TRIBOLIUM

Information Bulletin

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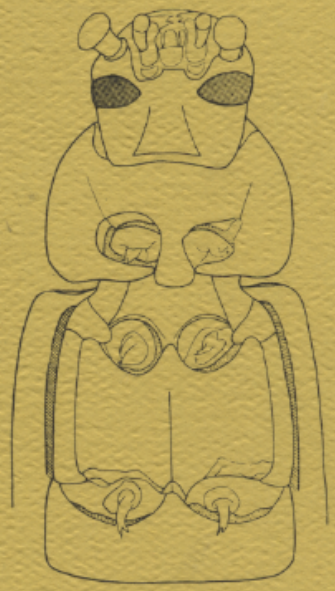

March 1966

MATERIAL CONTRIBUTED BY WORKERS ON TRIBOLIUM
AND OTHER COLEOPTERA

DEPARTMENT OF GENETICS, UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

TRIBOLIUM INFORMATION BULLETIN

Number 9

(Issued in 350 copies)

Editor: A. Sokoloff, Department of Genetics,
University of California, Berkeley, California

1966

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TRIBOLIUM INFORMATION BULLETIN

Number 9

March 1966

Foreword v

Editor's Random Notes vi

Announcements vii

Stock Lists 1

New Mutants 57

Notes - Research

Genetics of pearl eye in Carpophilus dimidiatus F., T. G. Amos and J. S. Scott 71

Response of Tribolium castaneum to adult feeding of n-methyl-n-nitroso-n-nitroguanidine, A. E. Bell, Lelia Brzostowski and R. D. Brock 71

Simplified bioassay method with adults of T. confusum Duv., B. Berck 74

Use of Tribolium species to assess fungistatic and insecticidal vapors generated by wheat in hermetic storage, B. Berck 75

An unsuccessful attempt to implicate infection as a contributing factor in the death of irradiated adult Tribolium, Howard S. Ducoff and P. Howell 76

Fitness modifications in populations of strains and species of flour beetles stressed with X-radiation and/or the insecticide, DDT, Howard E. Erdman 78

Failure of a radiation-protection effect of dimethylsulfoxide (DMSO) on female germ cells of flour beetles, Howard E. Erdman 80

Effect of removing or putting males to fecundated or virgin females, respectively, on the egg laying rate during a long period of time, in Tribolium castaneum, M. Espejo and F. Orozco . 80

Cytological study of Tribolium confusum inbred line CFI-11a, Shri B. Gupta 85

Notes - Research (continued)

Maxillopedia, a homeotic mutant in Tribolium castaneum affecting maxillary and labial palps, Marjorie Ann Hoy 85

"Abnormal abdominal sternites" (aas)--a possible phenodeviant in T. castaneum, Marjorie A. Hoy 86

Two homoeotic mutants affecting the urogomphi and the genitalia, Marjorie A. Hoy 90

"Reduced gin traps" (rgt)--a gene affecting only the pupae stadium in Tribolium castaneum, Marjorie A. Hoy and Barbara B. Daly 92

Preliminary observations on the incidence of Hymenolepis diminuta in the flour beetle, Tribolium confusum, as a function of age, Ronald J. Kelly, Dennis M. O'Brian and Frank F. Katz 94

Genotype-environment interactions in the outcome of "competition" in Tribolium. I. Population cage studies, I. Michael Lerner, Alexander Sokoloff and Frank K. Ho 96

Some further observations on Tribolium confusum populations, Daniel J. McDonald, Laura Fitting and Lila Weaver 105

Studies on the effects of ionizing radiations on Tribolium confusum, T. S. Mobley 106

Studies on the effects of methyl bromide fumigation on mycetomal microorganisms of Sitophilus granarius, A. J. Musgrave 106

Individual effect of the male on the egg laying rate of fecundated females in Tribolium castaneum, R. G. Ruano and F. Orozco 107

Addition to established linkage groups, A. Sokoloff 109

A behavioral difference of two mutant strains in Tribolium castaneum, A. Sokoloff 109

