Survival of *Tribolium castaneum* Herbst After Rocket Flight into the Ionosphere

ALEXANDER SOKOLOFF, Ph.D. and ROBERT R. SHRODE, Ph.D.

UNDER natural conditions, few insects are found at very large heights above the earth's surface. The pioneering work of Coad[2] demonstrated the existence of various arthropods up to a height of 14,000 feet. Hardy and Milne[5] pointed out that aerial plankton consists of weak fliers of small size with a large surface-weight ratio which could be picked up by wind currents and carried to considerable heights. Glick[3] showed that the number of arthropods decreases as altitude increases. More recently, Taylor[32] has shown that less than 2 per cent of insects flying up to one mile died in transit, and their sojourn through the air had no effect on their ability to reproduce.

With the development of high altitude balloons and modern jet aircraft, a number of studies have been made to determine the effect of the rarefied, cold atmosphere on the survival of a number of arthropods. Sullivan et al,[31] and Sullivan and Thompson[29] sent some eggs of the phalaenid *Prodenia ornithogalli*, the yellow striped army worm, on the external surface of jet aircraft to the stratosphere. The first flight was at 40,000 feet for 8 minutes at Mach 0.82 (465 knots true air speed) and an air temperature of –57°C.; the second flight was at 45-50,000 feet for 30 minutes, with 12 minutes above Mach 1 and an air temperature of –60°C. Both flights resulted in complete mortality of the phalaenid eggs. However, cold resistant eggs of *Malacosoma americanum* F., the eastern caterpillar, hatched normally under these conditions.

In another experiment, Sullivan and Smith[28] included houseflies (*Musca domestica*), *Xenopsilla cheopis* Rothsch., the oriental flea, and *Drosophila melanogaster* larvæ inside an air conditioned gondola in a balloon flight that maintained an altitude of 78,000-82,000 feet for 16 hours. The main purpose of the experiment was to obtain information on biological damage from cosmic and other radiations near the top of the atmosphere during a well-instrumented flight. Fleas exposed outside the gondola were killed by the low temperature. Succeeding generations of the fleas riding inside the gondola reproduced about normally, and no physical abnormalities were evident. The adults of *Drosophila* emerging from the larvæ failed to show any significant increase in gene mutation or in X chromosome breakage over the controls.[19]

Laboratory experiments by Packchanian to study the effect of simulated altitude on various insects have shown that most species survive two hours exposure at conditions simulating those encountered at 10,000 feet (–5°C., with 552 mm Hg); those exposed to conditions equivalent to 30,000 feet (–44°C. with 225 mm Hg) died after an exposure of 15 minutes although the parasites in these various insects survived the same conditions for from two to 24 hours.[10-12] The main altitude factor responsible for their death is temperature and not reduced pressure. Death increased directly with exposure time for a given altitude or vice versa.[13]

With the development of rockets of high thrust, it is now possible to send man and other organisms to higher and higher altitudes, and eventually to the moon and to the other planets.

From the W. H. Miner Agricultural Research Institute, Chazy, New York.

The present address for Doctor Sokoloff is Department of Genetics, University of California, Berkeley, California; and for Doctor Shrode, De Kalb Agricultural Association, Inc., Sycamore, Illinois.

Before sending man, however, it is necessary to assess the radiation hazard and the effect of weightlessness on organisms other than man. For this purpose, dogs and perhaps other organisms have been sent up in rockets by the U.S.S.R. and mice, rhesus monkeys and chimpanzees by the U.S.* The advantage in using mammals in pioneer space probes is that these animals are closest to man in their physiological reactions. The chief disadvantage is that the gestation period of both dogs and primates is rather long and the number of progeny relatively small, and genetic damage to these organisms may be difficult and relatively more expensive to determine.

In order to assess both genetic and physiological effects, it is more convenient to use some type of insect. Sullivan and Westlake[30] have stated: "An insect may be one of the most useful and convenient organisms for use in space biology"; Sullivan and McCauley[27] have restated this belief in the following words: "Insects have many qualifications that recommend them for use in measuring the effects of outer space on life." There is no further elaboration, since it is generally known that insects are small, they are prolific, and they have relatively short life cycles. Furthermore, a large number of insects are cheaply and easily maintained in the laboratory.

In choosing an insect for studies in space biology, there is always the problem of weighing the advantages and disadvantages that each species offers. *Drosophila melanogaster*, for example, has the advantage of a very short life cycle (10 days from egg to adult at 25°C.), and the unparalleled distinction of being the best studied organism from the standpoint of genetics. A serious disadvantage is that along with its short life cycle the length of life is also short (about 30 to 60 days) and the onset of physiological changes associated with age (for example, a lowering of fecundity) begin to manifest themselves within a relatively short period. Furthermore, this species of fruit-fly requires yeasts for food, and yeasts, in turn, require a relatively complex semisolid medium (consisting of water, agar, molasses and a mold inhibitor) for growth. A culture, unless maintained at relatively low temperatures, may become extinct in about one or two months if the flies are overcrowded owing to a depletion of the food supply and the accumulation of moisture on the inner surface of the culture bottle, on which the flies may get stuck and drown. The young stages of the fly can be collected, but only by rather elaborate techniques and some stages (the egg, for example) are too brief to be used advantageously in space research.

