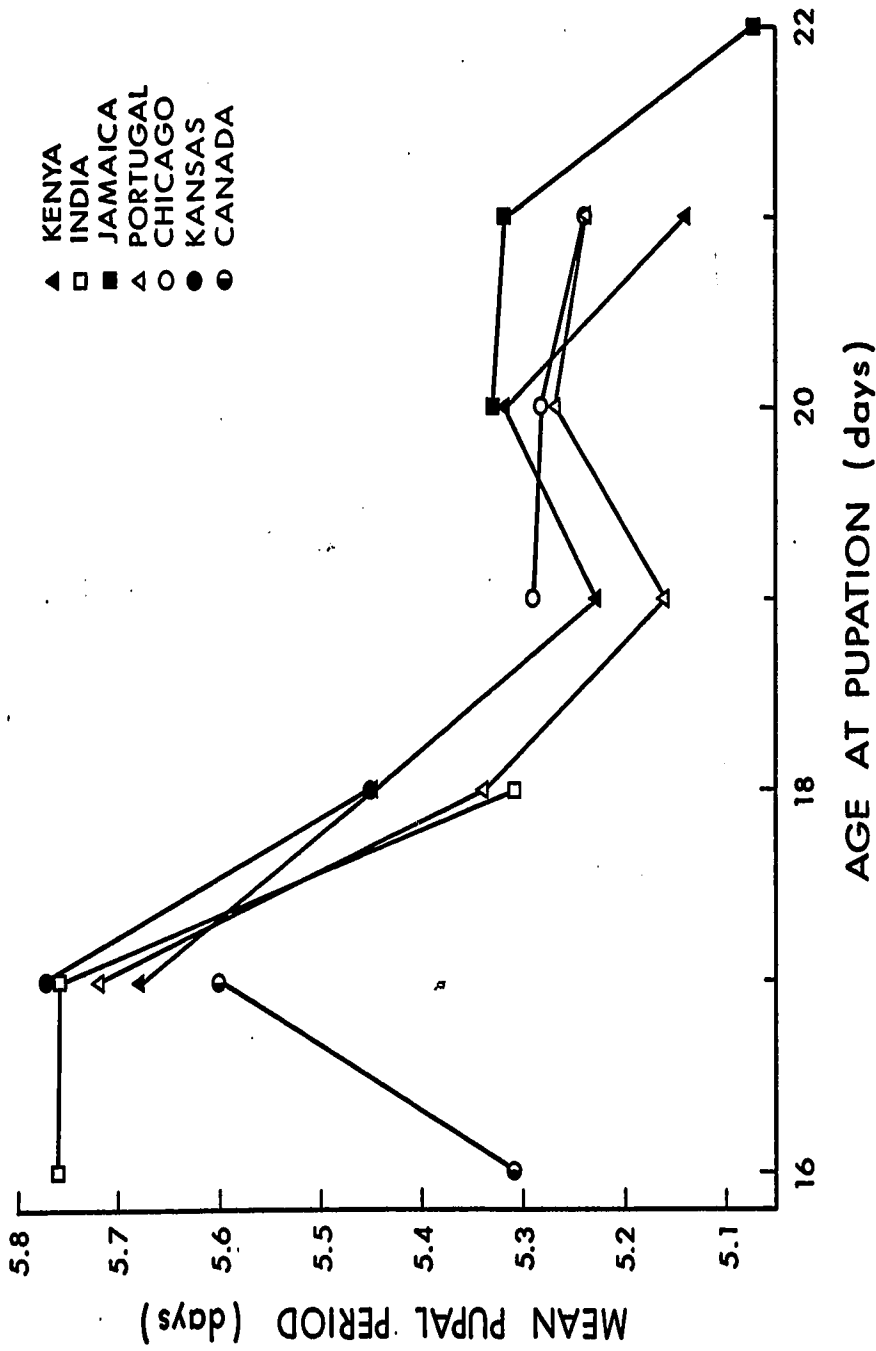
FIGURE 1 Relationship between age at pupation and mean pupal period of various geographic populations of *Tribolium castaneum* (for number of pupae and coefficient of variation see Table III).