"Competition" between Tribolium anaphe, T. castaneum, T. confusum, T. destructor and T. madens by pairs, A. Sokoloff and Frank K. Ho 110

Effect of removing the male on egg laying rate of fecundated females in Tribolium castaneum, MaP. Tagarro and M. Rico 120

Irradiation treatments, F. L. Watters 121

Notes - Research (continued)

Locomotor activity of spider beetles in wheat, F. L. Watters . . . 122

Notes - Teaching

Linkage group identification by the "dominant synthetic lethal" effect, A. Sokoloff 123

Notes - Technical

Techniques used in a genotype × environment interaction study with Tribolium castaneum, G. W. Friars 125

Cytological technique for the study of Tribolium chromosomes, Shri B. Gupta 127

Population cages, Nobuo Inouye 127

Food choice cages, Nobuo Inouye 129

Simplified techniques for obtaining Tribolium eggs, M. S. M. Stanley and A. W. Grundmann 130

Bibliography 131

Directory - Geographical 195

Directory - Alphabetical 214

FOREWORD

The present issue of TIB, although somewhat shorter than the previous one, still is of considerable length, indicating sustained research activities on the part of numerous individuals utilizing Tribolium. Actually, a number of individuals wrote that they had interesting information which should be included in the Bulletin, but either committee and teaching obligations, planning of or moving to new buildings, or lack of time or energy caused them to make only a token contribution for this year. It is hoped that such material will be made available for the next issue.

Owing to my absence from Berkeley during the academic year, it has not been possible for me to participate more actively in preparing this volume. Mesdames Pam Herman and Merlene Hohmann helped compile the bibliography, Mrs. Mary Ackerman revised the Berkeley stock list and gave valuable advice when such was needed in my absence. The main credit for putting this volume together should go to Mrs. Mary Jo Rawlins who typed the whole issue of TIB-9 and supervised the lay-out of the figures, and Mrs. Naomi Troth who helped to proofread the stencils before the issue went to press. The able assistance of all these persons is gratefully acknowledged.

The publication of TIB-9 was made possible by USPHS grant GM-08942.

A. SOKOLOFF

Berkeley, California
March, 1966

EDITOR'S RANDOM NOTES

Several features of the present number of TIB represent departures from previous issues. The section on Stock Lists has been greatly expanded. This has been deemed necessary because it is the current issue of TIB which is most useful to those seeking information on where to obtain particular species of beetles or strains. I have had to rely on lists published in previous issues since some contributors failed to submit stock lists. It is hoped that these individuals will check these lists and bring them up to date for the next issue in case there are errors or omissions.

Also, the section on New Mutants and a number of research or technical notes include illustrations of the mutants. Descriptions of certain mutants are satisfactory if the reader is well acquainted with the names of the body parts. A drawing, on the other hand, unmistakably identifies which particular parts of the body of the beetle are affected, and makes this clear to the reader even if he is not acquainted with the terminology.

We have been fortunate in having on our staff Mrs. Barbara B. Daly, who has skillfully drawn in India ink the illustrations accompanying the descriptions of new mutants in this issue of TIB. However, for those who may be less skilled in such drawings, I might point out that modern office equipment such as Thermofax generally makes it possible to produce, from a pencil drawing, a much darker figure which will photo-offset, obviating the necessity of having to ink the drawing in most cases.

* * * *

The illustration on the cover, prepared by Mrs. Barbara B. Daly, shows a normal beetle and a mutant called "Stumpy." This mutation has pleiotropic effects affecting most markedly the legs and antennae, but modifying also the appendages around the mouth. The larvae show a marked reduction of all of the appendages, but they manage to develop to the adult stage. The imago is of course helpless, and dies a few days after eclosion. This mutation is more severe in its effect than the pegleg mutant described by Lasley and Sokoloff in TIB-4, 1961.

ANNOUNCEMENTS

Andersen, F. S.
Statens Skadedyrlaboratorium
Skovbrynet 14,
Lyngby, Denmark

We have moved to new buildings. Please note our change of address from Springforbi to Lyngby.

