NOTES - RESEARCH

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*Quantitative differences between the "aureate" and normal phenotypes in T. castaneum

The "aureate" mutation produces beetles whose bodies appear more pubescent than the wild type. Counts of body hairs were made on typical adult beetles chosen at random from the au and normal stocks. Counts were made through a compound microscope at 150X magnification for sternites and 660X magnification for wings, using an ocular reticle calibrated for a square approximately 0.1 mm to a side for the sternite, and 0.024 mm for the wing counts. One of the membranous wings and the abdomen of ten beetles of each type had been temporarily mounted in paraffin oil. One square area from the central portion of each of the five visible sternites, and five contiguous areas along the main axis of the wing (near the distal tip to avoid veins) were chosen, and in each the number of bristles or hairs was determined.

The results are shown in Table 1. From the data in the table it can be seen that, although the difference is not significant for the membranous wings, it is significant for each of the abdominal sternites, the number of bristles being increased two- or threefold in the mutant.

Table 1. Numbers of setae in normal and aureate abdominal sternites and wings in T. castaneum (N = 10)

Apparent abdominal sternite	Normal	aureate
1	9.4 ± 0.2	30.6 ± 1.4
2	10.7 ± 0.4	27.4 ± 1.7
3	9.7 ± 0.4	28.3 ± 1.5
4	10.6 ± 0.3	27.4 ± 1.1
5	9.9 ± 0.4	23.7 ± 2.1
Total	10.06 ± 0.17	27.25 ± .69
Membranous wings	10.84 ± 2.36	11.10 ± 1.66

Thus, from our observations, the aureate gene seems to affect only the sclerotized portions of the exoskeleton. Scanning electron micrographs (see research note elsewhere in this bulletin) show that there is a definite increase of cervical setae (at the anterior margin of the prothorax, particularly at the gular border) and on the antennae. Most remarkable is the fact that the interommatidial bristles, which are single in normal beetles, are doubled under the influence of au.

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*Acarophenax tribolii--a parasitoid of Tribolium

In 1964 small mites were found infesting some of the Tribolium cultures at Berkeley. Since a general decline in the vigor of the cultures was associated with these infestations, it was necessary that some remedial action be taken. A review of the literature revealed that these mites, belonging to the family Pymotidae, had been described by Newstead and Duvall (1918) as Acarophenax tribolii. In addition to the taxonomic description they gave a generalized account of the life history and habits of these mites. More recently Cross (1965) reviewed the family Pymotidae and summarized what was known about the life history of Acarophenax tribolii by referring back to the paper by Newstead and Duvall (1918).

The adult mites are found attached to the bodies of both adult and larval beetles. Commonly 10 to 12 adult mites may be found on the bodies of the beetles. There is some question whether the mites feed on the adults and larvae of Tribolium, or whether the relationship is merely phoretic.

After several days the adult mites drop from the beetles and seek out a Tribolium egg. The mites attach themselves to the eggs, and in a day or two suck out the contents. During feeding there is marked enlargement of the body of the female (physogastry), and all power of locomotion is lost. Complete development of the immature mites takes place within the body of their mother. There is good evidence that mating takes place before the adult females leave the body of the mother. Since development takes only three or four days and commonly there may be 12 to 14 progeny per mother, it is understandable why infestations can build up rapidly.

Since establishment of new Tribolium cultures from small numbers of adults observed to be free of mites was unsuccessful in eliminating infestations, it was obvious that more drastic action would be necessary to clean up the cultures. A number of tests were conducted to determine if certain chemicals could be found that would control the mites without having an adverse effect on the beetles. These tests revealed that there was

selectivity in the insecticide endosulfan (Thiodan) and the acaricide Morestan when used in the flour at a rate of 300 ppm of the actual material. Because Morestan generally is regarded to be of lower toxicity to insects it was selected for use in cleaning up the cultures.

In actual practice it was found that Morestan did have a detrimental effect on the beetles particularly when they were held at higher temperatures. This difference can be explained by the fact that the initial tests were run with adults and mature larvae rather than the early larval instars. Nevertheless, it was possible to use Morestan to help free the cultures of the mites. This was done by placing beetles in flour treated with 300 ppm Morestan and holding them at a temperature of 20°C. After 10 days the beetles were removed from the treated flour and used to establish new cultures in clean flour. Through use of this treatment and greater care with the cultures it has been possible to hold the mites to a very low level.

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Further simplification of a bioassay method with adults of T. confusum Duv.

A simplified bioassay method used by the author for screening and validation tests with fumigant gases was described in TIB 9 (2). The method employed 100 cc. all-glass syringes (1) as combined test cages and micro fumigation chambers.

In the current work with phosphine (3), it was found that not all the syringes that had been tested were uniformly gas-tight at all positions of the syringe piston. In a search for alternative leak-proof test chambers, 125 ml. Erlenmeyer flasks and 4 oz. medicine bottles fitted with gas-tight stoppers and septums were much cheaper, more durable and easier to clean and use than the syringes. They were used in a simplified bioassay method for the simultaneous determination of LD_{50} and LT_{50} values (4). Changes in CO_2-O_2-N_2 relationships in these miniaturized test chambers are readily monitored. The main advantages are that a wide range of gas concentrations and temperatures can be tested in a relatively small space, and insect mortalities can be readily assessed over a wide time span. Thus, it was

possible to determine the LT_{50} of 0.002 mg. phosphine per litre air during a 14-day test with adults of T. confusum Duv.

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*Sex differences in recombination values for linkage group V of T. castaneum

In a study to establish the linkage relationships among the eye color mutants, ruby (rb) and maroon (m), and the jet (j) body color mutant of linkage group V in T. castaneum, it was noted that the recombination values were not equal for the two sexes. However, Lasley (1960, TIB, p. 14) reported evidence for equality of recombination in both sexes between jet and split in the same linkage group.

The phenomenon of unequal crossing over in reciprocal crosses was first pointed out by Sokoloff (1963, TIB 6; 1964) for markers of the seventh linkage group of T. castaneum (male rates higher than female rates) and was later studied by Johnson (1966) with reference to linkage groups IV and VII.

Preliminary tests (Dewees, 1965, TIB 8:72) indicated that rb was located about 27 units to the left of j near m. Due to the difficulties in classifying adult maroon beetles, a regular three-point linkage test of m, rb, and j was not undertaken. However, a modified two-point linkage test of m and rb in the trans phase was set up with the jet gene included as a marker on the ruby chromosome. The progeny were scored in the late pupal stage as either wild type or mutant with regard to eye color and the recombinants (wild type) were then test mated to rb j/ rb j beetles to determine the most probable sequence of the three loci. From 87 single pair matings of m rb +/m rb + ♀ × + rb j/m + + ♂, 60 wild type recombinants were recovered from a total of 8,085 progeny. In the reciprocal cross from 96 matings only eight out of 13,246 progeny had wild type eyes. The calculated recombination rates and their corresponding 95% fiducial limits are shown below.

Heterozygous parent	Recombination rate between m and rb	95% fiducial limit
Male	.0148	(.0113, .0191)
Female	.0012	(.0005, .0024)

Clearly, these rates are statistically different. The gene sequence was determined to be m - rb - j.

To further investigate this sex difference, regular two-point linkage tests of rb and j were conducted. Both cis and trans phases were examined in reciprocal crosses. Within each sex the recombination rates observed for the cis and trans phases were not significantly different at the 1% probability level, therefore the results presented below represent pooled estimates.

Heterozygous parent	No. of matings	Total No. of progeny	Recombination rates between rb and j ± s.e.
Male	19	1974	.3283 ± .0212
Female	20	3196	.2118 ± .0145

Here also it can be seen that the recombination rate in the males is significantly greater than the rate observed in females. Whether or not these two instances represent a general phenomenon for the fifth linkage group is not known, however further tests involving markers of this linkage group are being planned.

As for the possible mechanism responsible for sex differences in recombination rates, Sokoloff (1964) discusses evidence from other organisms that seems to indicate that such a phenomenon could result when the two sexes differ in the distribution of a single chiasma. However, an equally plausible explanation might be one in which there is a differential segregation of chromosomes in one sex, probably the female, such that the recombinant chromosome is more likely to be incorporated in the polar body during second division segregation thus lowering the number of recombinant progeny produced. This type of mechanism could very well account for the several cases in which male recombination rates are observed to be higher than female rates.

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Population performance of single--and mixed--species of flour beetles

Knowledge on the interaction(s) among environment, population structure and inter-species competition is needed to understand better species survival and population performance. Single--and mixed--species population of flour beetles were cultured at 25°C, 29°C and 35°C and censused monthly for eight months prior to chronic gamma irradiation. Results of the radiation will be reported later.

Tribolium confusum Duval ("Chicago Standard") and Tribolium castaneum Herbst (sooty) were sexed as pupae and held until sexually mature. Fifteen replicates of single species populations (begun with 20 pairs) and of mixed-species populations (begun with 10 pairs of both species) were censused as to numbers of larvae, pupae and adults. All live forms and eggs were returned to new food. Eggs were not counted and adults were counted as to species because of their color difference.

Population stability was achieved only at the high temperature. Adults' forms were most abundant and showed least variability, probably due to their longer life relative to the other life cycle stages.

Fitness, measured on total number of life forms, indicated that Tribolium confusum was better adapted when not in competition at 29°C; whereas Tribolium castaneum was better adapted at the temperature extremes. In mixed-species populations, Tribolium castaneum was progressively less fit than Tribolium confusum. Numbers of total life forms per population replicate after eight months were greatest at 25°C and least at 32°C, indicating the temperature effect on population performance.

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Ontogeny and X-radiation sensitivity of the flour beetle, Tribolium castaneum, mutant: sooty

Nonlinearity of radiation sensitivity during the life cycle reflects the differential sensitivities of dividing cells compared to nondividing cells and the degree of differentiation. This report defines the radiation sensitivities of the flour beetle, Tribolium castaneum (mutant: sooty) throughout its life cycle for specific stages which show developmental changes in somatic and gonadic tissues.

Approximately 100 individuals at each of nine different stages during the life cycle were X-rayed. X-rays were delivered at 1 kR/min by a generator operated at 30 ma, 250 kvp, 0.25 mm cooper + 1.0 mm aluminum filtration, and a 2.5 inch target-subject distance. Culture conditions were 30 ± 1°C and 70 + 10 per cent relative humidity.

Virgin adults were crossed after radiation. An exposure was considered as sterilizing if no progeny developed with 2 - 3 months and as lethal if individuals failed to mature or died within 3 - 4 weeks post eclosion. Exposures were performed at 1000 R increments, or in some cases, at 500 R increments.

The weakest link in the life cycle was the 1 - 3 hour old egg in which nuclei are rapidly dividing and undifferentiated.

The differential radiosensitivity between somatic cells and gonad anlagen diverged markedly during post-larval stages because:

(1) germ cells remained at a radiosensitive stage in cell division, and

(2) the increased differentiation of somatic tissue required greater exposures for lethality.

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Metamorphic and adult life span modifications of flour beetles, Tribolium confusum, X-irradiated as pupae

Adult life span in mammals and insects was decreased when immature stages were irradiated supposedly by induced mutations in somatic cells. When pupae are radiated one would expect any lethal effect on genetic material would be eliminated during metamorphosis into the adult, instead of occurring after the adult stage successfully developed. This investigation determined whether metamorphosis and length of life could be altered when flour beetle pupae were X-irradiated.

T. confusum Duval ("Chicago Standard" stock) pupae, age 25-26 days, were sexed and irradiated. The X-ray unit operated at 250 kv, 0.86 mm copper half value layer, 0.25 mm copper and 1.0 mm aluminum filtration. The anode was 2.5 in. from the subject and the apparatus delivered approximately 1 kr/min. Doses were 0, 1, 2.5, 5, 7.5, 10, 20, 30, 40, 50, 60 and 70 kr. Each of the five replicates at each dose contained ten pupae of each sex in 10 g food. Deaths were noted twice weekly. Culture conditions were 29 ± 1°C and 60-75 per cent relative humidity.

Lethality data for males and females were combined, because sex made no difference in flour beetles.