pupae affect the duration of the pupal period, in such a
manner that irradiated pupae required shorter times to
reach eclosion when compared with the unirradiated controls.
All irradiated pupae that succeeded in reaching eclosion took
five days in the pupal stage, except those that pupated
after 20 and 21 days from ovipositions. Some of their
pupae took longer than five days so that their average
duration of pupal stages exceeded five in these two groups
and were comparable to the unirradiated controls (Table IV).
These results are in contrast with those obtained by Beck
(1963) and Buckhold and Slater (1969) who reported delayed
eclosion in *T. confusum* after x-irradiation (3KR) of
young pupae. However, the differences between the present
results and their results may be due to the dose employed.
In the present study a much higher dose (10KR) has been used
and consequently the results obtained may reflect this
difference. In addition, it is of interest to mention that
the abnormal pupae that did not develop an adult wing and
abdomen, took a longer time to develop heads and legs than
the normal adults. These results indicate that the metabolic
events leading up to eclosion might not be able to proceed
until repair of somatic cell chromosome breaks take place
(Buckhold and Slater, 1969). Failure of this repair in the
imaginal wing disc cells and abdomen cells would consequently
account for these abnormal pupae.

TABLE IV

Means of Pupal Periods and Coefficient of Variation after
X-irradiation of Second-day Pupae of
Tribolium castaneum

	Age at Pupation (day)[a]					
	20			21		
Population	N	Mean	%C.V.	N	Mean	%C.V.
Kansas	24	5.08 (5.28)	8.0	53	5.08	5.2
Kenya	64	5.36 (5.32)	9.6	85	5.32 (5.14)	8.8
Chicago	23	5.22	8.1	59	5.17 (5.24)	7.3
Jamaica	43	5.26 (5.33)	8.4	50	5.22 (5.32)	8.0
India	16	5.38	9.3	29	5.10	6.0
Portugal	12	5.25 (5.27)	11.8	57	5.18 (5.24)	7.3

[a]All pupae irradiated before the 20th day age at pupation or
after 21st took five days to emerge into adults.

Values between brackets are for controls (same as in Figure 1).

SUMMARY

The effect of x-irradiation on day old pupae with a single dose (10KR) was studied in various wild populations of *Tribolium castaneum* and *T. confusum*. Developmental wing abnormalities and undifferentiated wing and abdomen were found to differ among populations and species. Populations originally from warm localities were more resistant to X-ray induction of developmental abnormalities than populations from cold localities. Also *T. castaneum* was more resistant than *T. confusum* in this respect. The length of pupal stage was also studied in *T. castaneum*. The pupal stage is decreased by increased age at pupation. The emerged adults after irradiation had shorter pupal periods than their controls. This finding is contrary to earlier records by other investigators of eclosion delay when lower doses of x-irradiation were used.

V. LACK OF GENETIC CORRELATION BETWEEN RATE OF DEVELOPMENT AND SURVIVAL AFTER X-IRRADIATION IN *Tribolium castaneum*

INTRODUCTION

In section I it has been found that there is an effect of "age at pupation" on survival (measured as percent adult emergence) after x-irradiation of second-day old pupae. Increasing age at pupation was positively correlated with increasing percent adult emergence. This effect was consistent for all populations of *Tribolium castaneum* which were studied and also for *T. confusum*. Time to pupation is also under genetic control (Dawson, 1965; Englert and Bell, 1970). These findings suggested that rate of development and survival after x-irradiation may have a common genetic basis. One way of examining this relationship is by computing the inter-population correlations between percent adult emergence after x-irradiation and mean time to pupation. This correlation is a genetic correlation in a broad sense. Section III indicated that, although the relationship between the two traits is positive ($r = 0.18$), nevertheless, it is not significant. A second approach to testing this relationship is to select for fast and slow developmental rates and to examine the resulting response in percent adult emergence after irradiation in later generations of selection. The purpose of the present investigation is to re-examine the relationship between survival after x-irradiation and age at pupation using this second approach.

MATERIALS AND METHODS

Mass selection for rate of development was started from a wild population of flour beetles, *T. castaneum* (Portugal). This population had been maintained as a closed population in the laboratory.