Preferable to *Drosophila*, we believe, are two species of flour beetles, *Tribolium castaneum* and *T. confusum*. The suitability of these cereal pests in laboratory experiments in population dynamics was pointed out by Chapman.[1] Park[14] has summarized the general biology of *Tribolium confusum* and listed the biological attributes of both *T. castaneum* and *T. confusum* in more recent papers (Park,[15] for example). Some of the attributes that make *Tribolium* useful test organisms in space research are the following:

1. The beetles are small. Pupal weights for *T. castaneum* are 2.59 mg for males and 2.72 mg for females (Tribolium Information Bulletin 3, p. 7). Data obtained by Good[4] indicate that *T. confusum* is somewhat heavier than *T. castaneum*. Imagoes of both species probably weigh less than the pupæ.

2. The two species have relatively short life cycles: at 29°C. they develop from egg to adult in about 30 days.[14] At 34°C., *T. confusum* completes its development in about 26 days and *T. castaneum* in about 23 days.[16]

3. The two species are long-lived. Observations by Good[4] on 25 pairs of beetles of each species indicate an average length of life of 634 and 447 days, respectively, for males and females of *T. confusum* and 547 and 226 days for males and females of *T. castaneum*. A few *T.*

*At this writing four human astronauts have already journeyed into space. Chronologically, the U.S.S.R. sent up Yuri A. Gagarin once around the earth and Gherman Titov orbited the earth 17 times in a 24-hour period. The United States has rocketed Alan B. Sheppard and Virgil I. Grissom.

confusum survived over two and a half years, and one male died after three years, 268 days. The oldest *T. castaneum* died at the end of two years, 159 days.

4. The two species are prolific. Park, De Bruyn and Bond[17] report the following values for the lifetime egg production of these flour beetles: *T. confusum* females produced an average of about 860 viable eggs in 180 days, and *T. castaneum* 614 eggs in 135 days in medium renewed at intervals of 30 days. Sokoloff[23] has observed that if medium is renewed every three or four days *T. confusum* can produce an average of 500 viable eggs in 60 days and *T. castaneum* 636 viable eggs in the same period of time. Observations by Good[4] point out that old females lay eggs but they are inviable. On the other hand, old males continue to produce viable sperm throughout their lives.

5. Both species of *Tribolium* can easily be reared in whole wheat flour supplemented with brewers' yeast. From this mixture the beetles obtain their food, and by breaking up the carbohydrates during digestion, the necessary water to survive. If the medium is not renewed adult beetles cease to reproduce or reproduce at a very low level. The flour will eventually consist of fecal matter, but before the medium is completely exhausted beetles can survive by reingesting the fecal pellets with whatever grains of undigested flour that may have become stuck to the pellets. After the flour is no longer usable the beetles can still survive for several weeks by feeding on the bodies of dead beetles, on larvæ or pupæ or by chewing off the antennæ or parts of the legs and elytra of other live beetles. A culture including both sexes will last unattended for eight months or possibly longer. If beetles are prevented from reproducing (by placing pupæ of the two sexes in separate containers), 5 grams of flour may be sufficient to maintain alive about 100 adults for at least five or six months.

6. Both species can resist starvation for prolonged periods of time. The resistance to starvation increases inversely with the temperature, and a high humidity (60 per cent) enables the beetles to survive a longer period than a low humidity (30 per cent). According to Good[4] adults of both species of *Tribolium* survive in an empty dish for about two weeks (with a maximum of 18 days) at 30°C. At room temperature during the winter the longest period recorded was 23 days. At 15°C. some survived for 27 days. At 10°C., several individuals lived over 40 days, and one *T. confusum* adult for 51 days. Larvæ were found to be slightly more resistant to starvation than adults at all temperatures studied.

7. *Tribolium* can withstand exposures to extreme cold. Payne[18] found that adult *T. confusum* can withstand undercooling temperatures of −4.5° C. and larvæ of −5.6°C. The mean freezing temperature of adult beetles was −2°C., and of larvæ −3.6°C. No similar data are available for *T. castaneum*. Since this species has a more southern distribution, it may not tolerate such low temperatures, but it can be placed in the refrigerator for a week and survive. At the other extreme, *T. castaneum*, if placed in a bowl of flour, can be introduced into an oven maintained at 62°C. for five hours and some beetles will survive (Sokoloff, unpublished).

8. *Tribolium* can withstand low atmospheric pressures. Kennington[6] subjected both species to atmospheric pressures simulating altitudes of 8,000 and 14,000 feet, but maintaining temperature and relative humidity the same as for the controls. There was no acclimatization or adaptation to low atmospheric pressures; there was a reduction in weight and in fecundity and a lengthening of the life cycle roughly proportional to the degree of reduction in atmospheric pressure. The reverse effect (i.e., effect of high atmospheric pressure) apparently has not been studied in beetles, but preliminary observations by Mr. Peter Hofmann in the senior author's laboratory indicate that both species can withstand pressures of 125 PSI for 24 hours.

9. Among insects, *Tribolium confusum* appears to be highly resistant to the stresses imposed by extreme forces of acceleration. Sullivan and McCauley[27] found that 50 per cent of the beetles survived when subjected to 20,600 g's

for 73 minutes. These authors refer to an anonymous writer who states that by comparison "man has structural damage when exposed to 18-45 g's for short duration."