Emergence of adults from pupae given 1 or 2.5 kR was comparable to that of controls. Metamorphic incompletion increased progressively for doses from 5 to 70 kR, but metamorphosis was not delayed. Partial metamorphosis occurred in some individuals given 5 kR and greater doses--that is, thorax and head were adult but the elytra, although pigmented, was pupal in size and position and the abdomen retained pupal characteristics. Some individuals given 30 kR and greater were unable to cast off their meconium. Death probably resulted from intestinal obstruction and not from starvation as heavily radiated adults ingest.

Reduction in length of life of adults radiated as pupae was a sensitive indication of radiation damage. Mortality increased progressively with increased exposure.

Exact cause(s) of death remain unknown because nuclear effects were difficult to differentiate from cytoplasmic effects. Death was probably due to chemical alteration of the cytoplasm because pupae developed adult structures. Antimetabolites could result from radiation-induced alterations in the chemistry of pigmentation. Other biochemical processes continue from juvenile stages to the adult and interruption of such processes could result in adverse effects on metamorphosis and adult life span when pupae are irradiated.

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Comparative studies with Tribolium. III. The productivity of T. castaneum and T. confusum on synthetic diets

A synthetic medium described by Naylor (1964) will allow development of T. castaneum and T. confusum, but has been shown to be a poor diet, in terms of productivity, compared to "natural" diets, such as wheat and corn flours (Sokoloff, et al., 1966b). However, the productivity of both species is raised approximately tenfold in Naylor's medium to which brewer's yeast has been added, and is comparable to productivity in commonly used diets, such as wheat + yeast and corn + yeast. Since yeast supplements are generally considered to be an important source of vitamins, it was considered desirable to examine the effect on productivity of varying the vitamin content of the Naylor medium. The vitamin mixture described by Naylor contains varying amounts of nicotinic acid, thiamine, pantothenate, riboflavin, pyridoxine, carnitine, folic acid and biotin, and the level of this mixture is 134 μg/gram of medium. Five media were tested containing different concentrations of vitamin mixture, and for comparison a sixth medium containing 5% brewer's yeast was used. Fifteen females and 10 males of each species

were transferred each day for four days into five grams of the test diets, and the adult progeny were counted during the following two months. Each species was replicated three times, and the average productivity replicate for the two species and the six diets as shown in Table 1.

Table 1. Supplement to Naylor's medium vitamin mixture μg/gram diet

Species	0	50	100	200	400	5% Yeast
T. castaneum	1	39	70	47	40	605
T. confusum	-	86	91	102	79	391

While it is apparent that the vitamin mixture is necessary for development in each species, there is little difference between the four diets in which the vitamin supplement was added. The differences in productivity between the two species is comparable with the conclusions of Sokoloff, et al. (1966b) that T. confusum performs better than T. castaneum on "poor" diets, and worse on "good" diets.

It was suggested by Sokoloff, et al. (1966a) that the two species T. castaneum and T. confusum may differ in their requirements for certain amino acids. Various combinations of eight amino acids, including those suggested by the above authors, were added to the Naylor medium and the productivity of both species was measured. The following concentrations of finely ground crystalline amino acies were used (Table 2).

Table 2. Amino acid concentrations used in enriching the Naylor medium

Amino acid	Concentration
alanine	2.5 mg/gram medium
aspartic acid	3.5 " " "
cystine	1.5 " " "
glycine	4.0 " " "
leucine	4.5 " " "
methionine	1.0 " " "
threonine	2.0 " " "
tyrosine	3.0 " " "

As before, 15 females and 10 males were transferred four times into five grams of fresh medium, and the total number of adults emerging in the subsequent two months were counted. Thirty-two diets were used, representing a 1/8 replicate of a 2^8 factorial design. None of the main effects were significant for either species, as illustrated by the analysis of variance table (Table 3).

Table 3. Analysis of variance

Source	d.f.	Mean Square CS	Mean Square CF
Main effects	8	197.8 n.s.	586.0 n.s.
Two factor interactions	20	203.9 n.s.	1,096.1 n.s.
Residual	3	61.8	218.2
	31		

These experiments show that the vitamins and amino acids which were tested are not limiting the productivity of either species in Naylor's medium, and the poor performance of T. castaneum and T. confusum is due to absence of other factors which are present in brewer's yeast.

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Literature Cited

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*Egg-laying rate of virgin females of Tribolium castaneum at different temperatures

To start quantitative genetics studies in connection with the genotype x environment interaction, some tests were run to determine the variation of the egg-laying rate in virgin females of Tribolium castaneum when they are exposed to different temperatures.

From some previous studies testing the egg-laying at 28°C, 32°C and 36°C during a four-day period comprised between the seventh and the eleventh days after adult emergence, we observed that both 28°C and 36°C were not as favorable environments as 32°C when egg-laying is the character studied.

We ran two experiments testing egg-laying during consecutive periods of 48 hours. The first test started at 24°C going two by two degrees up to 42°C and afterwards going in the same way down to 24°C again. The second one started at 42°C going down to 24°C and up to 42°C, also increasing or decreasing by two degrees in each 48-hour period. Temperatures greater than 42°C begin to be lethal.

At the same time some controls were maintained at 33°C in both tests to evaluate changes due to microenvironment variations through the testing periods, other uncontrolled effects and to take into account the decreasing effect of the females age after the first days of a greater lay. Such controls were females of the same age as the experimental ones.

Enough pupae from the "Consejo" strain of Tribolium castaneum were sexed to have 200 females emerging on the same day, at which time they were placed in individual vials. During the larval and pupal stage and until day 7 after adult emergence, they were maintained at a temperature of 33°C and a humidity of 70% RH; humidity was not changed in any case during the experiments. At day 7 after emergence fresh medium was put in each vial and at that time 100 females were shifted to 24°C conditions and 100 remained at 33°C. At day 9 eggs were collected by suction-sifting and counted, and the experimental females were changed to 26°C, leaving the controls at 33°C. The same procedure was followed every two days going up with the experimental temperatures until day 25, when experimental females were subjected to 42°C conditions. At day 27 and after egg collection, they were shifted to 40°C again and so on going down arriving at day 43 when they were placed in the 24°C conditions. The test finished at day 45 after egg collection and count.

The second test was run in the same way but starting with the 100 experimental females at 42°C and going down to 24°C and up to 42°C.

Table 1 gives for both tests the average egg-laying figures obtained

Table 1. Mean number of eggs produced by T. castaneum at different temperatures (°C) in consecutive periods of 48 hours.

Test 1					Test 2				
Testing temperature °C	m	Control at 33°C m	Deviation from control	Data adjusted to controls*	Testing temperature °C	m	Control at 33°C m	Deviation from control	Data adjusted to controls*
24	4.33	10.28	-5.95	6.68	42	1.12	6.66	-5.54	7.09
26	5.36	9.64	-4.28	8.35	40	4.80	11.96	-7.16	5.47
28	6.06	9.13	-3.07	9.56	38	9.73	11.39	-1.66	10.97
30	9.16	12.95	-3.79	8.84	36	12.98	12.28	+0.70	13.33
32	9.23	9.58	-0.35	12.28	34	13.41	11.29	+2.12	14.75
34	11.08	10.30	+0.78	13.41	32	14.41	14.54	-0.13	12.50
36	9.13	10.76	-1.63	11.00	30	10.77	12.31	-1.54	11.09
38	8.94	10.70	-1.76	10.87	28	9.78	12.73	-2.95	9.68
40	6.74	12.41	-5.67	6.96	26	8.01	13.68	-5.67	6.96
42	1.85	11.04	-9.19	3.44	24	7.94	16.02	-8.08	4.55
40	0.84	10.81	-9.97	2.66	26	9.30	15.12	-5.82	6.81
38	5.27	9.96	-4.69	7.94	28	10.57	14.04	-3.47	9.16
36	12.11	12.16	-0.05	12.58	30	12.62	15.81	-3.19	9.44
34	14.20	11.68	+2.52	15.15	32	14.05	14.56	-0.51	12.12
32	13.51	12.25	+1.26	13.89	34	20.24	16.10	+4.14	16.77
30	10.84	13.22	-2.38	10.25	36	17.44	17.04	+0.40	13.03
28	8.29	10.10	-1.81	10.82	38	16.76	18.09	-1.33	11.30
26	8.67	12.71	-4.04	8.59	40	9.71	16.73	-7.02	5.61
24	4.86	13.34	-8.48	4.15	42	1.77	16.58	-14.81	-2.18

* Standard error for corrected figures: ± 1.83

from the experimental and control females at specified temperatures. Deviations from controls and corrected figures with the average for all the controls in both tests are also given. Table 2 includes the figures averaged for every temperature disregarding any possible influence or effects of the previous temperatures.

Table 2. Figures for each temperature, averaging data in Table 1

Testing temperature °C	m	Control at 33°C m	Deviation from control	Data adjusted to controls*
24	5.71	13.21	-7.50	5.13
26	7.84	12.79	-4.95	7.68
28	8.68	11.50	-2.82	9.81
30	10.85	13.57	-2.72	9.91
32	12.80	12.73	+0.07	12.70
34	14.73	12.34	+2.39	15.02
36	12.91	13.06	-0.15	12.48
38	10.17	12.53	-2.36	10.27
40	5.52	12.98	-7.46	5.17
42	1.58	11.43	-9.85	2.78

* Standard error for corrected figures: +0.92

In order to see the results more clearly, the corrected figures for both tests are plotted in graph 1. Graph 2 presents four second order parabolas adjusted to the points of graph 1 by the least squares method. Equations of such parabolas are:

Test 1, increasing temperatures: $y = -0.18 + 0.25\,x - 0.36\,x^2$

Test 1, decreasing temperatures: $y = 0.60 + 0.16\,x - 0.51\,x^2$

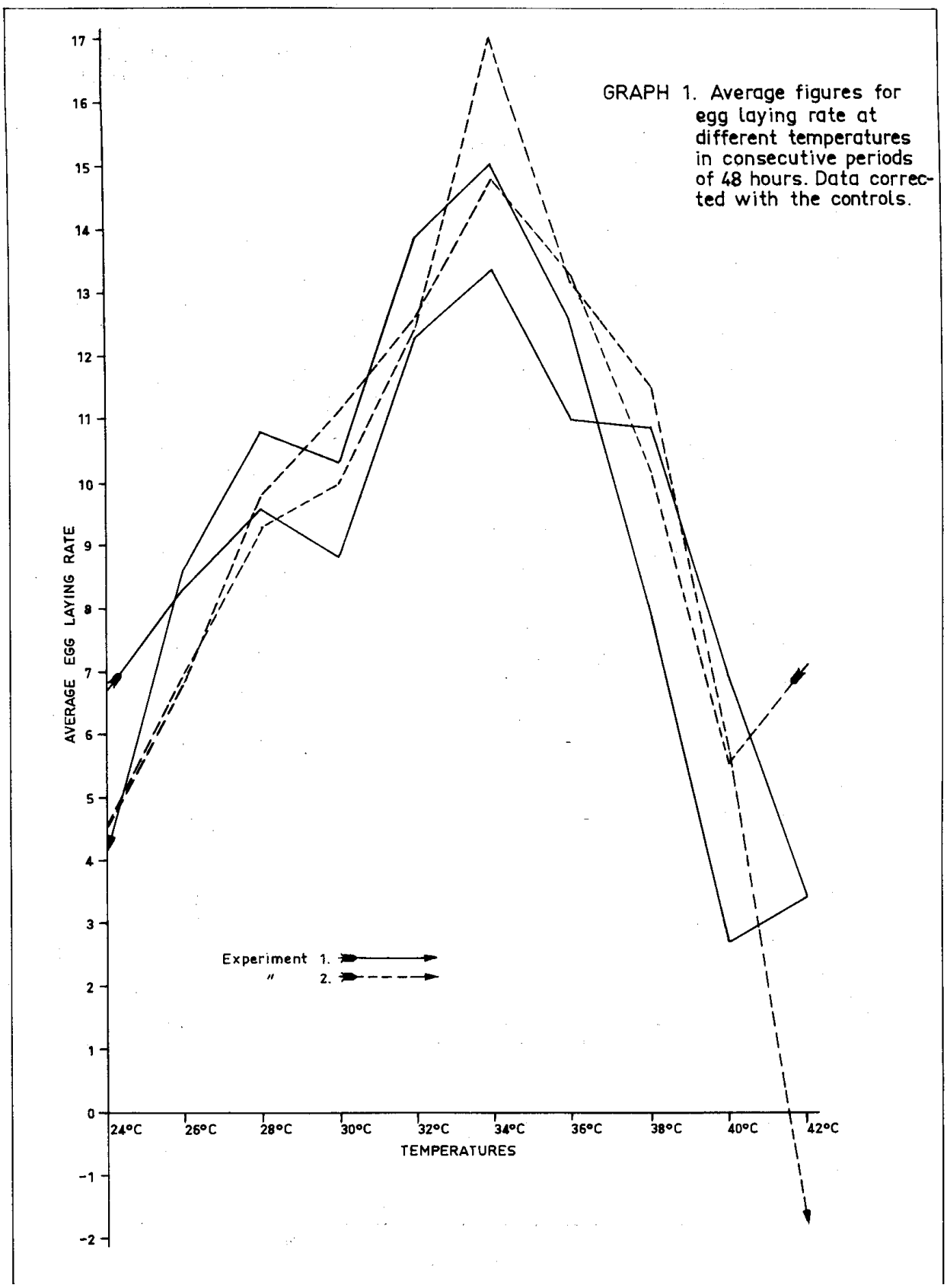
Test 2, increasing temperatures: $y = 1.45 + 0.36\,x - 0.62\,x^2$