Stanley, J.
Department of Genetics, McGill University
Montreal, P.Q., Canada

In June of 1965, the autotrephon laboratory was dismantled and moved to the new 10 million dollar Stewart Biological Sciences Building. The new laboratory is much larger and it and its preparation room are fully air conditioned with filtered air. The apparatus is now being reassembled but this is a large operation because the apparatus is being spread out for better access and all the D.C. circuits, motors, etc., must be converted to A.C. This involves about 20% of the circuits. Some of the circuitry is being cleaned up and improved and some new blowers are being installed. If other duties permit, it is hoped to have the apparatus running perhaps by Spring of 1966, and certainly by the Fall.

NEW MUTANTS

A. Anthonomus grandis (Curculionidae)

*REPORT OF A. C. BARTLETT

walnut (w)--Bartlett, October, 1965. A body color mutation in Anthonomus grandis Boheman. Lighter in color than slate or ebony, but closely resembling the slate heterozygote. Autosomal recessive, complete penetrance and good expressivity. In some crosses the female appears somewhat darker than the male. Most easily classified in young adults, since body color of wild-type boll weevils darkens with age. Isolated in the F_2 of a pair of weevils obtained from Acala, Mexico. Relationship to other markers now under study.

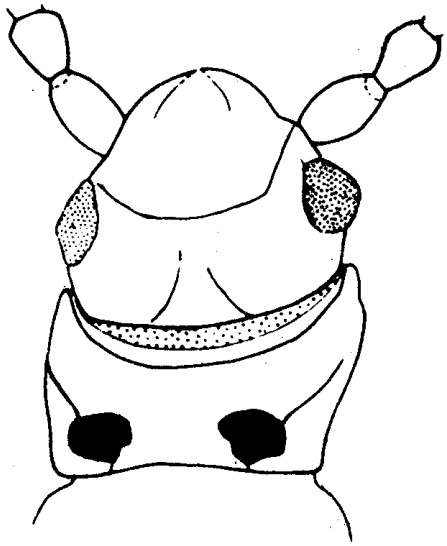
B. Carpophilus dimidiatus F. (Nitidulidae)

See research note by Amos and Scott.

C. Cryptolestes turcicus (Cucujidae)

*REPORT OF MARJORIE A. HOY

deformed prothorax (dfp)--Hoy, 1965. A number of stocks of C. turcicus yield relatively large numbers of adults with abnormal prothoraces. The prothorax is reduced in size, especially along the anterior ventral margin (see figure illustrating dfp prothorax). The deformity is so extreme that the head is frequently deflected ventrally, giving the beetle a characteristic appearance. These beetles are often found dead in the stock. If pupal cases are dissected that contain dead adults, almost all are dfp beetles. Crosses of dfp × +/+ and of dfp × dfp yield very few dfp progeny, suggesting that dfp is a semi-lethal and incompletely penetrant gene.



D. Dermestes maculatus (Dermestidae)

*REPORT OF D. D. SHAW AND J. J. WELCH

1. creased sternites. Spontaneous in a culture originating from Australia. Third to fifth abdominal segments have grooves on the ventral side extending inwards from the lateral margins. In occasional extreme individuals the grooves extend from margin to margin. An autosomal recessive not linked to fuscous. Linkage tests with rufous not yet completed.

2. deformed antennae. Spontaneous in culture from South Africa. Expression variable, antennal club usually double but sometimes only one or two segments are swollen and deformed. Genetics not yet studied.

3. fuscous (fu). Autosomal semi-dominance suspected last year (TIB-8, p. 42) has been confirmed. Markers for second linkage group.

4. pearl (p). This mutant reported in TIB-3, p. 42 has proved to be a sex-linked recessive. It cannot therefore be a reoccurrence of Philip's "white eye."

5. light antennae. Spontaneous in a culture from the Indian region. The three segments forming the antennal club distinctly paler than normal, sometimes colorless. Genetics not yet studied.