10. Information is available on the effects of irradiation on the fecundity and fertility of *T. castaneum* and *T. confusum*.[9,17,22,23] These studies indicate a lowering in fecundity and fertility with a consequent gradual increase in dominant lethals roughly proportional to the degree of irradiation until at 6000 r almost 100 per cent of the eggs fail to hatch.

11. Since beetles live in particulate medium, it it possible to collect any desired stage with the minimum of effort by sifting the flour from the culture. This feature is particularly advantageous when it is desired to collect large numbers of eggs laid within a short period of time.

The disadvantageous features in using these organisms in space research are the following:

1. The genetic information on *Tribolium* is superficial at the present time. In *T. castaneum* about 30 different mutations are known, of which five are sex-linked. Of the autosomal mutations known about one-third are not useful because of poor penetrance or variable expressivity. In *T. confusum* fewer genes are known (about 15) and about half are not useful for the same reasons or because of poor viability of the mutants.

2. *Tribolium* has very small chromosomes making cytogenetic studies difficult.

3. *Tribolium* is susceptible to the attack of a number of protozoan parasites. Consequently, a constant watch must be maintained to insure that the stocks remain parasite-free. A parasitized culture should not be used for space research since parasites reproduce rapidly and may eliminate virtually every member of the colony within a short time. Predatory mites, particularly *Pyemotes* (=*Pediculoides*) *ventricosus* may occasionally parasitize *Tribolium*[4] or other flour beetles.[25]

4. The active stages (larvæ and adults) of *Tribolium* are cannibalistic. Both stages may eat live eggs or pupæ, and both stages may eat dead juvenile and adult beetles even in the presence of fresh food.

5. Both species of *Tribolium* secrete quinones through several odoriferous glands[8] with which they condition the medium. Occasionally, if large numbers of adults of either species are placed in an empty covered dish, they may become excited releasing the quinones in large enough concentrations to kill all the individuals in the dish. This situation can be avoided by always placing the beetles in flour.

Despite the seemingly serious drawbacks listed, the advantages far outweigh the disadvantages and we believe that flour beetles are among the best organisms available for space research, particularly if the flight is prolonged and especially in assessing the effects of radiation on biological material leaving the protective cover of the earth's atmosphere.

An opportunity to test these organisms "in the field" arose on December 4, 1959, when Sam, a much publicized rhesus monkey, was sent 55 miles into space in a Meteor capsule lifted off Wallops Island, Virginia, by a Little Joe rocket. Accompanying Sam were other organisms. The purpose of this paper is to report on the results of our experiments with *Tribolium castaneum*, in the hope that other investigators may consider using flour beetles in future space probes.

MATERIALS AND METHODS

The material used in this investigation was a strain of *Tribolium castaneum* derived (in 1955) from a stock maintained by Professor Thomas Park for some 15 years in his laboratory at the University of Chicago, Chicago, Illinois. Available for inclusion in the "biopack" were the following numbers of the various stages of the beetle: 400 male and 400 female adults not more than 10 days old; 400 pupæ of both sexes; 100 "inactive" larvæ approaching the pupal stadium; 400 active large larvæ and 400 small larvæ. The rest (about 8500) were eggs not less than 16 hours and not more than 40 hours old by the time the weather cleared and the rocket was fired.

The adults and the juvenile stages up to two days prior to their sojourn to Virginia had been reared in an incubator maintaining a constant temperature of 29°C. and 70 per cent relative humidity. The eggs were obtained from several hundred adult beetles kept in the bathroom of

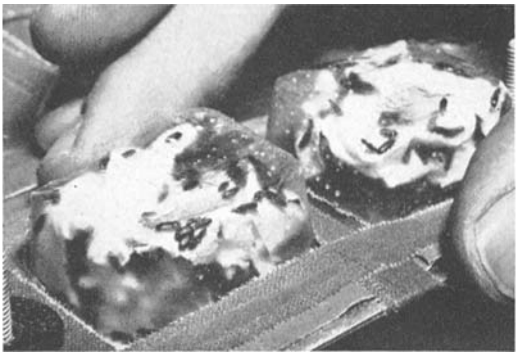

Fig. 1. Plastic boxes containing the adult stages of *Tribolium castaneum*. Each box contained 400 imagoes and some tissue paper. (SAM · Official Air Force Photo).

a motel where a radiant heating floor provided a temperature of 78°F. Humidity was not recorded.

The small and large active larvæ were placed (along with one-half of a sheet of toilet paper rolled into a loose wad to provide the larvæ with clinging surface) in one dram glass vials closed with a plastic cap previously puctured with a small hole for ventilation. The other juvenile and the adult stages were introduced, along with some tissue paper, in plastic containers commonly used in restaurants to dispense grape jelly and other jams. These inexpensive containers are rigid enough, they are light in weight, and they possess a flange by means of which the container can be securely taped (topside down) on any flat surface. After the beetles were introduced into the containers the plastic cover was resealed with Scotch tape, and the sides of the container punctured with a fine needle to permit free exchange of gases. These containers were subsequently affixed with adhesive cloth tape on a metal plate bearing several bolts for the attachment of metal brackets which helped in securing the various containers with biological specimens to the metal plate (Fig. 1). Because the beetles can withstand long periods of starvation, no food was included in any of the containers.