Test 2, decreasing temperatures: $y = 0.70 + 0.58\,x - 0.44\,x^2$

Using the corrected figures it is concluded:

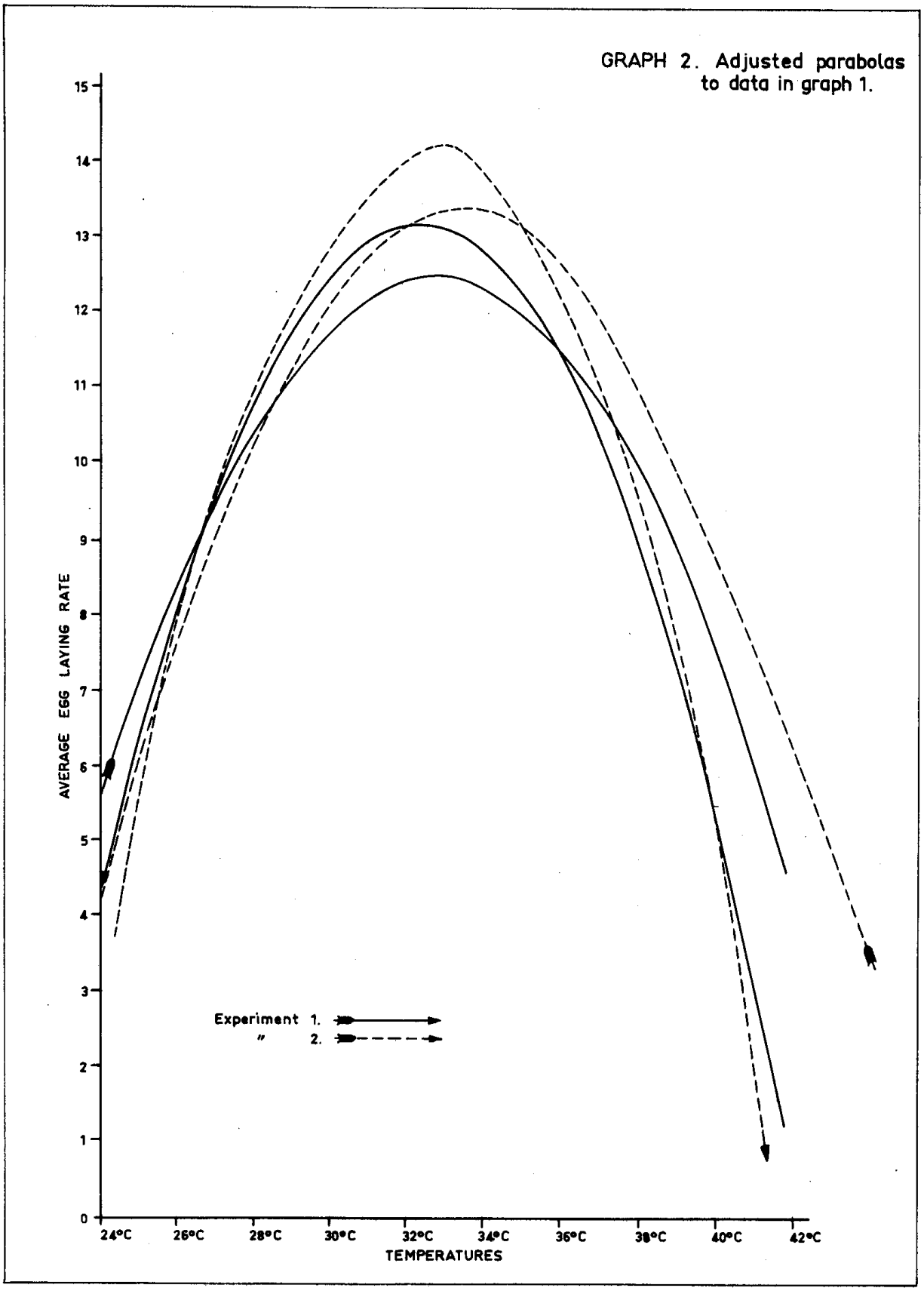
(a) In our strain, temperatures around 33 or 34°C are the optimum for egg-laying. Temperatures above or below those seem to be worse; the greater the distance from the optimum the smaller is the average egg-laying rate.

(b) Response to the same temperature does not seem to be the same when



GRAPH 1. Average figures for egg laying rate at different temperatures in consecutive periods of 48 hours. Data corrected with the controls.

GRAPH 2. Adjusted parabolas to data in graph 1.



progressive temperatures are increasing or decreasing, having gotten an average of 8.90 and 9.29 eggs respectively. Test 1 (starting at 24°C) has an average figure of 9.04 and test 2 (starting at 42°C) 9.15 eggs. In any case these differences do not appear to be significant, nor is the interaction of those possible effects. We reach the tentative conclusion when we see both data and graphics, that in general laying is greater with decreasing temperatures.

(c) It is interesting to note that even though the differences are not significant we are tempted to conclude that after the females have been submitted to a very high temperature the laying rate afterwards is larger, because the combination of test 2 (starting at 42°C) and decreasing temperatures (coming from 42°C) has the best effect. This could be explained by assuming that at 42°C the females experience greater stress retaining eggs which, after some days, they lay in excess.

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Algae flour as nutrition for Tribolium confusum

Algae can be cultivated in heavy water resulting in fully deuterated species. In an attempt to determine the effect of deuteration on higher organisms I planned to use deuterated algae as nutrition for Tribolium confusum. As a preliminary study Scenedesmus obliquus grown in water culture was processed into a flour and fed to Tribolium to determine the feasibility of using this algae as deuterated nutrition for the beetle.

The preliminary survey used 10 adults/vial containing two grams of algae flour prepared by desiccating and grinding water grown algae. The amount of materials used was limited by the ability to produce the algae. Various concentrations of the algae flour were prepared using flour media to dilute the algae.

The populations were surveyed with the following results (see Table 1).

Algae as presently processed would not make a good "carrier" of deuterium for studies with Tribolium. The flour beetle should be able to support itself in a high concentration of algae before an evaluation of the effect of deuterium is attempted. A more carefully controlled evaluation of the effect of algae on Tribolium is presently being planned, and modifications are being made in the preparation of the algae flour.

I feel bacterial toxins were probably present in the previous algal preparations and future techniques will attempt to separate the algae from other microorganisms and their metabolic products.

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*The use of vital dyes for marking Tribolium eggs in fresh and aged flour

The development of a technique for marking eggs with dyed flour (Rich 1956) has facilitated the measurement of oviposition and cannibalism rates in a population. This technique has been used advantageously by Sonleitner (1961), and Ho and Dawson (1966). In the studies of Rich, and Ho and Dawson, 0.5 per cent neutral red dye concentration was used. Sonleitner preferred to use only 0.2 to 0.3 per cent dye concentration. For coloring the eggs, commercial flour is considered the most ready-to-use choice. If whole wheat flour is used, however, it should be sifted through a number 5 bolting cloth sieve. This insures the eggs to be thoroughly coated with the fine-colored flour particles. To prepare the flour, dye should be mixed and ground into the flour, then sifted through the number 5 bolting cloth sieve and stored in a high humidity condition for at least a week before using. Marked eggs are obtained by allowing beetles to oviposit in the dyed medium. Eggs are removed from such egg farms using the number 2 or number 5 bolting cloth sieves.

In some population studies, more than two or three strains or species of eggs may be used in an experiment. The dyes for marking eggs, other than the neutral red mentioned may be needed. For this purpose, the safety range of Bismarck brown y, methylene blue and methyl green in Tribolium studies has been observed. All these dyes are well-known stains which have been used in histology and bacteriology for many years.

In addition, the aged-colored flour and the fresh-colored flour were tested for a nutritive comparison. The fresh-colored flour in this study was prepared and stored in the incubator at 29°C, 60% RH with a cheese cloth cover over the bottles one week before using; the aged-colored flour was prepared and stored in the same manner, after which the cheese cloth was replaced with an air tight cover and stored in room condition for more than three years. T. castaneum and T. confusum synthetic strain beetles, two weeks old, were used as the parents of the egg farms. In the colored flour testing series, five jars included one control and four colored flour for each species. After three days, 500 eggs were collected from each jar; 100 eggs were placed in each vial containing eight grams of colored or white flour. A total of 25 vials for each species were used. The cultures were introduced into an incubator maintained at 29°C and 60% relative humidity. The adults were censused 45 days later. These experiments were carried out in the same manner for both fresh and aged flour. The results are summarized in Table 1.

It is evident that no appreciable effect is produced when Tribolium are reared in the colored flour from egg through adult stages. The beetle emerging rate from five replicates of each colored flour was satisfactory. It is

Table 1. Per cent of Tribolium adults emerged from 500 eggs in fresh colored, aged colored and commercial white flour (five replicates for each test)

	T. castaneum		T. confusum	
	Fresh flour	Aged flour	Fresh flour	Aged flour
Neutral red	81.00	62.20	85.00	82.00
Bismarck brown y	86.60	85.00	83.60	80.00
Methyl green	93.20	81.00	82.00	67.50
Methylene blue	74.40	80.50	84.00	76.00
Control	79.00	82.10	78.60	80.50

very interesting to know that flour aged more than three years can be used almost as effectively as the fresh-colored flour for both species.

It should be pointed out that the emerging rate of beetles in vials containing neutral red in the aged flour for T. castaneum, methyl green in the aged flour for T. confusum, methylene blue in the fresh flour for T. castaneum, and methylene blue in the aged flour for T. confusum, was slightly lower than the emerging rate of the controls.

The results indicate that the vital dyes used are not poisonous to Tribolium when being used for egg coloring at a 0.5 per cent or less concentration in the flour.

The observation also suggests that all the dyes used in this study give brightly colored, easily distinguishable eggs.

This work was supported in part by USPHS grant GM-08942.

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*Identification of eye mutants in Tribolium larvae

The previous studies (Good, 1936 and Ho, 1960) indicated that the arrangement of the ommatidia (facets), the shape and size of the compound eyes, and the distance between eyes in the pupal and the adult stages can be used to identify the species of Tribolium castaneum and T. confusum. In the larval stage no compound eyes have developed. However, the setal map of the larva has been used advantageously for their species identification (Ho, 1967 in press).

Tribolium has been used for genetic studies only recently. At present, over 150 mutants are described, including 11 eye mutants in T. castaneum and nine in T. confusum (Sokoloff, 1966). To distinguish the wild type and eye mutant larvae, examination of their stemmatal characteristics has been considered a practical technique. In general, three types of stemmata (eyespot) are present in Tribolium larvae. In wild type and in some mutants with deformed eyes (microcephalic, and Bar eye) the stemmata appear black; in the eye mutants red, pink, eyespot, light eyespot, ruby spot and dirty pearl eye of T. confusum, they are brown. There are no visible stemmata in pearl, platinum eye, Microphthalmic, frosted, and chestnut eye mutants, probably because the lack of pigment renders these stemmatal structures invisible or because the stemmata are too small or absent. In the squint (sq) beetle no ommatidia form, with the result that the ocular diaphragm, unaffected by sq, forms in the pupa and lies exposed in its normal position (Sokoloff and Dawson, 1963; Sokoloff, 1966). The sq gene apparently suppresses the formation of the stemmata as well, since these structures are not visible in the larva. In microcephalic adults, the eyes are variably reduced and they bear very few facets. In some cases, the eye on one side may be absent. If these beetles are selected, they produce progeny with both eyes missing (Sokoloff, 1966). Therefore sometimes, in a microcephalic stock, three types of larvae may be found: those with stemmata missing on either side, those with stemmata missing on both sides, and those with stemmata present on both sides. If the stemma is present, it is always black. The color of the stemmata in larvae usually corresponds with the color of the compound eyes of the resulting pupae and adults. The data are summarized in Table 1.