6. pink. Spontaneous in culture of unrecorded origin. Eyes pink at center surrounded by a broad pearly-green area. Genetics not yet studied.

7. red. Spontaneous in a culture of unrecorded origin. Eyes a uniform pale red. Genetics not yet studied.

8. rufous (ru). Described in TIB-8, p. 42. Has proved to be an autosomal recessive not linked to fuscous. Linkage tests with creased sternites are underway.

9. ridged elytra. Spontaneous in a fuscous × pearl cross. Two distinct ridges extend across each elytra near the scutellum. Genetics not yet studied.

10. white tip. Spontaneous in culture from Sudan. The fifth abdominal segment in normal beetles has ventrally two bands of white hairs enclosing two marginal spots leaving a black area in the middle. In this mutant the central area is covered with white hairs. This may be a reoccurrence of "white tip," an autosomal recessive with poor penetrance described by Philip (1940).

E. Latheticus oryzae (Tenebrionidae)

*REPORT OF MARJORIE A. HOY

incomplete meso-metathoracic suture (ims)--Hoy, 1965. A single male was found in a stock containing r te (fas) that had a meso-metathoracic abnormality resembling ims in T. castaneum. The gene ims is a recessive with incomplete penetrance. No pure stock has been developed. No linkage data are available.

F. Tribolium castaneum

*REPORT OF C. E. DYTE, R. W. LEMON AND DOROTHY G. BLACKMAN

1. long abdomen (la). Autosomal recessive isolated from P.I.L. stocks at University of St. Andrews. Most easily identified in pupal stage where abdomen is elongated, and not curving as in wild type. The abdominal segments are somewhat longer than usual, so that in adults the abdomen is longer than elytra and tends to curve upwards. Apparently not linked to pearl, black, sooty, chestnut or antennapedia. Linkage tests to be continued.

2. pearl-like. Autosomal recessive isolated from P.I.L. stocks at University of St. Andrews (see TIB-8, p. 27). Larvae and pupae show lack of pigment as in pearl, but the adult develops slight coloring of similar shade to chestnut though not as dark. Not allelic with pearl or chestnut.

3. mahogany (my). Autosomal recessive isolated from P.I.L. stocks at University of St. Andrews (see TIB-8, p. 27). Body color deep reddish brown not fully pigmented until five weeks. Larvae and pupae no different from wild type. Not allelic with black, sooty. Crosses made so far suggest mahogany may be linked to sooty. Three point crosses not yet carried out.

4. jet (j^k). Autosomal recessive isolated from a strain from Kingston, Jamaica, at University of St. Andrews (see TIB-8, p. 27). This is a reoccurrence of jet and has been named Kingston jet.

*REPORT OF D. C. ENGLERT

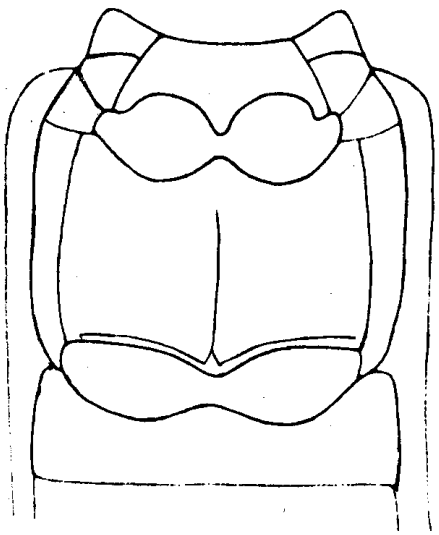
mottled (mt).--Englert, 1962. Autosomal recessive, spontaneous in a control stock of pupation time selection experiment, derived from the Purdue University + foundation stock. Incomplete penetrance with variable expressivity. Dark red brown pigmented "blotches" appearing internally in areas of the stink glands of prothorax and abdomen; other common degrees of expression include very dark pigment "spotting" appearing to be incorporated in the exoskeleton over the prothorax region, particularly along the edges. Character is best viewed from ventral

surface, but can be classified with reasonable success from dorsal viewing. Degree of penetrance varies from 60-98% depending upon genetic background and selection intensity. This mutant appears to be similar to "melanotic stink gland" (msg) reported by Sokoloff and Hoy (see New Mutants section, TIB 8, p. 55). To date allelism tests have not yielded conclusive results, but indications are that they may be similar occurrences of the same mutation. (If so proven, then mt will be changed to msg^{62}.) Linkage relationships are still being investigated.