The eggs were glued to two photographic plates measuring two by three inches in the following manner: One of the surfaces of the polyethylene enclosing the track plates was covered by a rectangular piece of graph paper (100 squares to the square inch) of the same size as the track plate and held in place by double-sticky Scotch tape. A peripheral strip 1/4 inch wide was covered with masking tape to provide a lip by which the track plate could be handled and taped to the underside of the metal bracket. The eggs were sprinkled on the sticky 1.5 by 2.5 inch surface left exposed. After all the eggs were sprinkled on the two plates, each plate was tapped vigorously on the edges to dislodge any

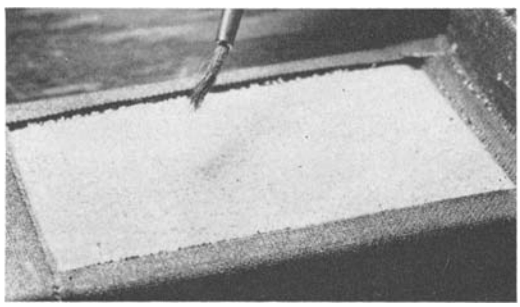

Fig. 2. One of the two egg-bearing track plates (still taped to a metal bracket) after recovery of the Mercury capsule. Note the faint lines of the graph paper in areas not covered by the eggs. (SAM Official Air Force Photo).

eggs loosely glued to the tape. The eggs were resprinkled over and over until all the eggs remained attached after three vigorous taps. Each track plate accommodated over 4000 eggs.

After all the eggs were securely glued, the track plates were taped to the underside of a metal bracket by means of an adhesive cloth tape and the bracket bolted onto the metal plate designed to carry the biopack. Figure 2 shows one of the egg-bearing track plates still taped to the bracket after recovery of the capsule from the sea.

The plastic containers holding the male and female adults were placed under one of the track plates. The male and female pupæ were placed under the second track plate.

Figure 3 shows the whole biopack. The barrel-like container held the rhesus monkey and the instruments designed to telemeter the monkey's reactions while in flight. Attached to it in parasitic fashion is a metal plate holding the packages containing the various other biological materials. The beetles were placed on the far left, between the first three upper and three lower bolts. The whole assembly was placed inside a Mercury capsule.

It is evident that when organisms are taken outside the laboratory it is impossible to control the environmental conditions. Controls in this experiment were of two types: "primary controls" were contemporary beetles from the same stock as the rocketed beetles but kept in the laboratory; "secondary controls" experienced the same changes in environmental conditions enroute from the laboratory to the firing area in Wallops Island. Subsequently, however, the secondary controls and the experimentals were exposed to different conditions. The latter, because of adverse weather conditions which caused postponement of the rocket for 29 hours, were kept in the Mercury capsule. The temperature within the capsule was 68°F. The secondary controls, on the other hand, were kept in an adjacent unheated building. The temperature was not recorded, but it was probably higher than the outside temperature which was close to 45°F.

After recovery, the biopack was placed in a cabin of the ship that was to transport it to Newport News, Virginia, and subsequently it was moved by truck to Langley Field, Virginia. In the meantime, the secondary controls had been taken by car to the same military installation. Thus, for a period of about 48 hours the two sets of beetles experienced different environmental conditions.

After the experimentals were returned to us, the graph paper, while still on the track plate, was punctured with a fine needle in three predetermined places. The puncture was deep enough to pierce the black polyethylene wrapper of the track plate and to allow some light to penetrate, affecting the silver emulsion under the wrapper. Thus, identifiable coordinates were established both on the track plates and on the graph paper, and in the event of a hit by a cosmic particle its location and the position of the eggs around it could be identified.

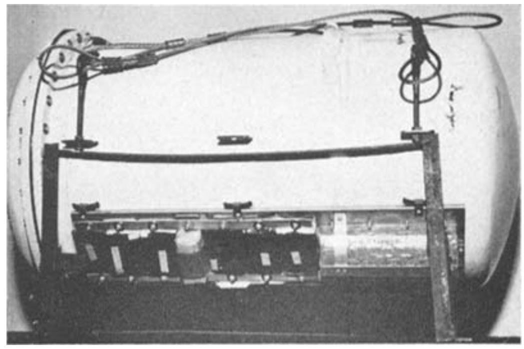

Fig. 3. Arrangement of the whole biopack. The flour beetles were located between the leftmost 3 pairs of bolts. (SAM Official Air Force Photo).

The track plate was sent off to the School of Aviation Medicine, Brooks Air Force Base, Texas, for processing. In the meantime, upon return to the laboratory, the graph paper from each track plate was cut into 15 squares one-half inch to the side, and each square identified by a number. After counting the eggs, each square was thoroughly sprinkled with flour to enable the larvæ to crawl away from the egg-shells without getting stuck on the tape, and introduced in separate vials containing standard medium (consisting of 19 parts by weight fine whole wheat flour and one part brewer's yeast). All vials were placed in an incubator maintained at 29°C. and 70 per cent relative humidity. Dead larvæ stuck on the tape covering the graph paper or dying in the medium were recorded two weeks later. Beetles surviving to the imago stage were counted three to four weeks thereafter, and pair mated in separate vials (the number of pair matings from each square being determined by the smaller number of beetles of either sex, any extra beetles of either sex being discarded).

Upon notification that one of the squares had been hit by a cosmic particle, the vials containing beetles from all but two squares (the one recording the hit, and another on the opposite side of the same plate to be used as a control) were discarded. From the remaining vials, five pairs of beetles were bred by single pair matings for three generations and scored for visible and sex-linked lethal mutations. If the sex-ratios indicated the possible presence of a sex-linked lethal, further matings were carried out to confirm their presence. Contemporary beetles that had never left the laboratory served as primary controls for this part of the study.