The stemmata are located near the antennae from the first instar through the early part of the last larval instar. They are paired and more or less fused, and are located on each side of the head. In the later part of the last larval instar, the stemmata migrate along the head margin to a position near the vertex, and gradually become lighter, finally disappearing when the larva nears pupation (Ho, 1961). In these last larval stages the mutant may not be distinguishable from wild type.

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Table 1. The type of stemmata of Tribolium larvae

Species	Wild type or eye mutants	Symbol	Type of stemmata
T. castaneum	wild type	+	black
	Bar	Be	black
	red	r	brown
	pink	p^{PK}	invisible
	pearl	P	invisible
	ivory	i	invisible
	squint	sq	invisible
	Microphthalmic	Mo	invisible
	chestnut	c	invisible
	platinum eye	pte	invisible
	microcephalic	mc	usually invisible; if present, black
	glass	gl	undetermined (specimen not available)
T. confusum	wild type	+	black
	red	r	brown
	pink	pK	brown
	eyespot	es	brown
	light eyespot	es^{lt}	brown
	ruby spot	rus	brown
	dirty pearl eye	dpe	brown
	pearl	P	invisible
	frosted	fro	invisible
	ruby	rby	undetermined (specimen not available)

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*Susceptibility of Sitophilus granarius to moderate cold

This experiment was performed following the failure in several successive years to establish populations of granary weevil in freshly-harvested wheat and barley stored in farm bins. Cultures were prepared by groups of 800 adult weevils on successive lots of 320 g of Manitoba wheat for one day at 25°C and 70% R.H. Each culture was split into 32 samples and stored at 25°C and at a predetermined age each sample was moved into a room controlled at 15°C and left there for eight weeks. In all, 24 samples of every age from 1 to 39 days old at 25°C were exposed at 15°C and a similar number kept as controls. On return to 25°C the samples were sieved regularly and the fecundity of a sample of the emerged adults was measured for one week. Very young eggs and the pre-pupal stage seem to be susceptible to exposure at 15°C, a temperature close to the developmental threshold.

Age at exposure days	No. emerged	% alive after 6 weeks	Eggs per beetle	Age at exposure days	No. emerged	% alive after 6 weeks	Eggs per beetle
1	119	98	8.1	11	687	98	6.8
2	187	99	9.9	12	754	98	8.3
3	360	99	8.1	13	691	99	8.7
4	463	98	9.4	14	607	98	7.5
5	651	94	7.8	15	622	91	6.3
6	669	99	9.0	16	592	89	6.8
7	717	100	9.4	17	568	70	3.6
8	662	99	8.1	18	512	63	3.9
9	608	99	8.3	19	462	64	2.4
10	625	99	7.9	20	404	44	1.5

Age at exposure days	No. emerged	% alive after 6 weeks	Eggs per beetle	Age at exposure days	No. emerged	% alive after 6 weeks	Eggs per beetle
21	316	37	0.7	31	551	56	4.3
22	272	45	1.4	32	640	72	5.6
23	244	27	1.0	33	728	84	4.8
24	221	33	0.4	34	707	84	6.8
25	238	26	0.8	35	728	90	5.8
26	297	20	1.9	36	751	--	--
27	353	30	3.1	37	731	--	--
28	404	35	3.0	38	708	--	--
29	499	50	4.3	39	723	--	--
30	497	58	5.6	Control	804	100	8.4

Predominant stage in each group

1 - 6	egg	26 - 28	prepupa
7 - 10	larvae 1	29 - 34	pupa
11 - 14	2	35 - 37	adults in grain
15 - 19	3	38 - 39	adults emerged
20 - 25	4		

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Quinoid secretions in Tribolium confusum

We have most recently perfected a micro-polarographic method for the quantitative determination of simple alkyl-parabenzo-quinones in the odorous secretion of the flour beetle T. confusum. The method is capable of measuring quantities of quinone as small as 1×10^{-4} mgs, making it an excellent tool for the study of quinones in individual specimens of insects--even in melanotic stink gland mutants msg (Sokoloff, 1964) which bear the chemicals at as low as the submicrogram level. The procedure also furnishes conclusive information on the minor amounts of hydroquinone that invariably appear to accompany the quinone in the insect secretion. Furthermore, data of electrochemical significance concerning the reversibility of the redox process and the extent of electron transfer can readily be obtained by evaluating the analytical evidence from these extremely small

amounts of test substance. These special aspects, and details of our new method will be discussed elsewhere.

In essence, the beetle to be studied is crushed in 500 microliters phosphate buffer solution, pH 7, and the mixture is subjected to conventional direct current polarographic electrolysis. Light is carefully excluded from the sample to minimize possible photo-reduction. The resultant polarogram shows the amount of quinone (cathodic wave height), the amount of hydroquinone (anodic wave height), and the half-wave potential. The latter provides evidence on the identity of the insect chemicals in comparison with synthetic alkyl-parabenzoquinones, i.e., in this instance with 2-ethyl-1,4-benzoquinone, and 2-methyl-1,4-benzoquinone. We have used a Leeds and Northrup Polarotron P-40 and a Sargent Model III Polarograph. The sample holder for the Polarotron was redesigned with an electrolysis chamber of 250 microliters minimum capacity, and with a bridge containing de-aerated electrolyte.

Typical polarograms of the very small amounts of quinone and hydroquinone determined in mutants msg are depicted in Fig. 1. Computed quinone and

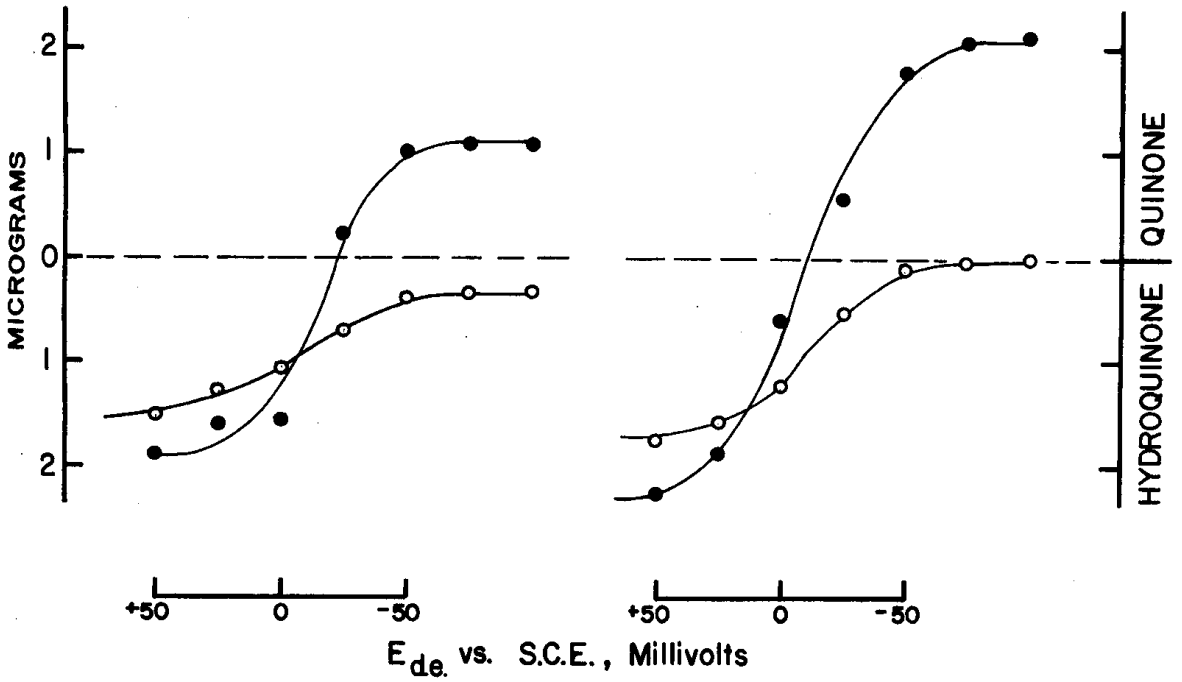

Fig. 1. Polarograms of quinoid secretions in melanotic stink gland mutants T. confusum msg. Each curve refers to an individual insect from the culture. Data obtained in supporting electrolyte .05M alkali phosphate buffer pH 7.0, at 25°C. Drop time, 3.58 sec; mass flow Hg, 2.33 mgs/sec. Corrected for residual current.

hydroquinone values for both the wild-type and the mutant insects are listed in Table 1, for ten individual beetles in each case. An average amount of

Table 1. Quinoid compounds in T. confusum, micrograms/insect. Ten specimens each of wild-type and mutant cultures. (Q, Quinone; HQ, Hydroquinone)

Wild-type culture			Mutant msg		
Q	HQ	Total	Q	HQ	Total
57.9	1.2	59.1	None	1.1	1.1
69.0	2.4	71.4	1.1	1.9	3.0
53.1	1.5	54.6	None	1.8	1.8
36.6	2.4	39.0	2.2	2.3	4.5
62.1	3.0	65.1	None	1.3	1.3
31.2	2.1	33.3	0.3	1.4	1.7
35.1	2.7	37.8	None	0.5	0.5
40.2	6.0	46.2	None	0.1	0.1
64.8	5.4	70.2	1.8	2.9	4.7
75.6	3.3	78.9	2.8	2.9	5.7
Average Q: 52.6			Average Q: 0.8		
Average HQ: 3.0			Average HQ: 1.6		

52.6 micrograms quinone per beetle was thus found for the wild-type culture which was several months old. This result is somewhat higher than that previously arrived at with a more indirect analytical procedure. The latter has disclosed progressive biosynthesis of quinone in T. confusum as the beetles grow to maturity, from zero in newborn adults to approximately 35 micrograms per beetle at the age of four weeks (Ladisch, 1965).

The present data for mutants msg confirm the findings of Engelhardt, Rapoport, and Sokoloff (1965) with respect to the greatly reduced amount of quinone in these beetles. In contrast, hydroquinone was not detected in the mutants by these authors, who extracted the volatile compounds by passing a stream of air over the insects and condensing the sublimed matter in cold traps. This method would appear unsuitable to demonstrate hydroquinone in insects as this compound will not sublime under these conditions due to its low vapor pressure of a few microns Hg at the temperatures employed. Information on the vapor pressure of insect quinones may be found in a recent article by Ladisch and Suter (1965). Evidently, as is now shown, there is more hydroquinone than quinone in the glands of these beetles (Fig. 1, Table I).

Hydroquinone has not been previously detected in wild-type insects by Loconti and Roth (1953), Ladisch and McQue (1953), and Ladisch (1963;

1965), all of whom have used sublimation methods in the isolation of the beetle quinones. Sixteen hundredths of a microgram of hydroquinone per beetle was reported by Engelhardt et al. (1965) for wild-type insects. Yet, this compound was most likely a degradation product formed in vitro from the quinone after collection, and is probably not related to hydroquinone existing in the glands of the beetles. We have found insect quinones from T. confusum to convert photochemically with ease to hydroquinone. In aqueous solution at pH 7, and exposed to light of 750 foot-candles intensity for one hour, these quinones undergo complete reduction. The solution is stable when stored in darkness. We shall discuss this phenomenon in detail elsewhere. Employing the described direct method of analysis, we have now found an average of 3.0 micrograms of glandular hydroquinone per insect of the wild-type strain (Table 1).

The presently described analytical procedure is easy to perform and most reliable. It has proved to be of special benefit in our current study of quinoid agents which we have been able to detect in several genera and numerous species of the family Tenebrionidae.