*REPORT OF MARJORIE A. HOY, BARBARA B. DALY AND A. SOKOLOFF
(See also research notes by Mrs. Hoy. Ed.)

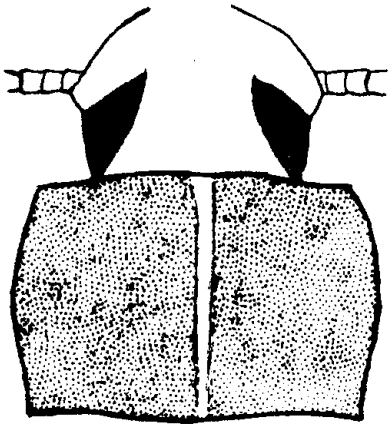
1. aureate (au)--Hoy, 1965. Four males and three ♀♀ adults were found in a stock containing Be, s, and "reduced heads," that had a very striking and unusual condition. These beetles have a dense covering of golden "hairs"--at least twice as many hairs as normal beetles. The covering of golden hairs on au beetles is easy to see under low magnification. Beetles with au are not as shiny as wild type beetles. One of the au beetles was also sooty but body color does not interfere with the identification of au. The golden "hairs" are spectacularly beautiful against the darker ground color of the chitinous exoskeleton. After careful examination, it became evident that the "ground substance" of the exoskeleton is more finely pitted or punctate. Present available data suggest that au is an autosomal recessive with complete penetrance. There does not appear to be any variation in expression. Larvae and pupae with au are also identifiable: they are less shiny than normal larvae and pupae and have more hairs than usual. The new hairs are short to medium in length.

2. Blunt abdominal and metathoracic projections (Bamp)--Hoy, 1965. One non-virgin female in a stock containing lmg showed a condition resembling ims (see figure). In addition, the medial abdominal