Four replications were originated from the same sample of 100 unsexed adult beetles mass mated for one generation. Eggs were collected for 24 hours during 4 successive days to initiate the four replicates. In generation 0(within each replication) three lines were started (fast, intermediate and slow) by the first, intermediate and last 15 male and 15 female pupae:

G0   Fast←——→Intermediate←——→Slow

G1   Fast←—— ——Intermediate—— ——→Slow
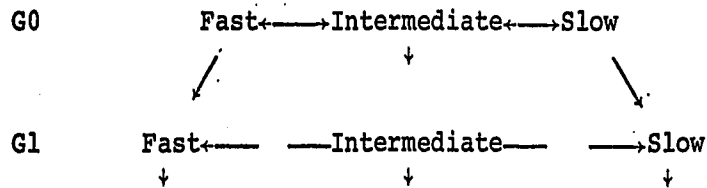
In each generation the same constant number of 15 males and 15 females were used as parents for the next generation for any selected line. A similar number of males and females was randomly chosen within the control (unselected) lines after all larvae had pupated. Selection intensity was about 19% which is considered to be reasonable for minimizing the effect of inbreeding (Falconer, 1964).

Rate of development was measured as follows: parents (15 pairs) not less than seven days old were introduced into a plastic box (5 x 3.5 x 2.5 cm.) containing 25g whole

wheat flour supplemented with 5% brewer's yeast. After

three days the adults were transferred to fresh flour and

the original box was discarded. The beetles were left to

lay eggs for 24 hours and then discarded. Starting on the

14th day after oviposition pupation was checked at 24

hour intervals until the 30th day. The few larvae which had

not pupated by that time were discarded. At the same time

every day, the flour was sifted; the pupae were removed, sex-

ed and the remaining larvae were returned to the box.

Developmental time was measured as the number of days

required for the egg to develop to pupa. The choice of

the pupal stage is favoured by the ease of sexing the pupa

and of collection of virgin females.

After collecting eggs for the 7th generation, another

24 hours' egg sample was collected from the three selected

lines and the control for x-irradiation as indicated in

Section I. Male and female pupae produced daily were irra-

diated separately.

The environmental conditions were constant. All

cultures were maintained at 33 ± 1°C and 70 ± 2% relative

humidity. The same batch of flour was used and sifted in

the same manner throughout the course of the investigation.

Standard procedures were followed for removing cultures from

the incubator.

RESULTS AND DISCUSSION

Effect of Selection:

The main purpose of the selection for time to pupation was to produce strains with different rate of development and not to study the quantitative aspects of selection *per se* (see Englert and Bell, 1970). Hence, the results of selection will be presented only for the generation that has been tested for X-ray sensitivity.

The result of the effect of the seventh generation of selection for fast, slow and intermediate age at pupation is presented in Table I and Figure I along with the un-selected line. Analyses of variance on the mean age at pupation (Table I) indicate that there is a pronounced response to selection for both fast and slow lines. Stabilyzing selection for the intermediate phenotype did not produce any difference in mean time to pupation from the unselected line. No detectable difference between the samples of pupae used for x-irradiation and corresponding nonirradiated samples was found (except for the unselected and intermediate lines, Table I). Taking all lines together the time to pupation for both sexes was similar, however, when the early and late pupated lines were analysed separately a significant sex difference appeared. For both of these two lines males developed faster than females. The early line exhibited a mean time to pupation of 17.11 days for females and 16.98 days for males (three hours difference);

TABLE I

Analyses of Variance on Mean Age at Pupation Among
Different Lines of *Tribolium castaneum*

| Source of Variation | All Lines | | Fast & Slow | | Unselect. & Intermed. |
	d.f.	m.s.	d.f.	m.s.	m.s.
Line (L)	3	98.2417**	1	266.9783**	1.1213
Sample (M)	1	1.6965	1	0.0001	3.4257*
LM	3	0.8610*	1	0.2945	0.5592
Replicate (R)	3	0.9529	3	0.5183	1.2976
LR	9	0.9034*	3	1.3243*	0.5229
MR	3	0.0071	3	0.0070	0.0300
LMR	9	0.1731*	3	0.1974*	0.2918*
Sex (S)	1	0.1892	1	0.4680*	0.0048
LS	3	0.1229	1	0.0851	0.0001
MS	1	0.0930	1	0.1582	0.0011
LMS	3	0.0789*	1	0.0914	0.0790
RS	3	0.1029	3	0.1065	0.0260
LRS	9	0.0285	3	0.0176	0.0382
MRS	3	0.0736*	3	0.0602	0.0353
LMRS	9	0.0167	3	0.0167	0.0115