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We thank Dr. Alexander Sokoloff, University of California, for a culture of mutants T. confusum msg. This study was supported in part by a grant from the Damon Runyon Memorial Fund for Cancer Research. We shall present a more comprehensive treatment of this subject, including potential environmental health aspects of quinoid toxicants from stored-food insects at the April 1967 annual meeting of the Pennsylvania Academy of Sciences.

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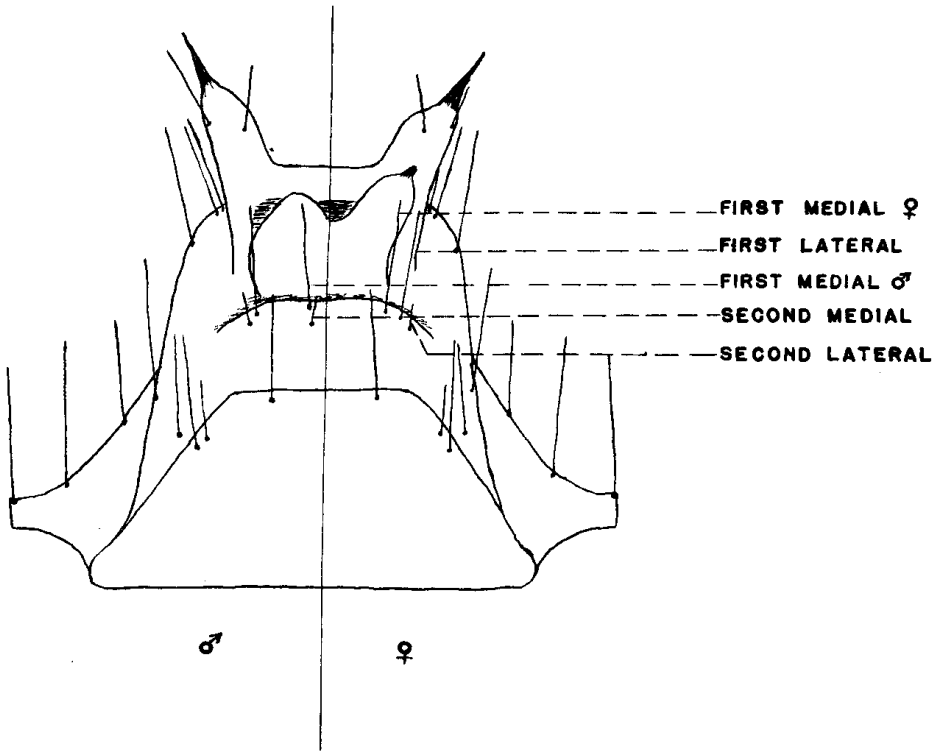
*Sexual dimorphism in the pupal setae of Tribolium

The setae of Tribolium larvae and pupae have been examined by several people. The variation that has been noted has dealt with specific differences (Mertz, 1961; Ho, 1960, 1964). No known intraspecific difference has

been reported previously. This is a bit surprising considering the mutants affecting seta number and shape in Drosophila. The following is a description of a pupal sex difference in the position and number of setae in Tribolium castaneum Herbst.

On the posterior margin of the eighth sternite of the pupae are a series of setae, the pregenital setae (Fig. 1). The most lateral pairs are the long first lateral and the short second lateral. They are slightly lateral and cephalad of the genital lobes and are present in both sexes in this position. The second lateral is sometimes absent. Medially to these pairs of setae are the first and second medial setae. The second medial is the smaller and more sephalad of them. In males (left half of Fig. 1) both of the medials may be present although usually only the first medial is found. They are approximately equally spaced between the laterals. In females only the first medial is present, and it is about as far from the first lateral as the second lateral is.



COMPOSITE MALE-FEMALE TRIBOLIUM CASTANEUM
SHOWING PUPAL SEX DIFFERENCES IN SETAE

Fig. 1

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*Regression of the sex ratio on maternal grandfather's age

In 1961 Cavalli-Sforza proposed a method of estimating the spontaneous mutation rate for sex-linked lethals by the regression of the sex-ratio of still (a) and live-born (b) children on the age of the maternal grandfather at the time of the mother's birth. The rationale of these methods was given by him in the original paper as well as in a 1962 publication. Both he and Krebhiel (1966) found significant positive regressions on samples of humans by method (a). For method (b) Cavalli-Sforza (1961) obtained, on material involving some 67,000 women, a negative regression which was not significantly different from zero. He demonstrated that (b) would be a less sensitive measure of the effect, and proposed a massive study on a scale sufficiently large to obtain significant results. The results have so far not been published.

Meanwhile, we have undertaken a similar study of our synthetic stock Tribolium castaneum and T. confusum with method (b). Two separate experiments with the first of the species, and a single with the second, containing ten replicates each (five males mated to 10 stock females per replicate) were performed. In them, daughters were obtained from males at monthly intervals (with some minor exceptions), and ten daughters per replicate were mated to ten stock males and permitted to produce pupae, which were then sexed. The age of the grandfathers spanned the period of 2 to 24 months, and any of those that died were replaced by males of the same age. All the stocks were maintained at 29°C and 70% relative humidity. The total number of pupae examined was over 150,000.

The pooled data for the different experiments are presented in Table 1 separately for the two species. It is apparent from mere inspection that no linear regression is detectible in T. confusum. The data for T. castaneum are not as unequivocal, and hence a chi-square test was carried out:

Regression chi-sq.	0.70 for 1 d.f.
Residual chi-sq.	20.23 for 20 d.f.
Total chi-sq.	20.91 for 21 d.f.

The discrepancy in the total is likely due to rounding off figures in the calculation. There does not seem to be a significant negative regression, although the possibility of deviations from chance in some other manner is not excluded.

Thus it seems that the extent of our material bears out Cavalli-Sforza's suggestion that method (b) is not very sensitive, so that on the basis of our data, no estimate of mutation rate is possible. They are, nevertheless, presented here, since if other attempts at studying the same problem are made it may be possible to use them in combination with other material.

Table 1. Sex ratio and age of grandfather

Age of maternal grandfather at the time of mother's eclosion	T. castaneum		T. confusum	
	No. of pupae sexed	% males	No. of pupae sexed	% males
2	3341	51.39		
3			3883	49.70
4	5663	49.87	4750	50.57
5	5808	49.69	5658	48.30
6	5839	48.36	3899	49.81
7	2628	50.02	4067	49.79
8	6049	50.24	5252	49.95
9	6305	48.96	5371	50.12
10	6761	50.39	4718	50.59
11	5961	50.39	4876	51.35
12	5659	49.48	4983	50.41
13	6946	49.55	4587	49.86
14	7803	49.97	2204	49.09
15	6423	49.76		
16	5238	49.77		
17	5619	49.47		
18	2152	50.65		
19	4100	49.02		
20	1750	48.34		
21	1710	49.53		
22	1488	47.51		
23	666	48.65		
24	1307	48.13		
Total	99216	49.61	54248	49.99

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Evaluation of an anti-feeding compound as a protectant against stored-products insects

An anti-feeding compound, 24055 discovered by American Cyanamid Company was initially reported to inhibit feeding by certain phytophagous chewing insects. This interesting property suggested that the compound might be exploited as a protectant against stored-products insects. Laboratory experiments were conducted to determine whether 24055 applied to the outer surface of cotton sacks prevented penetration and oviposition by flour beetles and grain beetles. The compound was used as a 25% wettable powder in water and applied to empty sacks at different rates of application.

Adults of Tribolium confusum, Cryptolestes turcicus and Oryzaephilus surinamensis did not penetrate or lay eggs through the mesh of treated sacks filled with flour. They died or became moribund within one week of exposure. This compound was shown to cause mortality of flour beetles by volatile action and by contact action, the former having the greater effect. Its ability to protect packaged cereal products is currently being investigated. Its anti-feeding and insecticidal properties are being examined in relation to reproduction and mortality of treated adults.

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The distribution of Oryzaephilus mercator Fauvel in Canada

Since late summer 1966, we have received an unusually large number of reports of the merchant grain beetle, Oryzaephilus mercator Fauvel occurring in packaged cereal products. This species had not been reported in

Canada before 1952. It was recorded from Ottawa in 1952 and Vancouver in 1954.

This beetle superficially resembles the saw-toothed grain beetle, O. surinamensis and was considered by many workers to be a variant of the latter species. However, Howe (1953, 1956) and Slow (1956) confirmed Fauvel's earlier finding (1889) that O. mercator was a separate species. We know that the merchant grain beetle has frequently been erroneously identified as the saw-toothed grain beetle. Consequently, we suspect that the former species is distributed in Canada far more widely and for a longer time than indicated by the two reported records mentioned above.

The increasing frequency of reports of the merchant grain beetle suggests that it is becoming firmly established as a pest of stored products in households, retail stores, and storage warehouses in Canada. It has been found in flour, rolled oats, biscuit products, peanuts, walnuts, puffed rice, cake mix, chocolate bars, dried fruits and vegetables, flax seed, Oriental foods, and copra.

A survey is being conducted to determine whether this species enters the country on certain imported products from which it may spread to other food commodities via normal handling, shipping, and storing operations.

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*Factors involved in the survival of Tribolium confusum populations

Flour beetle populations can persist for long periods of time in small volumes of unrenewed flour. One factor that may influence the longevity of a population under these conditions is the effectiveness of the self-limiting mechanisms that restrict population size, for it seems likely that a smaller population would be able to persist longer than a larger one. However, there may be other factors influencing longevity and these might be revealed by a close study of populations from genetically distinct strains that differ in several population characteristics. In these first experiments two strains were used, McGill black and a wild type (red) strain. One hundred single pair populations of each strain and another hundred populations of mixed pairs, one red adult and one black, were started in 5 gms of flour medium. These three population types are referred to hereafter as the R, B and H (heterozygous) populations. The populations were censused at two-week intervals and since the medium was never renewed they eventually became extinct. Table 1 shows some of the data obtained during weeks 10 through 28 inclusive. Since the adult and egg numbers of the H populations fall between the R and B populations it is possible that these characteristics are determined by the body color genes which exhibit no dominance.

Table 1. Mean characteristics of red, black, and heterozygous populations

	Population type			Tests of significance between:		
	Red	Heterozygous	Black	R and B	R and H	H and B
Live adults	97.6 ± 19.5	81.2 ± 17.2	71.1 ± 13.8	t = 11.0 P < .001	t = 6.3 P < .001	t = 4.5 P < .001
Egg numbers	20.5 ± 7.3	28.7 ± 10.0	42.6 ± 13.6	t = 14.2 P < .001	t = 6.6 P < .001	t = 8.2 P < .001
Weeks duration	37.4 ± 6.5	45.1 ± 5.4	39.9 ± 5.6	t = 2.9 P < .005	t = 9.1 P < .001	t = 6.7 P < .001

Since the H populations, although bigger than the B populations, survive several weeks longer, it is clear that factors other than the adult population size can influence survival in a depleted environment. However, within each population type there is a negative correlation between adult population size and duration. Table 2 shows this as well as the fact that the egg number is

Table 2. Correlations between adult numbers (A), egg numbers (E), and weeks duration (D)

	Population type					
	Red		Heterozygous		Black	
Total correlation coefficient						
r_{AE}	-.70	P < .001	-.41	P < .001	-.49	P < .001
r_{AD}	-.72	P < .001	-.88	P < .001	-.67	P < .001
r_{ED}	.49	P < .001	.37	P < .001	.32	P < .001
Partial correlation coefficient						
$r_{AD \cdot E}$	-.97	P < .001	-1.01	P < .001	-.76	P < .001
$r_{ED \cdot A}$	-.02	P > .10	.06	P > .10	-.02	P > .10
$r_{AE \cdot D}$	-.94	P < .001	-.42	P < .001	-1.46	P < .001

apparently not a factor in determining duration. When the effects of the negative correlations between eggs and adults are eliminated the partial correlation coefficient between eggs and duration are not significant. Since the level of adult numbers is established early in the population's life, it is possible that this level can influence the population's chance of surviving a period of stress, generated by depleted resources. Since, in the present instance, a smaller population has a greater chance of surviving, the results suggest a way in which natural selection acting on the whole population as a unit might establish self-limiting population mechanisms. In another experiment, using the same strain, when the population reaches its peak number of adults at eight weeks, all the adults are discarded then and every fourth week thereafter. Table 3 shows some of the