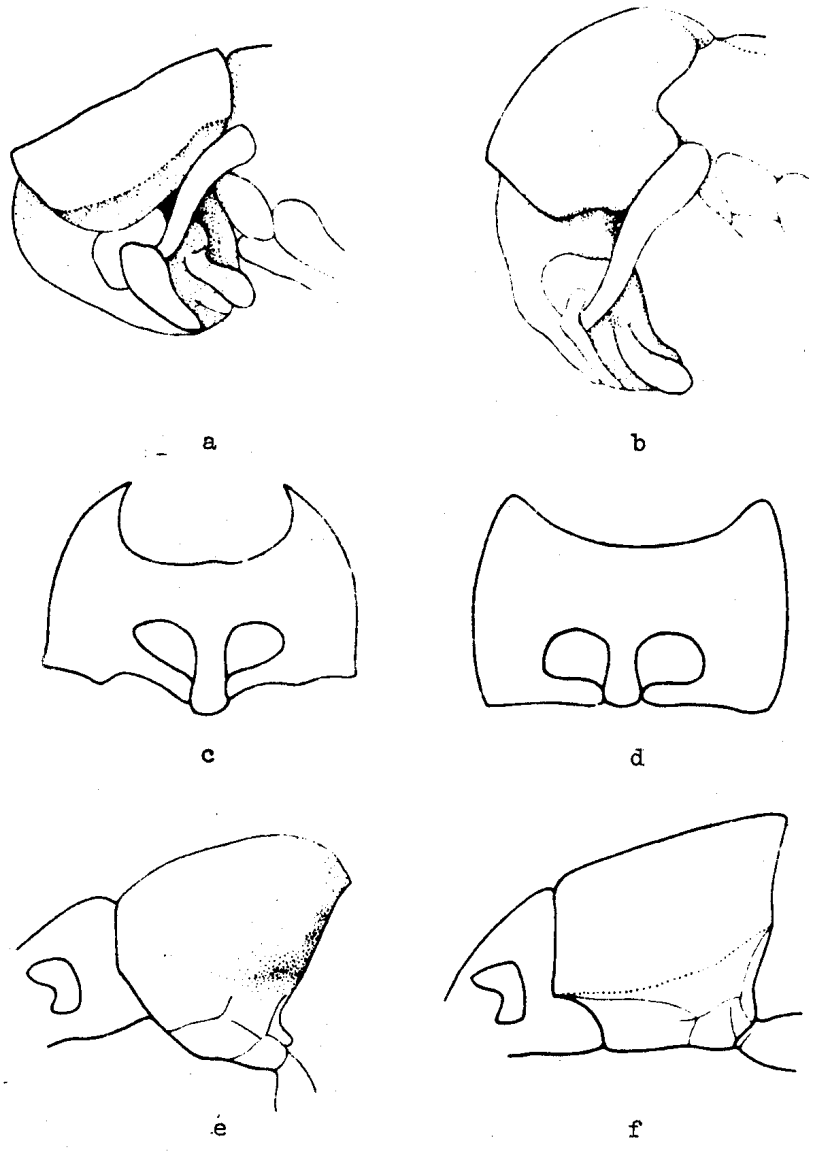
projection was blunt. When bred to normal males, this female produced 16 males and females exhibiting Bamp, and 39 normal sibs. A cross of Bamp ♂ with three b/b virgins gave 42 Bamp ♂♂ and ♀♀ with variable expression, and 129 normal sibs. No Bamp × Bamp crosses have been made to determine whether a recessive lethal effect is produced, but it is clear that this autosomal dominant has reduced viability.

3. Nonpunctate prothorax (Npp)--Hoy, 1965. In a cross to determine the heritability of a femoral abnormality (which proved to be a teratology) a number of beetles were found with the prothorax exhibiting a lack of punctate indentations on the midline but no change in pigmentation. The available data suggest this is a dominant gene with no recessive lethal effects.



4. padded prothorax (pdp)--Hoy, 1965. Spontaneous in a rough (ro) stock. One male and three female pupae were found with the phenotype shown in the accompanying figure. In both the pupa and the adult the prothorax appears puffed or padded (b, e) and the ventral aspect of this segment may be deformed (contrast c with the normal d). The pdp condition appears to be a phenodeviant. Despite selection for the strongest expression for six generations the stock exhibits only 30% strongly affected and 20-25% weakly affected beetles. The rest appear like the normal beetles shown in a, d and f.

padded prothorax



a. normal pupa
b. pdp pupa
c. pdp adult
d. normal adult
e. pdp adult
f. normal adult

5. paddle-1 (pd-1)--Hoy, 1965. A number of beetles from a stock containing depressed prothorax had an antennal gene resembling paddle (pd). A cross of pd-1 ♀♀ × 3 py pd ♂♂ yielded all paddle F_1 progeny. Expression of paddle-1 is very similar to paddle in the males; in females, however, expression of paddle-1 is somewhat different from paddle. Paddle-1 ♀♀ have a stronger fusion of club and funicular segments.

6. prothoraxless-1 (ptl-1)--Hoy, 1965. A number of beetles from a stock containing aa and p were isolated with abnormal prothoraces resembling ptl. A stock has been established that has very strong expression of ptl-1. Crosses were made with ptl and results suggest that the two genes are allelic. The new allele, ptl-1, however, has stronger expression and somewhat better penetrance than ptl, making it a little better to use as a marker for chromosome IX.