 * P<0.05

** P<0.01
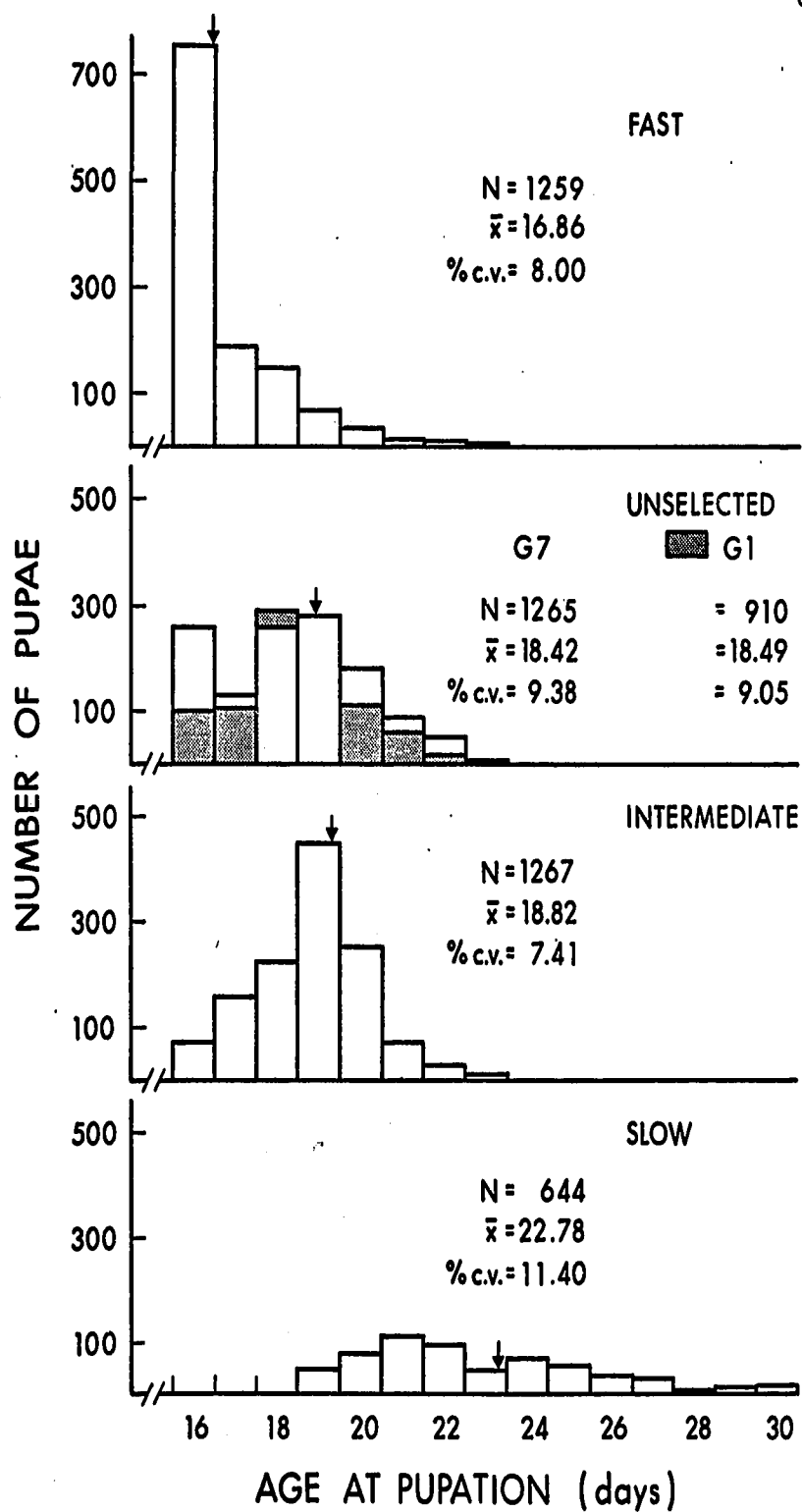
FIGURE 1 Distribution curves for time to pupation of selected and unselected lines before selection and after seventh generations of selection.