Table 3. Correlation between the total number of live adults produced (T), the duration of the population in weeks (D), and the mean number of adults at the peak period, weeks six and eight (P)

	Means			T tests		
	Red	Heterozygous	Black	R and B	R and H	H and B
Total live adults produced	166	157	148	$< .0001$	$< .01$	$< .005$
Weeks duration	41	51	47	$< .005$	$< .0001$	$P = .11$
Peak adults $\bar{x}$ 6-8 wks.	92	76	74	$< .0001$	$< .0001$	$P = .38$

Total correlation coefficient	Red		Heterozygous		Black	
r_{TD}	.43	$P < .01$	.31	$P < .01$	.54	$P < .01$
r_{PD}	-.043	$P > .1$	-.24	$P < .02$	.05	$P > .1$
r_{TP}	.39	$P < .01$	.06	$P > .1$	.34	$P < .01$
Partial correlation coefficient						
$r_{TD \cdot P}$	.528	$P < .01$	.345	$P < .01$	.593	$P < .01$
$r_{TP \cdot D}$	.519	$P < .01$	.157	$P > .1$	.443	$P < .01$
$r_{PD \cdot T}$	-.305	$P < .01$	-.287	$P < .01$	-.214	$P < .05$

data. Again, for reasons not yet known, the H populations survive longest although not significantly longer than the B populations. Within each population type the total number of adults produced is positively correlated with duration. This can be interpreted to mean that populations differ in their efficiency in converting flour into beetles, and the more efficient populations have an increased chance of surviving. However, the rate at which this conversion takes place should have some effect on duration. Those populations producing more beetles early in the population's life should exhaust the environment sooner. Consequently, a negative correlation might be expected between the peak number produced at weeks 6 and 8 and duration. However, since the peak number is part of the total number, and the total is positively correlated to duration, the negative relationship between peak number and duration only emerges in the partial correlation coefficients. Other characteristics, such as the mean weight of the individuals in the population, measured as the weight of pupae, appeared to have no effect on population survival.

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*Cytochemical studies of Sitophilus granarius mycetomes

Cytochemical studies of the outer membranes and chromatinic material in the inherited, supposedly symbiotic mycetomal micro-organisms of Sitophilus granarius have revealed the presence of considerable amounts of DNA occurring as either consolidated dots or a spongy network. There is also evidence that, at times, the organisms are surrounded by cell walls.

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*Mycetomes as possible obligate symbiotes of Sitophilus

The peripheral membranes of the micro-organisms of the mycetocytes of adult midgut caecae and of larval mycetomes of Sitophilus granarius (L.), GG strain, have been examined with an electron microscope. The majority of the mycetocytes were depleted of intracellular organelles but contained large numbers of mycetomal micro-organisms, most of which exhibited only one peripheral membrane. Some mycetocytes, however, had well-developed ultrastructure and harbored mycetomal micro-organisms which showed two peripheral membranes, namely a cell wall and plasma membrane. Intermediate conditions also occurred.

It is suggested that the absence of host-provided membranes around the micro-organisms categorizes them as obligate symbiotes.

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A quantitative approach to the feeding of Hymenolepis diminuta eggs to the flour beetle, Tribolium confusum

All stock cultures were maintained at 30°C, 92 to 96% relative humidity and 12L:12D light conditions. The medium consisted of 95% bleached Gold Medal Wondra(R) flour and 5% National Active Dry(R) yeast and was changed at least every two weeks. Eggs collected from a population of beetles four to six weeks following their eclosion were used to set up the experimental groups which consisted of virgin adults maintained in individual vials throughout the duration of the experiment.

At six to eight weeks of age, the experimental beetles were starved for a period of five to six days prior to being allowed to feed for a 48-hour period on a known number of eggs. The eggs were obtained from five to ten freshly obtained terminal gravid segments of H. diminuta and embedded in a 17% gelatin, 2% sucrose medium. The solvent for preparing the gelatin and sucrose consisted of three parts distilled water: one part supernatant of a mammalian saline extract of freshly-obtained homogenized gravid segments. This extract was centrifuged for a period of five minutes at 1800 rpm and the supernatant was removed. The eggs and diluted supernatant were added to the gelatin sucrose mixture just prior to gellation. After examination of the surrounding feeding area and feculae for eggs or egg shells, it appeared that no eggs or embryos were passed through the digestive tract during the 48-hour feeding period, and the 24-hour period after replacement on the regular diet. Thus, it was possible to determine the number of eggs consumed per beetle by subtracting the number remaining at the end of the 48-hour feeding period from the initial number. Selective staining with Trypan Blue (0.02 to 0.04% in mammalian saline) in addition to observational studies indicated over 90% viability of the eggs freshly obtained from gravid segments and those kept for the 48-hour period in the gelatin medium. In addition, eggs maintained for 48 hours in the gelatin medium did not take up the stain at a faster rate than freshly obtained eggs, indicating no decrease in the relative viability of the eggs. The majority of beetles that fed consumed between one and 60 eggs, 21 to 29% becoming infected, with a mean of 1.5 to 3.0 cysticercoids per infected beetle.

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The behavior and biology of flour beetles, genus Tribolium as studied in laboratory gradients of temperature and humidity

Under the supervision of Dr. F. L. Waterhouse, Natural History Department of Queens College, University of St. Andrews, Dundee, Scotland, Dr. A. K. Onyearu has recently completed a three-year research program on the behavior and biology of Tribolium spp. including some genetic strains and geographical populations of Tribolium castaneum, Hbst.

The laboratory studies were mainly conducted in a gradient environment. The equipment for this has been described earlier (Graham, Onyearu and Waterhouse, 1965).

Tribolium populations employed in these investigations include:

1. Seven geographical stocks of Tribolium castaneum obtained from:

   Umuahia - (Eastern Nigeria)
   Ibadan - (Western Nigeria)
   Kano - (Northern Nigeria)
   Kenya - (East Africa)
   Kingston - (Jamaica, West Indies)
   Rangoon - (Burma)
   Tokyo - (Japan)

2. Three genetic strains of Tribolium castaneum namely:

   Black, Pearl and Mahogany. The last is a recent find described elsewhere (Onyearu and Graham, in press).

3. Laboratory strains of:

   Tribolium confusum duVal
   Tribolium anaphe Hint.
   Tribolium madens Charp.
   Tribolium destructor Uytt.

The following aspects were covered in these investigations:

1. Adult distributions in the gradient environments.

2. Oviposition as a functional aspect of adult orientation on the gradients of temperature and humidity.

3. Developmental periods in gradient environments as well as under the uniformly controlled conditions of the C. T. Room.

Results of these investigations have served to emphasize the need for relating experimental conditions and experimental animals to those occurring in the field to which the original ecological problems relate. Details of these studies formed the theme of a Doctoral thesis recently submitted to the University of St. Andrews (Onyearu, 1966). Further publications now under preparation are to be made elsewhere.

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The scanning electron microscope

The scanning electron microscope utilizes a fine, moving beam of electrons (diameter as small as 50 Å) to probe the specimen for a variety of kinds of physical, chemical and electrical information. As the scanning beam is swept across the specimen, the electron beam of a standard cathode ray tube is driven in synchrony with it and the brightness of the cathode ray beam is modulated by the signal from one of several detectors. The secondary radiation produced by the interaction of the scanning electron beam is not imaged but is only used to convey information about the particular point being bombarded. The image is a result of the synchrony between the scanning beam in the microscope column and the beam of the cathode ray tube. The resulting 1:1 correspondence of points on the specimen with points on the face of the cathode ray tube serves to identify the location of the information seen by the detectors. The separation of information and localization allows many different kinds of information to be gained while utilizing the localization possible with an electron beam. Different kinds of secondary radiation are produced by the interaction of the electron beam with the specimen and each carries a particular kind of information. For example, visible light may be produced and give information about the molecular structure of the biological material itself or the location of light producing stains within the specimen. Operated in this mode, the scanning electron microscope could be described as a high resolution fluorescence

microscope (Pease et al. 1966a). Secondary electrons are another form of radiation produced by the interaction of the scanning beam with the specimen. Since production of these secondary electrons is a function of the angle between the scanning beam and the surface of the specimen, stereoscopic information can be obtained. The scanning electron microscope operated in this mode can be described as a high resblution stereoscopic microscope (Hayes et al. 1966). In addition, ultraviolet radiation, characteristic X-rays, backscattered electrons, energy loss electrons and specimen current are all produced in the specimen and might be used to build up the scanning electron microscope image. The principles of the instrument and several of its modes of operation have been recently reviewed (Oatley et al. 1965).

The present paper deals with an attempt to visualize living Tribolium by using the scanning electron microscope in the secondary electron mode of operation. There are several advantages both physiologically and morphologically if the specimen can be viewed while it is living. Morphologically, the reduction in the possibility of artifacts and the simplicity of sample preparation are important. Physiologically, the possibility of observing "on-going" processes would be most valuable, as well as utilizing the tiny electron beam as a micro-radiation source in radiobiological studies (Pease et al. 1966b).

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Fig. 1. Larval leg in T. confusum under small magnification (left). The same leg focusing on the tarsus and the claw (right).

Fig. 2. Upper left: T. confusum female pupa under small magnification. Upper right: detail of the abdominal sternites under moderate magnification. Lower left: detail of the head and prothorax. Lower right: detail of the central area in the preceding photograph showing detail of some cranial bristles.

Fig. 3. Upper left: T. confusum adult showing half of the head with a portion of the right antenna, the whole eye, the mandible, the maxillary and labial palps and the labrum and the frons. Upper right: the tip of the antenna showing sensillae. Lower left: detail of the ommatidia showing single interommatidial bristles. Lower right: detail of sensillae of maxillary palp.

Fig. 4. Tip of maxillary palp in T. confusum showing arrangement of sensillae. On the right can be seen the tip of the labial palp. In the background are seen a few ommatidia.

Fig. 5 and 6. Contrast between normal (left) and "aureate" mutation (right) in T. castaneum. Fig. 5, upper left: cephalic, cervical and prothoracic setae in the normal beetle. Upper right: the same area showing great increase in bristle number in the mutant. Lower left: fourth apparent abdominal sternite in the normal beetle. Lower right: same area showing great increase in bristle number (see Ackermann research note in this issue).

Fig. 6, upper left: ventral aspect of anterior portion of prothorax showing arrangement of normal bristles. Upper right: the same area in the "aureate" mutant. Middle left: normal eye. Note single interommatidial bristles. Note also that at this magnification nearly all the ommatidia of the eye are seen in focus. Middle right: the eye in the "aureate" mutant. Note that the interommatidial bristles may be doubled. Lower left: normal maxillary palp showing arrangement of sensillae and the bristles. Lower right: sensillae and bristles in the distal segment of the maxillary palp in "aureate". The sensillar number is not affected, but the bristle number is increased.

Fig. 7. Labial and maxillary palps in the mutant "maxillopedia" (mxp) in T. castaneum. Upper left: specimen with right maxillary palp, right and left labial palps deformed, (compare with Fig. 3, upper left). The right maxillary palp is not segmented, and it bears a claw-like structure at the tip. The right labial palp is badly deformed. The left labial palp terminates in a claw. Upper right: the same left maxillary palp in detail. The claw points to the sensillae which have formed in this area of the "maxillopede". Middle left: detail of the right labial palp. Note tubercles. Middle right: another mxp mutant showing a

deformed maxillary palp. The two claws are prominent, but the tarsal segments are fused into a continuous mass. Lower left: a modified maxillary palp in mxp showing how closely this appendage may be modified into a leg-like structure. This appendage clearly consists of a femur-like segment; a tibia-like segment showing the distal spines and the tarsal segments, of which the proximal one is discrete, but the others are fused. Only one claw developed in this specimen. Lower right: the tip of the tibia and the tarsus in the second pair of legs of a normal beetle. Note the tibial spurs at the distal end of the tibia and the tarsal segments.