TABLE I. INVENTORY OF T. CASTANEUM LEAVING AND RETURNING TO THE LABORATORY (SECONDARY CONTROLS—TAKEN TO WALLOPS ISLAND BUT NOT SENT ON THE FLIGHT. EXPERIMENTALS—SENT ON THE ROCKET FLIGHT.)

Secondary Controls				
Stage	Number taken	Number returned Live	Dead	Remarks
Adults	225	219	1	5 lost (Dead beetle was mutilated)
Pupae	100	95	5	
Medium larvae	100	86	6	8 lost
Small larvae	100	78	12	10 lost

Experimentals				
Stage	Number sent on flight	Number returned Live	Dead	Remarks
Adult ♂♂	400	398	1	1 lost
Adult ♀♀	400	397	0	3 lost
Pupae	400	388	12	(1 accidentally crushed)
Large larvae	100	96	4	(all had pupated; 2 had been cannibalized)
Medium larvae	400	387	0	13 lost
Small larvae	400	381	0	19 lost

Beetles derived from astronaut pupæ were also bred for three generations since the track plate indicated traversal of the cosmic particle through the container holding this stage of *Tribolium*.

The juvenile and adult stages sent on the flight were subjected to the following observations:

1. The number of adults surviving the sojourn into space was noted, and the adults observed for any abnormal behavior.

2. Mortality of eggs, larvæ and pupæ was compared with that of the controls.

3. A large number of adults taking the flight and adults derived from rocketed pupæ were selected to determine whether their flight into the ionosphere had had any effect on fecundity and/or fertility.

Further details on methods used will be discussed under the section on results as the need for further elaboration arises.

RESULTS

Survival of Juvenile and Adult Stages After Rocket Flight.—Table I presents an inventory of beetles of all stages (except the eggs). Examination of the figures in Table I is sufficient to convince one that only sampling differences in viability occurred. Of the 800 adults sent on the flight, only one died, whereas in the secondary controls only one dead beetle was observed among 220 beetles returned. Among the pupæ used as secondary controls, 5 per cent failed to survive to the imago stage. This compares with the 3 per cent mortality observed among the rocketed pupæ. Among the large larvæ sent into space, four deaths were observed, and two of these resulted from cannibalism on the part of some larvæ before pupation took place. The deaths among the medium- and small-sized larvæ, if anything, were greater among the secondary controls than in the experimentals. The known deaths were certainly no more numerous than would be observed in handling under laboratory conditions groups of beetles of comparable size from the same stock. It seems likely that most of the shortages in numbers returned to the laboratory were made up of live beetles accidentally lost in the process of removing them from their containers under somewhat suboptimal conditions.

Fecundity of Beetles Taking Flight and of Those Remaining on Ground. — Fecundity was measured as the number of eggs laid during a 24-hour period by flight beetles and control beetles. Both single-pair and mass matings were made for this purpose. For brevity, the four kinds of beetles, with respect to treatment, will be referred to as F (adult flight beetles), P.C. (primary control beetles, i.e., contemporary beetles of the same stock as the flight beetles but kept at the laboratory), S.C. (secondary control beetles, i.e., contemporary beetles from the same stock as F beetles, taken to Wallops Island but not sent on the rocket flight), and S.P. (beetles which survived the rocket flight as pupæ).

Single-pair matings were made using only F and P.C. beetles which were adults at the beginning of the experiment. No attempts were made to pair-mate S.C. and S.P. beetles since these had not been sexed and the females were no longer virgin when fecundity experiments were begun. The matings made were as follows: ten replicates each of F ♂ x P.C. ♀; P.C. ♂ x F ♀; F ♂ x F ♀; and P.C. ♂ x P.C. ♀. The average numbers of eggs laid in 24 hours was 11.9 for F females and 10.75 for P.C. females indicating no significant difference in fecundity between beetles retained in the laboratory and those experiencing the rocket flight. These conclusions have been derived from the analysis of variance shown in Table II.

TABLE II. ANALYSIS OF VARIANCE OF 24-HOUR EGG PRODUCTION OF T. CASTANEUM FEMALES IN SINGLE-PAIR MATINGS

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Total	39	658.78	
Mating types	3	51.48	17.16
F ♂ x P.C. ♀ vs. P.C. ♂ x P.C. ♀	1	22.05	22.05
P.C. ♂ x F ♀ vs. F ♂ x F ♀	1	16.20	16.20
F ♀ vs. P.C. ♀	1	13.23	13.23
Matings within type	36	607.30	16.87

TABLE III. ANALYSIS OF VARIANCE OF FERTILITY IN THE SINGLE-PAIR MATINGS (TRANSFORMED TO ARCSIN $\sqrt{\%}$)

Source of variation	Degrees of freedom	Mean squares
Total	39	
Mating types	3	530.03
Matings within type	36	342.68

The lack of significance of differences between mating types actually makes unnecessary the evaluation of the three mean squares based on individual degrees of freedom. They are shown here simply as a matter of interest. The first two demonstrate the fact that treatment of males (voyaging to space versus remaining in the laboratory) had no significant influence on the fecundity of females to which they were mated, and the third indicates that similar treatment of females had no significant influence on their own fecundity.