whereas the late line showed 22.9 days for females and 22.65 for males (eight hours difference). These results are in agreement with those found by Soliman and Hardin (1971b) using various wild populations of the same species.

Since replication and sex did not have any effect on the mean time to pupation in general (Table I), the data from the four replicates and the two sexes were pooled for distribution curves (Figure 1). The observed response was greater for late pupation, with an average response of 4.36 days, as compared to an average response of -1.56 days for early pupation. These results fit well with those of Englert and Bell (1970) who reported an average response of 4.69 days and -1.99 days for both lines, respectively, after six generations of selection. (The slight difference between the two results may be due to the slightly higher selection intensity (18%) applied by these authors.)

The successful response to selection for differences in time to pupation is expected in *Tribolium* as indicated in all previous selection experiments for the same trait by Englert and Bell (1964, 1970), Dawson (1965) both using synthetic stocks of *T. castaneum* and Wool (1969) using wild strain and black mutant strain of the same species.

The results from the present study (Table I and Figure I) support the model of genetic homeostasis proposed by Dawson (1965) and confirmed by Englert and Bell (1970) for maintenance of developmental rate at intermediate optimum in

Tribolium. This model predicts that:

1. genes for developmental rate are maintained at interme-
   diate frequencies.

2. due.to dominance selection progress will be greater in
   the slow direction than in the fast direction.

3. reproductive superiority of intermediate phenotypes.

4. increase in variance for slow development due to loss
   of heterozygosity; and decrease in variance for
   intermediate selected line due to stabilizing selection.
   (The use of coefficient of variation, Figure 1, is to
   eliminate any possible scale effect due to the
   dependence of the variances on the means).

Distribution curves for the first and seventh generation
of the unselected line are given in Figure 1. A slight
skewness is observed for the seventh generation, without
altering the mean or the variability. This deviation from
normality might be accidental. On the other hand the ob-
served skewness of the fast time is more pronounced. A similar
observation has been reported by Hardin and Bell (1967) for
lines selected for 13-day old larval weight in *T. castaneum.*

Correlated Response in Radiosensitivity:

The mean and standard error of the angular transformation
of the percent survival and percent split wing of the x-irra-
diated four lines and their four nonirradiated controls (after
seven generations of selection) are presented in Table II.
Analyses of variance on all the data are given in Table III.

TABLE II

Mean and Standard Error of the Angular Transformation of the Percentage Survival and Percentage Split Wing of the X-irradiated Lines of *Tribolium castaneum* and their Nonirradiated Controls

	Fast		Unselected		Intermediate		Slow	
	Control	Irrad.	Control	Irrad.	Control	Irrad.	Control	Irrad.
Survival:								
N^1	693	615	654	636	603	663	283	388
Mean²	90	36.56	85.28	26.43	84.68	33.44	90	36.61
S.E.		2.49	1.48	1.33	1.74	1.74		3.07
d³	53.44 (64.51)		58.85 (73.52)		51.24 (60.80)		53.39 (64.43)	
Split Wing:								
N^4	684	204	635	108	571	181	280	123
Mean²	5.33	19.94	7.41	25.73	11.17	21.80	2.75	10.55
S.E.	1.56	4.14	1.26	1.50	1.12	2.32	1.68	4.12
d³	14.61 (6.36)		18.32 (9.88)		10.63 (3.4)		7.80 (1.84)	

[1]Total sample size (number of pupae used).

[2]Each mean is based on eight percentages (4 replicates and 2 sexes).

[3]Difference between control and irradiated line. Values between parenthesis are the back-transformed values.

[4]Total sample size (number of normal adults used).