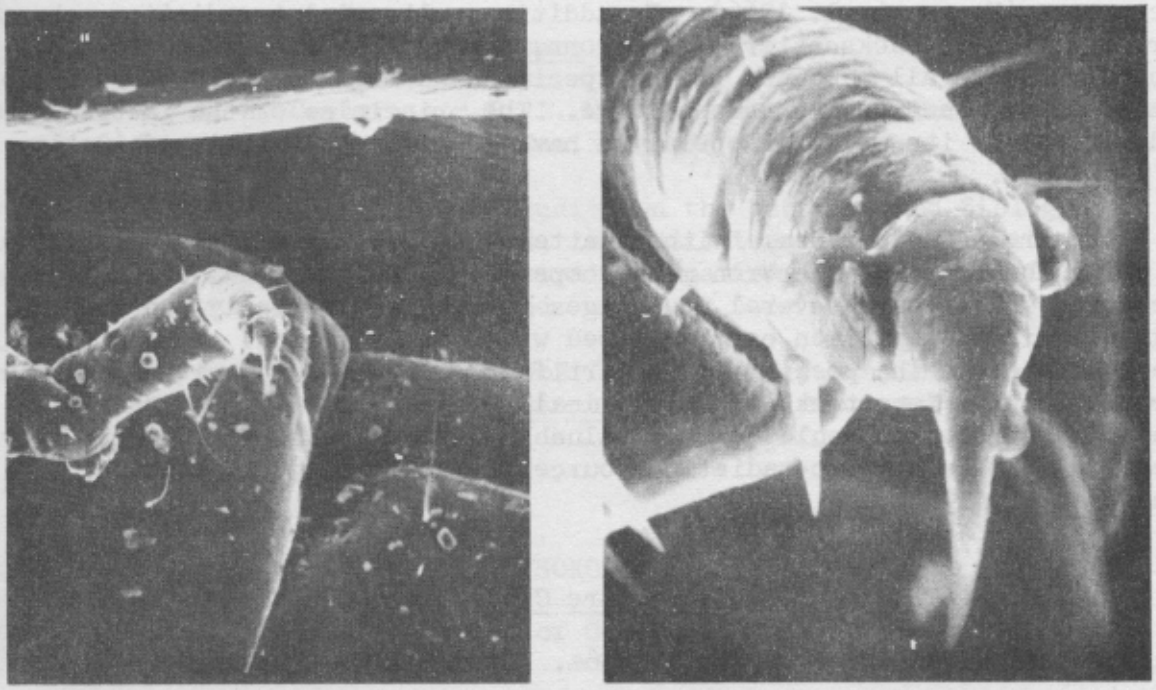

Fig. 1

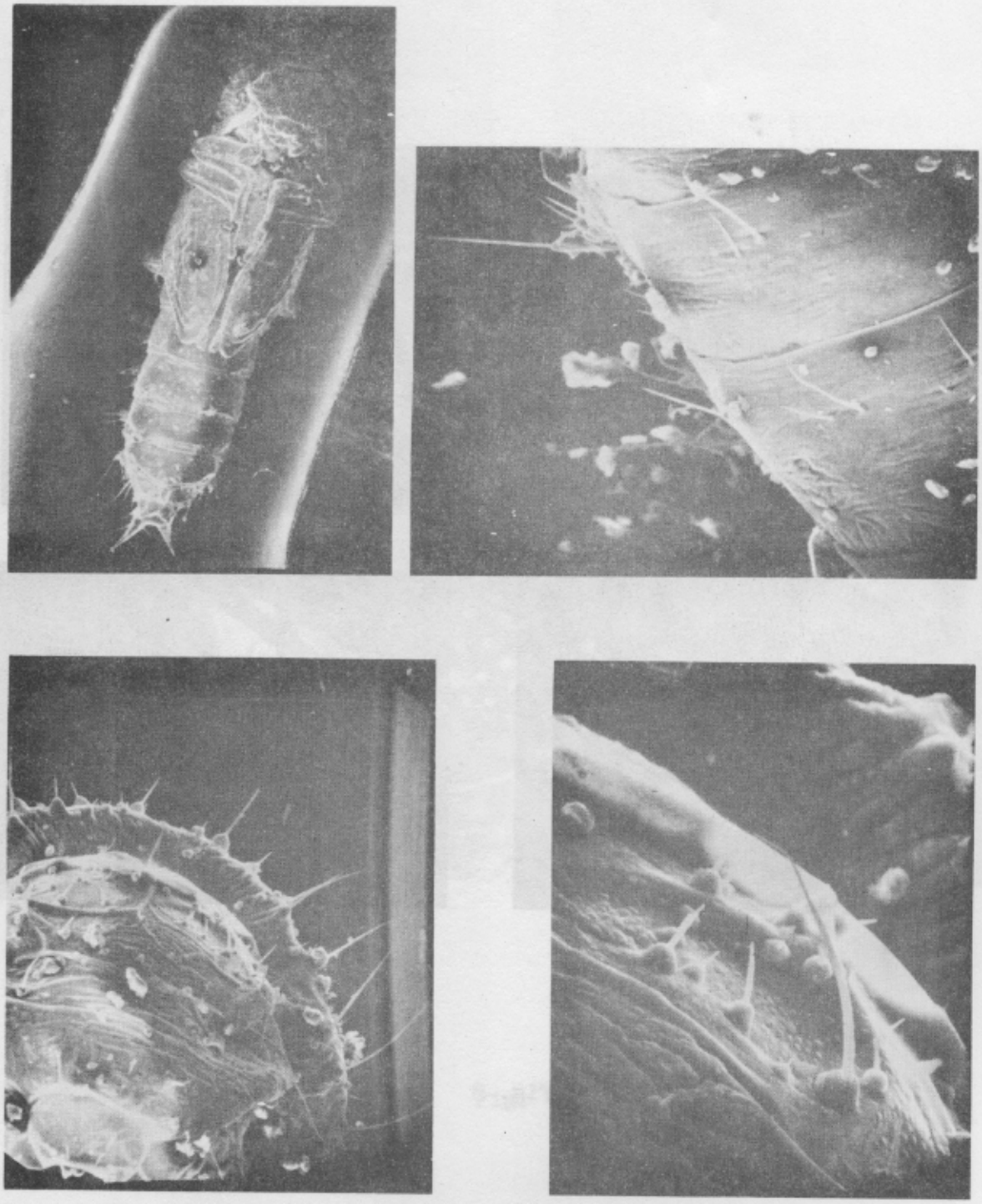

Fig. 2

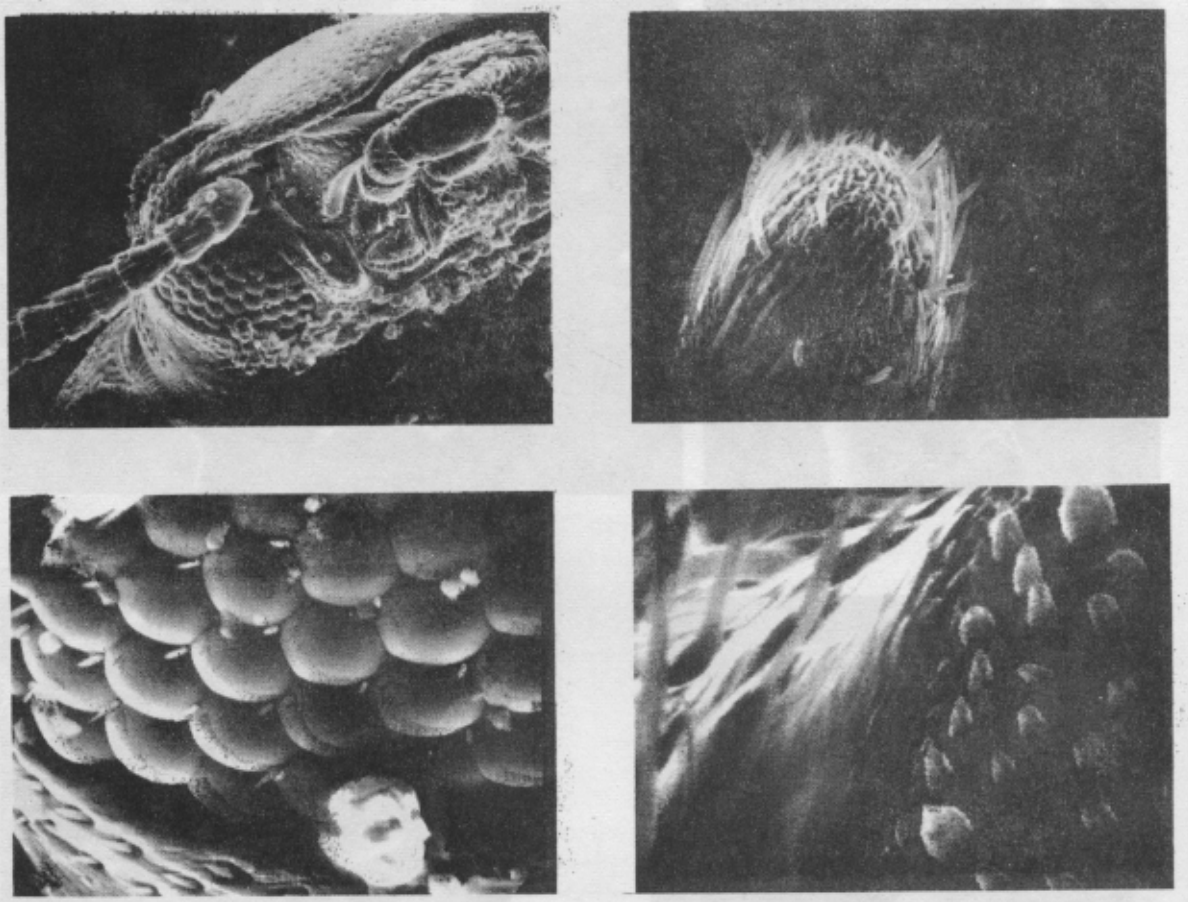

Fig. 3

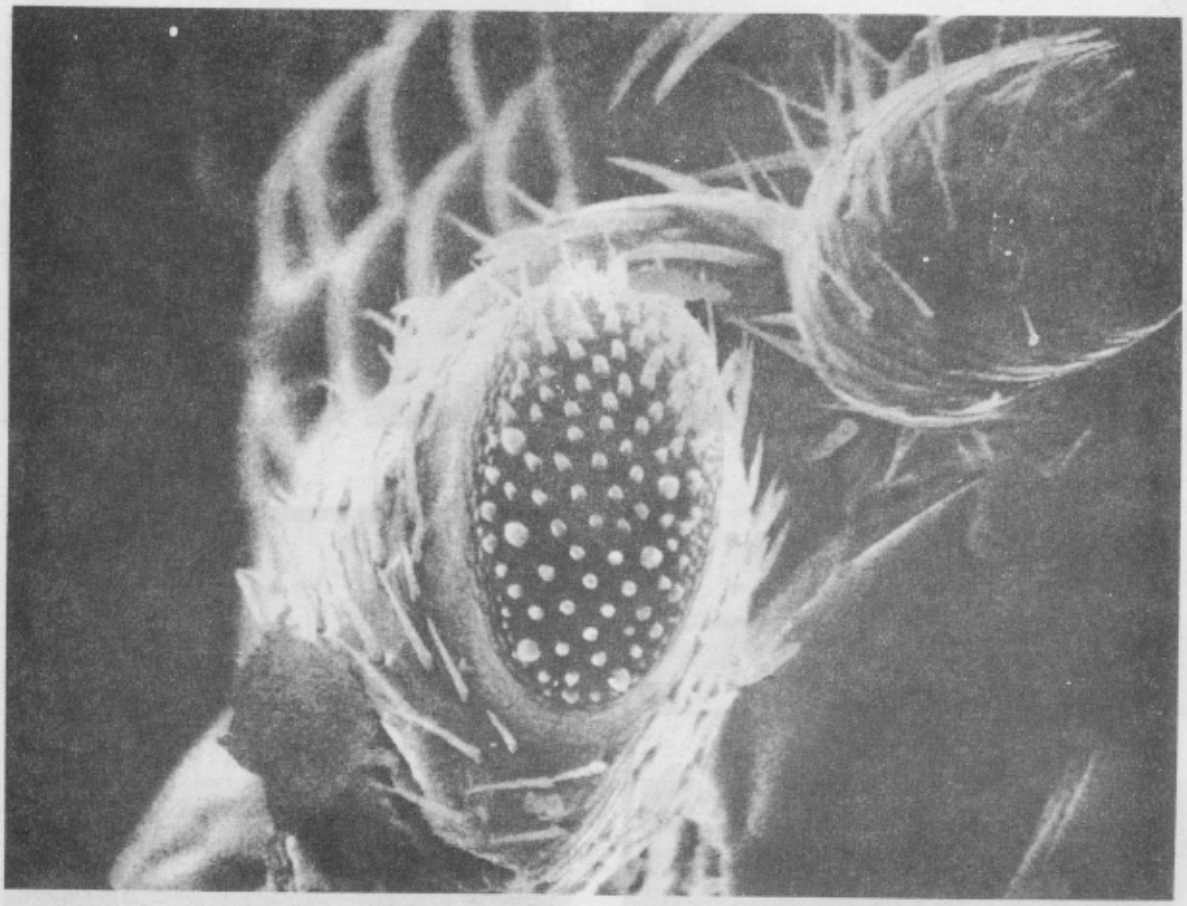