TABLE IV. RESULTS FROM MASS CULTURES. (FOR EXPLANATION SEE TEXT.)

Mating ♂ ♀	Number of females	Ave. No. egg per female	Ave. No. larvae per female	Per cent eggs hatching	Per cent larvae surviving to adults
F x P.C.	50	5.08	3.92	77.2	100
F x P.C.	50	5.34	4.52	84.6	99.6
F x F	49	5.37	4.31	80.3	100
F x F	49	6.29	5.45	86.6	98.1
F x F	49	4.00	3.51	87.8	100
F x F	49	3.98	3.02	75.9	99.3
P.C. x F	50	5.34	4.48	83.9	100
P.C. x F	49	5.35	4.67	87.3	97.4
P.C. x F	50	5.02	4.10	81.7	99
P.C. x P.C.	50	5.60	4.68	83.6	98.7
P.C. x P.C.	50	4.90	3.76	76.7	99.5
S.C. x S.C.	42	5.81	3.71	63.9	100
S.C. x S.C.	68	5.35	4.22	78.9	98.3
S.P. x S.P.	39	6.38	5.46	85.6	98.6
S.P. x S.P.	45	5.47	4.73	86.5	99.5

The eggs derived from these various matings were allowed to hatch as larvæ in order to get an estimate on the fertility of the eggs laid by females voyaging to space and compared with the fertility of eggs laid by females never leaving the laboratory. As with egg production, there were no significant differences in hatching percentage as shown by the analysis of variance given in Table III. The mean hatching percentages of F females and P.C. females were 90.3 and 86.5, respectively. This, again, indicates that the beetles voyaging to and returning from space will produce as fertile eggs as those remaining in the laboratory.

A summary of information concerning mass matings is given in Table IV. Within the body of the table are indicated the types of matings involved. The number of repetitions of a given type of mating gives an indication of the number of replications. The number of females used in each replication is indicated. The number of males used is not indicated in the table and it is not important, since one male can inseminate a female three times per hour. It is sufficient to state that the number of males used was quite variable, but never less than 20 and not more than 50. The eggs were collected for a period of 24 hours, counted, and introduced into creamers with standard medium. The creamers were placed in incubators maintained at 29° C. and 70 per cent relative humidity. Larvæ emerging from the eggs were counted at the end of three weeks. Adults metamorphosing from these larvæ were counted about four weeks later.

TABLE V. ANALYSIS OF VARIANCE OF 24-HOUR EGG PRODUCTION IN MASS CULTURES

Source of variation	Degrees of freedom	Mean square
Total	14	
Mating types	5	0.32
Matings within type	9	0.52

Since values could not be computed on an individual basis as in the case of single-pair matings, the analysis had to be based on averages. Hatching percentage for each culture was computed by dividing the average number of larvæ per female by the average number of eggs per female. This gives estimates of the fertility of the eggs laid by females in the various classes. Taking the figures at face value, it is seen that the lowest value obtained (63.9 per cent) was in one of the samples in the secondary controls. The second sample in this class, however, had a fertility value of about 79 per cent. Primary control (P.C.) females produced eggs which were about equally fertile when mated to P.C. males as when mated to F (i.e., beetles sent on the rocket flight). Similarly, females sent on the rocket flight produced eggs of about equal viability when mated to males that also were sent in the rocket as when mated to males that had never left the laboratory. Nearly all of the larvæ emerging from the eggs usually develop into adults in normal laboratory cultures. The data from the various matings show that nearly all of the larvæ succeeded in becoming imagoes regardless of the previous history of their parents.

The pupæ of *Tribolium* are fairly soft and one might suppose that the stresses of rocket flight would result in a higher pupal effect and that the physiological effects from such an experience might be reflected in such things as fecundity. This supposition is not borne out by the last two entries in Table IV: female imagoes emerging from pupæ taking the flight produced, on the average, as many eggs as the controls. The fertility of these eggs was about the same, and the number of larvæ reaching the adult stage was not significantly different.

TABLE VI. ANALYSIS OF VARIANCE OF HATCHING PERCENTAGE (TRANSFORMED TO ARCSIN $\sqrt{\%}$) IN MASS CULTURES

Source of variation	Degrees of freedom	Mean square
Total	14	
Mating types	5	26.87
Matings within type	9	15.15

Tables V and VI contain the analyses of variance for percentages of egg production and for percentages of eggs hatching into larvæ (fertility). No analysis of variance is needed to corroborate the more evident fact that the mortality of larvæ is small and not significantly different when the various matings are compared: Table IV shows that nearly all these percentages were 100 in mass cultures. The list of percentages from 40 single-pair matings (not presented here) is very similar. Only three of the forty values differed from 100, being 75 and 92.3 respectively, for two of the P.C. ♂ x P.C. ♀ matings and 90 for one of the P.C. ♂ x F ♀ matings. These three percentages represent the survival percentages of original total numbers of 12, 13 and 10 larvæ, respectively.

From these data, one can safely conclude that, with respect to fecundity, fertility and percentage of adult hatch the adult beetles and pupæ (adults emerging from larvæ journeying into space were not studied in this regard) were not adversely affected by the environmental extremes encountered during the rocket flight.