TABLE III

Analyses of Variance on the Angular Transformation of the
Percentage Survival and Percentage Split Wing after
Single X-ray Exposure of Four Lines of
Tribolium castaneum

Source of Variation	d.f.	Mean Square	
		Survival	Split Wing
Line (L)	3	207.9**	348.3*
Treatment (T)	1	47025.0**	2632.5**
LT	3	41.6	84.5
Replicate (R)	3	54.9*	29.1
LR	9	31.0	22.5
TR	3	35.6	44.5
LTR	9	38.3	47.0
Sex (S)	1	28.0	119.1
LS	3	27.9	44.4
TS	1	28.7	25.8
LTS	3	22.6	9.5
RS	3	8.6	13.5
LRS	9	17.8	57.4
TRS	3	13.4	41.9
LTRS	9	12.2	75.5

* $P<0.05$

** $P<0.01$

In general, the X-ray treatment has a highly significant effect on the percent survival and percent split wing of all four lines. Moreover, distinct divergence among the selected lines in their response to x-irradiation is evident for both traits.

Survival:

The effect of x-irradiation on the survival of the selected lines was more pronounced than for the unselected line. Unexpected is the low survival after irradiation (60.8%) of the line selected for intermediate time to pupation. The similarity between the fast and slow selected lines (64.51 and 64.43%) over the line selected for intermediate rate of development may be attributed to: (i) some fixation of genes resulting from the unavoidable amount of inbreeding that accompanied selection; and (ii) increase in homozygosity (Dawson, 1968).

The similar percent survival after x-irradiation of the two selected lines for slow and fast development, disproves the hypothesis proposed earlier (Section I, Figure I) that the genetic mechanisms involved in X-ray sensitivity (measured as percent adult emergence) and age at pupation are correlated. These results indicate that the previously observed consistent positive correlation between percent adult emergence and age at pupation within the eight wild populations of *T. castaneum* is mainly of physiological nature. This conclusion is also supported by the lack

of interpopulation correlation between the two traits
(Section III)

Split Wing:

The present study indicates that the four lines differ
in their percentages of spontaneous and induced split wing
(Table III). Although various environmental conditions
could influence the frequency of split wing, it was found
to be under genetic control (Sokoloff, 1966).

In the present study there are two causes that are
affecting the frequency of split wing: selection and
irradiation. Selection for time to pupation has resulted
in a significant effect on the frequency of split wing
(Table II and III). The unselected line has a higher
frequency of split wing (7.41%) than the selected lines
for fast (5.33%) or slow (1.68%) time to pupation. This
difference may be due to fixation of genes that reduce the
frequency of this trait in these two lines. These results
are different from those reported by Dawson (1968) who
studied the frequency of abnormal adults (including split
wing) after selection for rate of development in *T. casta-
neum*. He found that the unselected line has the least percent
of adult abnormality (14.8%) than either fast (23.8%) or
slow (18.4%) selected lines. Dawson explained his results
as being due to the superiority of heterozygous genes
favoured by natural selection. In the present experiment
selection for intermediate time to pupation (Table II)

showed higher frequency of split wing (11.17%) than the two
selected lines or even the unselected one. The low
frequency of split wing for the slow line may be due to
accidental fixation of some homozygous dominant allele.

The effect of x-irradiation on the selected lines
(Table II) indicates that x-irradiation has increased the
frequency of split wing for all lines. The small differ-
ence between the nonirradiated and irradiated intermediate
line (3.4%) and the nonirradiated and irradiated unselec-
ted line (9.88%) may be due to better developmental homeo-
stasis and not to genetic homeostasis, since the nonirradiated
intermediate line showed higher frequency of split wing
(11.17%) than either the unselected line (7.41%) or the
fast line (5.33%).