Fig. 4

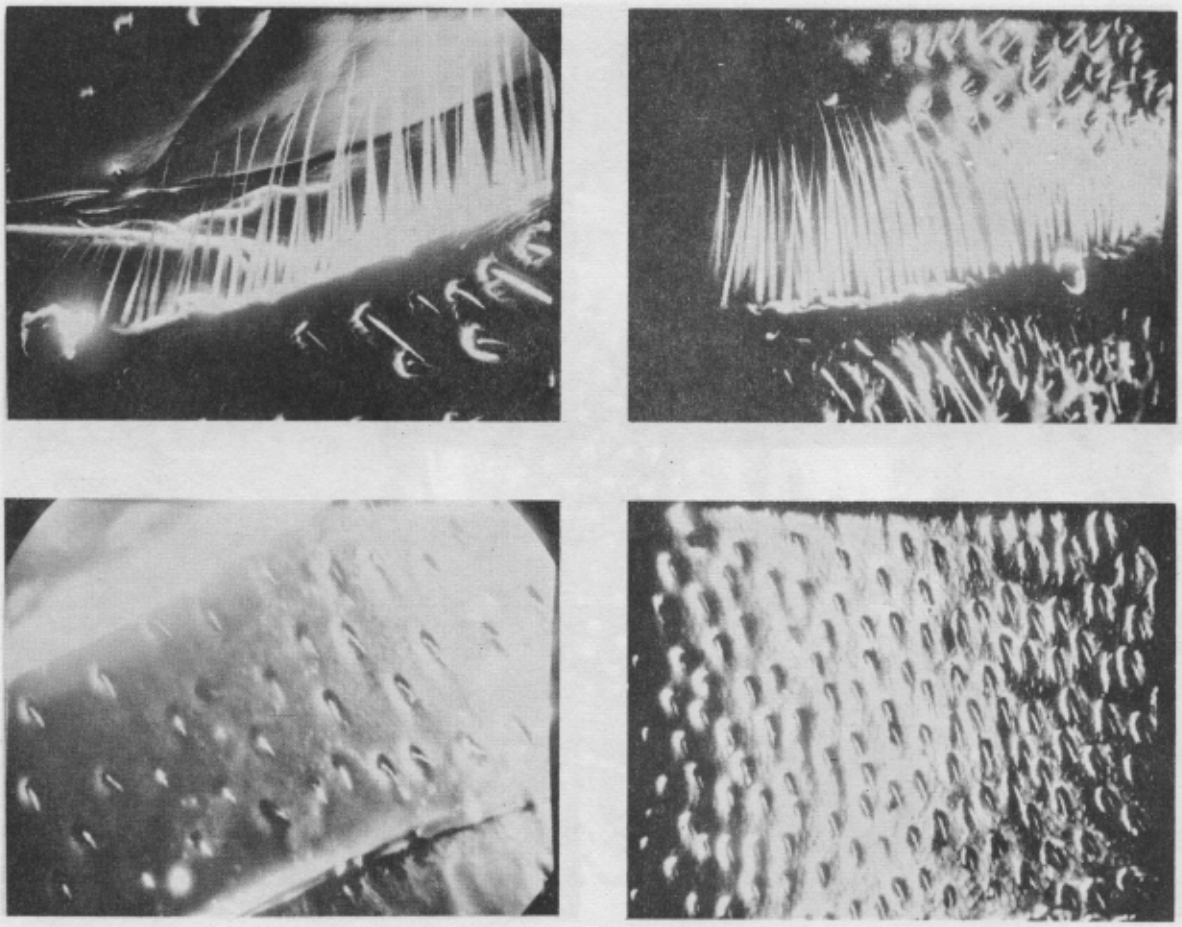

Fig. 5

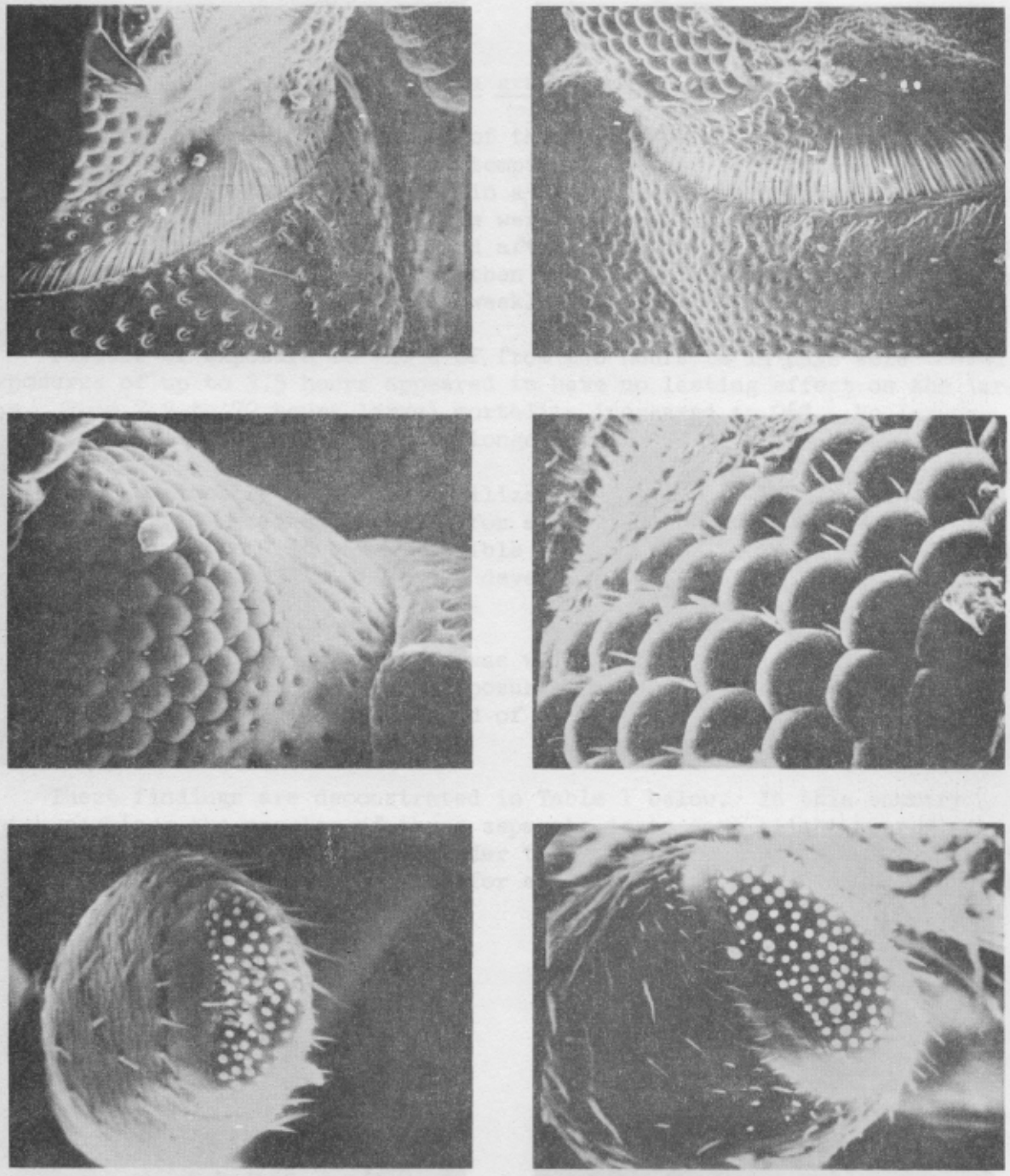

Fig. 6

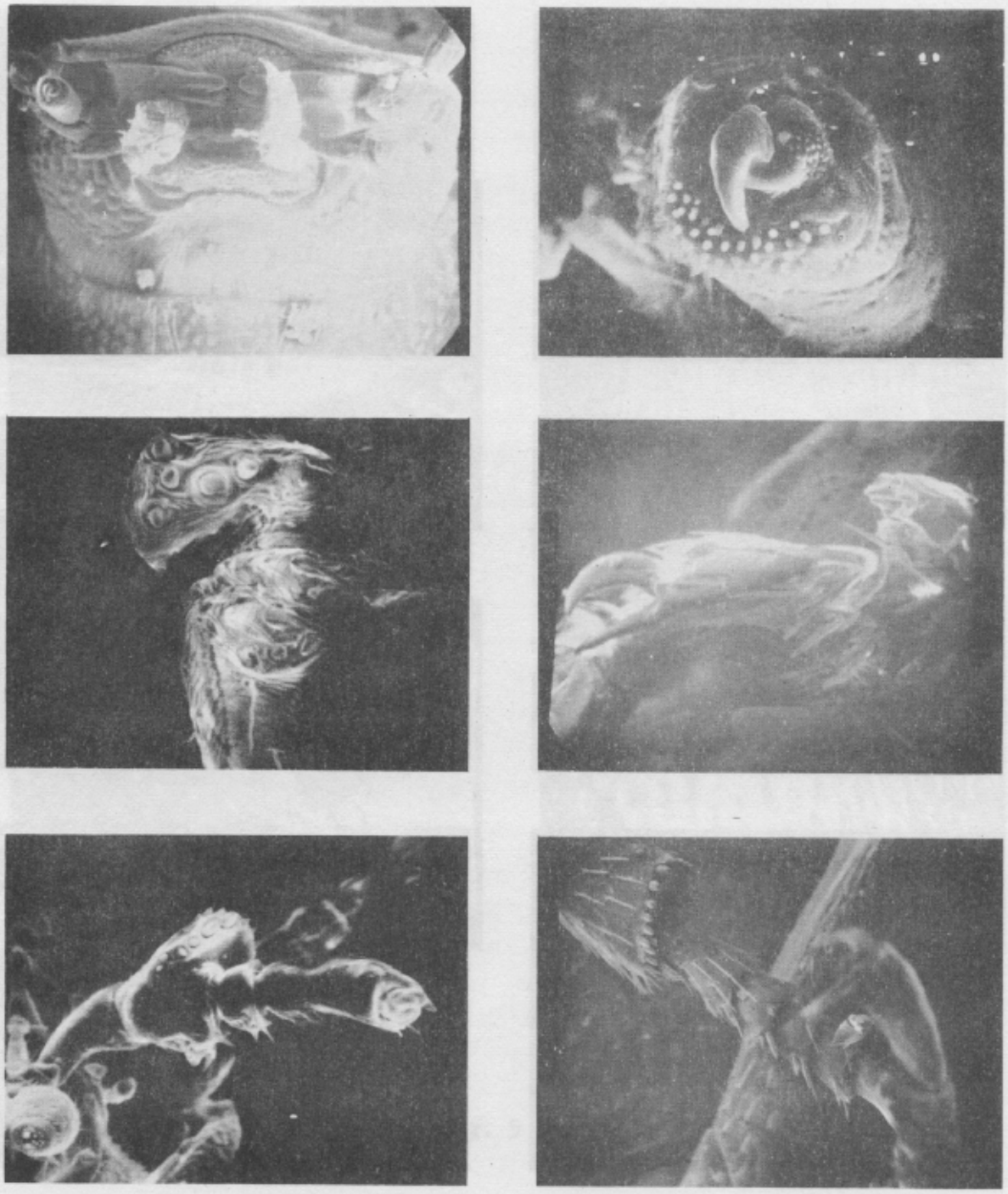

Fig. 7