Fate of Eggs Sent Into Space. — Table VII summarizes the fate of eggs journeying into space. Of the 8500 eggs included in the track plates only about 56 per cent were returned to us. Part of these eggs probably were lost while the track plates were being taped on to the remaining biopack assembly; but the majority, it is felt, must have been lost while the material was being transported (by truck) from the "field" laboratory to the firing area, and subsequently from the dock to Langley Field since the track plates were not covered. Of the returned eggs 91.6 per cent were viable and produced larvæ, but only 70 percent survived to the imago stage. These figures, however, do not indicate an excessive mortality since in experiments where adult productivity has been studied (from eggs reared under uniform laboratory conditions), values of 70 per cent adult hatchability are not unusual.

TABLE VII. INVENTORY OF EGGS SENT INTO SPACE AND THEIR FATE AFTER RECOVERY

Number sent into space	8500
Number recovered	4840
Total dead eggs	406
Number hatched as larvae	4434
Per cent surviving to larvae	91.61
Number hatched as adults	3384
Per cent eggs surviving to adults	69.92
Per cent larvae surviving to adults	76.32

Of greater interest, perhaps, is the fact that one of the track plates bearing eggs was hit twice by heavy primary cosmic particles. The report from the USAF Aerospace Medical Center (ATC), Brooks Air Force Base, Texas, reads as follows:

Plate No. 59-12-4R-10

Examination of this plate revealed two primary traversals,

(a) One track, left by a particle whose charge (or atomic number) was estimated at Z = 20, was located in the bottom left portion of the specimen area. Using an estimated limit of error inherent in the experiment, the portion of the specimen through which we feel the particle traversed is outlined on the enclosed graph. (The pencil area encircled in red.) [See Figure 4 for a schematic reproduction of this graph.]

Fig. 4. Schematic representation *(at lower left)* of the area of the egg-bearing track plate hit by a cosmic particle whose atomic number was estimated at Z=20. The stippled border represents area of the track plate not bearing eggs of *T. castaneum*.

(b) The second track, left by a particle of charge estimated at Z = 14 (Silicon), entered the *top* of the track plate in the bottom left portion such that it missed the specimen entirely. The angle formed by the track left by the particle and the plate surface was so shallow (approximately 48' of arc) that the particle never actually traversed the bottom emulsion on the track plate but left the plate through its side.

The area hit by the heavy primary cosmic particle (Fig. 4) was included in square number 10. In this square a total of 93 eggs were recovered. Of these, 71 per cent were viable producing larvæ, and 69 per cent of the eggs produced adult beetles. Although the *in ovo* mortality was much greater in this square than in the rest of the squares (the average larval hatchability being 91.6 per cent), the number of eggs surviving to the adult stage is no different from the average value of adults obtained from the eggs in the remaining squares (70 per cent). The mortality of the eggs observed in square number 10, therefore, cannot be attributed to the heavy primary cosmic particle, but must have been due to the natural mortality of these eggs.

If information on the location of the area hit by a heavy primary cosmic particle were immediately available, it would have been possible to isolate the larvæ emerging from eggs included in this area. Unfortunately, this information was considerably delayed, and by that time all the imagoes had emerged from all the eggs. Beetles from all the squares had been pair-mated (to the extent of the number of pairs available) in vials. With the information at hand, only the pair-mated beetles from squares number 10 (the one receiving the hit) and number 6 (on the side opposite to the area hit) were retained. Square 6 yielded enough beetles to mate 58 pairs; 74 pairs were mated from square 10. These matings represented the P_1 generation. The F_1 of each pair were sexed and counted, and from each P_1 pair four F_1 pairs were mated to obtain F_2. The F_2 were again sexed and counted. All F_1 and F_2 beetles from the two squares were scored for any abnormalities and any significant deviations in sex ratio.

TABLE VIII. HERITABLE AND TERATOLOGICAL ABNORMALITIES OBSERVED IN ROCKETED BEETLES (EXPERIMENTALS) AND IN LABORATORY STOCKS (CONTROLS). FOR FURTHER DETAILS, SEE TEXT.

Heritable abnormality	Controls	Experimentals
Autosomal		
blistered elytra	x	x
droopy elytra	x	x
warped elytra	x	x
deformed legs	x	x
bronze		x
prothoraxless	x	x
tiny	x	x
Sex-linked		
lethal	x	x
divergent elytra		x
Teratologies		
bow antennae	x	x
serrated antennae	x	x
short antennae	x	x
microphthalmic-like		x
deformed thorax	x	
split thorax	x	x
black-spotted elytra	x	x
short elytra	x	x
wrinkled elytra	x	x
black-spotted abdomen	x	x
twisted abdomen	x	x

If any beetles with detectable abnormalities were found, they were bred in order to establish whether the abnormality was heritable or the result of a developmental accident. If any significant deviations from the one to one sex ratio were observed in any crosses, the females were bred again to confirm the presence of a sex-linked lethal. As controls, bettles derived from the same stock as that from which the rocketed bettles were derived (but which had never left the laboratory) were pair-mated. Both the rocketed beetles (experimentals) and the laboratory beetles (controls) were bred for a sufficient number of generations to establish whether the abnormalities were heritable.