The data as presented indicate that there are two
physiological mechanisms that are responsible for the
observed frequencies: 1) induction mechanism due to the
administration of x-irradiation and 2) repair mechanism
due to slowing of the rate of development. One way of
studying this repair mechanism is by subtracting the
frequency of split wing of the nonirradiated line from that
of the irradiated line for all the selected lines. These
results are presented in Table II and graphically in Figure
2. From this figure it is clear that increases in the
rate of development are associated with increases of the
frequency of split wing. These results indicate that the
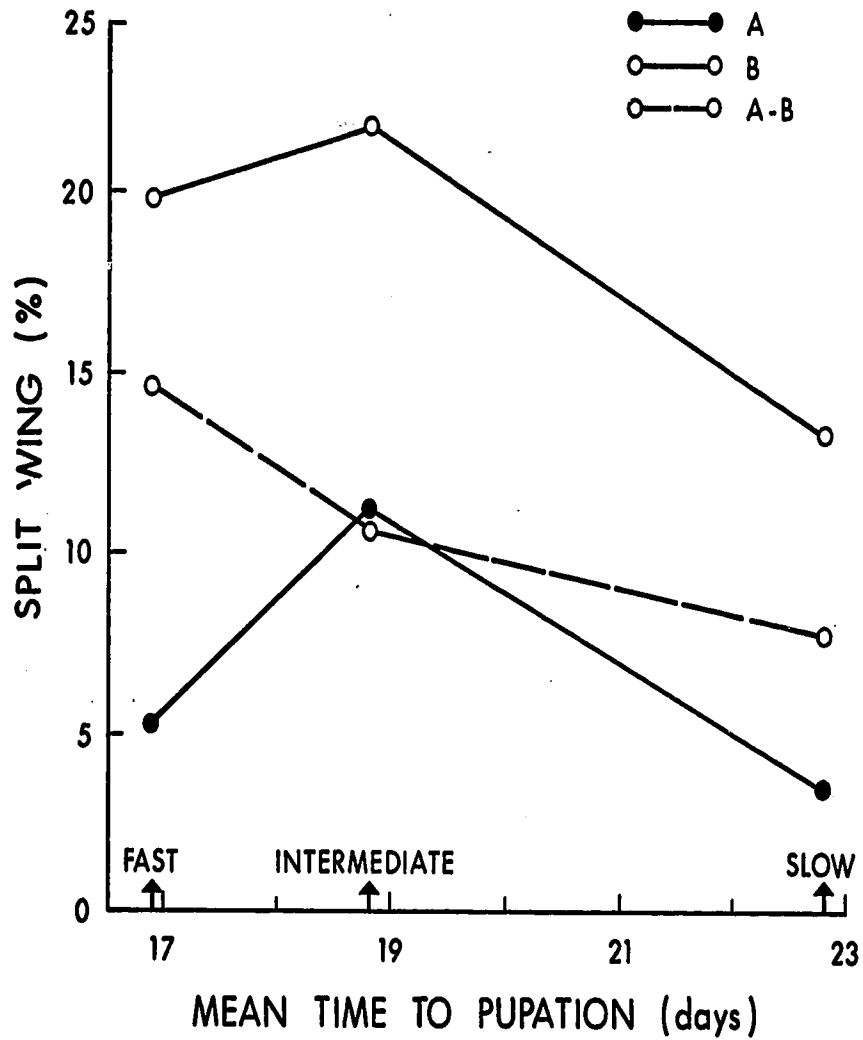
FIGURE 2 Relationship between mean time to pupation and mean percent split wing (Angular transformation) of x-irradiated and nonirradiated lines selected for fast, intermediate and slow rate of development in *Tribolium castaneum*. A = Nonirradiated (selection), B = Irradiated (selection and irradiated), A - B = Irradiation.

repair mechanism may be more efficient in the slow line,
and hence contradict the observation that deceleration of
development by other means, e.g., temperature, results in
reducing rate of recovery (Yang and Ducoff, 1971).

SUMMARY

Artificial selection for fast, slow and intermediate
time to pupation in *Tribolium castaneum* (Jamaica) was
successful in producing lines which differed from the
unselected line for this character. After seven genera-
tions of selection all four lines were compared with respect
to their percent survival and percent split wing after
exposure to a single dose of X-ray (10KR). Although the
four lines differed in both traits, no correlated response
between percent survival or percent split wing and time to
pupation was observed. However, there is an indication that
rate of development may be negatively associated with rate
of repair for the induction of split wing. There was no
difference between males and females in their response to
either selection or x-irradiation.

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