Table VIII shows a tabulation of the heritable abnormalities observed in the two sets of beetles. It is evident that most of the heritable abnormalities observed in beetles derived from eggs sent into space are the result of genes carried in a heterozygous condition in the stock from which the eggs were derived: "blistered elytra" is a recessive gene with expressivity subject to modification owing to the presence of modifiers[24]; "droopy elytra," "warped elytra" and "deformed legs" are recessive genes with poor penetrance and variable expressivity[20]; "bronze" is the heterozygous manifestation of the semidominant gene black[26]; "prothoraxless" is a semidominant, recessive lethal, gene of variable expression overlapping wild type affecting the prothorax and/or the first pair of legs[7]; "tiny" results from the action of several autosomal genes affecting body size (Sokoloff, unpublished). Of the sex-linked genes divergent elytra is an incompletely recessive gene new to *Tribolium*. It must be a spontaneous mutation and not the result of the unusual experience undergone by the beetles, since it was discovered among the imagoes emerging from the pupae rocketed into space. In regard to the lethal observed in the experimentals and in the controls, it is not possible, at the present time, to state whether they are independent lethals or the same. More than likely, since the two sets of beetles were obtained from the same stocks, they may be the same lethal. Suffice it to say, for the present, that these mutations were observed in the F_1 of both the controls and the experimentals and therefore could not have occurred as a result of the rocket flight. As to the teratological specimens observed, none is really new. They have been observed from time to time among many thousands of beetles examined and (so far as known) they have no genetic basis (Sokoloff[21] and unpublished data).

DISCUSSION

In the brief thirteen-minute flight of the Mercury capsule the biological materials were lifted 280,000 ft. into space and landed 200 miles off the coast. Aside from the gravitational forces induced by the forces of acceleration, abrupt changes in atmospheric pressure and temperature occurred. According to the Altitude, Pressure and Temperature Tables worked out by the Guardite Company, Division of American-Marietta Company, Wheeling, Illinois, as the rocket ascended it passed through various atmospheric layers in which the temperature drops from 59°F. at sea level to —69.7°F. at 37,500 ft. This temperature remains constant up to a level of 80,000 ft. and from this height to 170,000 ft. above sea level the temperature rises until it is 49°F., almost the same as that at sea level. From here the temperature again drops gradually until it reaches a cold belt 250,000-300,000 ft. above sea level which is at —105.3°F. At higher altitudes than this the temperature gradually rises until, at 1,800,000 feet, it reaches 2238°F. The atmospheric pressure gradually drops from 759.99 mm Hg at sea level until, at 280,000 feet it is reduced to 3.716×10^{-3} mm Hg. Such conditions obviously are inimical to life, and living material can survive only if protected in an air-conditioned capsule where atmospheric pressure and temperature conditions remain constant and close to those encountered on the surface of the earth. The fact that Sam, the rhesus monkey, survived this flight attests to the perfect functioning of the capsule, and therefore it is not surprising that the flour beetles also survived.

The purpose of this experiment was threefold: (1) it tried to determine whether flour beetles are suitable organisms for space research; (2) it tested whether the various forces exerted on the flour beetles during the rocket flight had any possible effect on the organisms's physiological functions and (3) it tried to find any evidence for damage to the genetic material as the result of the flight.

In regard to the first point, we believe that the results presented above point to the fact that these insects are eminently suitable for space research. Although the flour beetles had no food or water during the whole experiment, which lasted for over 28 hours owing to adverse weather conditions, the mortality of the rocketed beetles was no greater than of the controls. The pupae continued developing so that by the time they were returned to us some imagoes were beginning to emerge, and some of the last instar larvae had become pupae. The larvae of various sizes also seemed no worse off for their experience.

The second point has been answered in the negative by the normal development of the juvenile stages and the additional demonstration that the adult beetles sent into space reproduced normally.

The approach in answer to the third question has been to test the adults emerging from eggs sent into space, and particularly by mating for several generations the beetles emerging from eggs near the area of a track plate recording a hit of a heavy primary cosmic particle. This experiment failed to show any increase in mutation rate in beetles derived from eggs making the flight into space as compared with contemporary beetles from the same stock never leaving the laboratory.

Since various types of irradiation are known to induce genetic damage, the question remains whether the heavy primary cosmic particle recorded by the track plate actually hit any eggs. Despite the fact that the whole surface of the graph paper over the track plate was covered with eggs there were some spaces between the eggs. Thus, the heavy primary cosmic particle could have missed the eggs entirely. On the other hand, the particle could have hit an egg and damaged it genetically, but we failed to detect the damage because the egg fell off the track plate before it reached the laboratory. Finally, the possibility remains that the egg hit by the heavy primary cosmic particle was among the eggs recovered, but the damage to the egg was so severe that it failed to develop. This egg, with the methods at our disposal, would be indistinguishable from those dying from natural causes.

SUMMARY

After surveying the literature on aerial and space ecology involving insects and discussing the advantages and disadvantages in utilizing flour beetles of the genus *Tribolium* in space research, data are presented on the fate of some 11,000 specimens (eggs, larvae of various sizes, pupae, and adults) sent up 55 miles into space in a Mercury capsule lifted off Wallops Island, Virginia, by a Little Joe Rocket. There was no increase in mortality in any of the stadia of the insects rocketed into space over the controls. There was no physiological damage on the reproductive capacity of the flour beetles (as tested by fecundity and fertility) resulting from any of the physical forces experienced by the adults during the flight. Although hits of two cosmic particles were recorded by the track plates bearing eggs, no increase in mutation rate was observed in beetles emerging from eggs near the area hit by the cosmic particle. It is concluded that flour beetles, because of the vast information available, are the best organisms to use in space research, particularly if the space flight is prolonged for several months.

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