7. reduced pleurosternal suture (rps)--Hoy, 1965. A number of adults from a stock containing Be; ppas were discovered with abnormal prothoraces. The anterior ventral margin of the prothorax is deformed along the pleurosternal suture line. Beetles may be found with the entire ventral margin of the prothorax reduced, with greater indentations where the pleurosternal suture meets the anterior margin. (See figure IA and B). This is an autosomal recessive gene with variable expression but complete penetrance. Expression of rps varies according to the background into which it is mated. Backcrosses yield progeny with good expression of rps, while $F_1 \times F_1$ crosses may result in beetles with very weak expression (i.e. only slight indentaticns of the anterior margin of the prothorax in the vicinity of the pleurosternal suture), but penetrance seems complete. No linkage information is available.

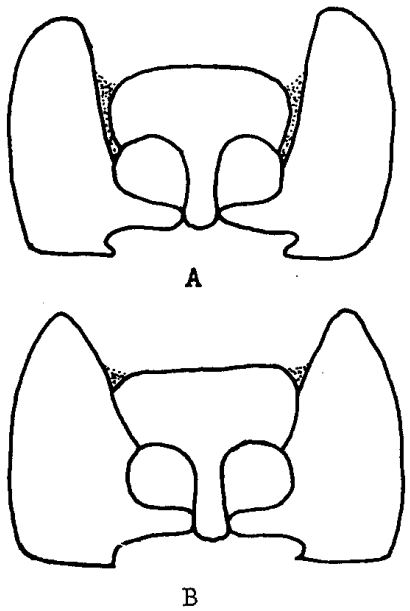
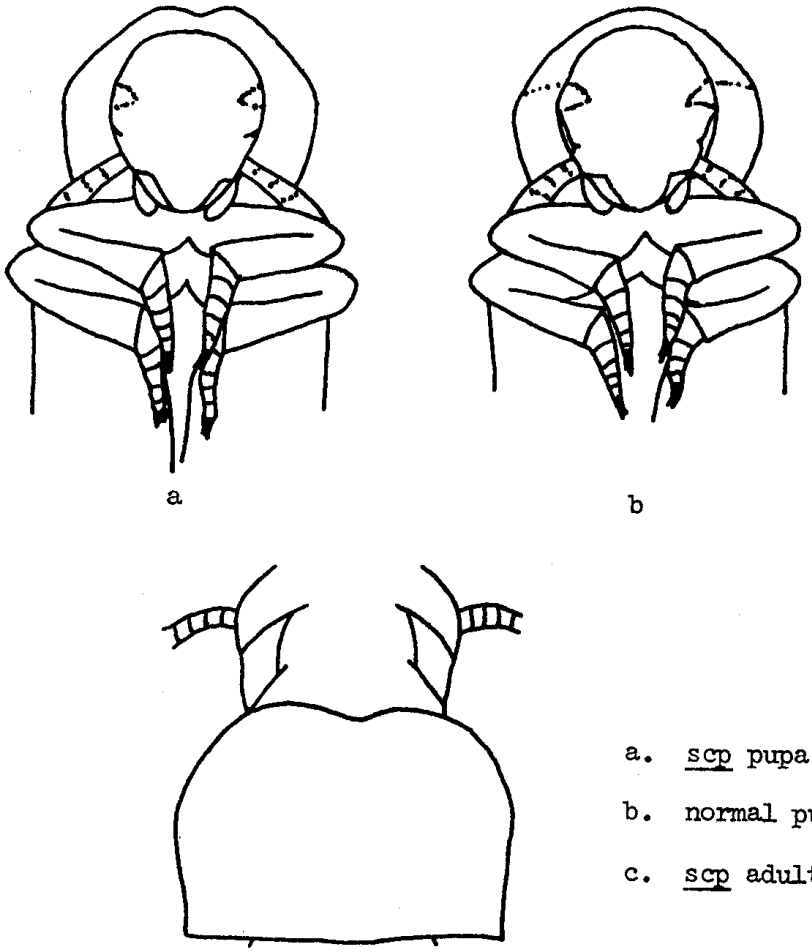


Fig. I. Strong (A) and moderate (B) expression of rps in T. castaneum (ventral view of the prothorax).

8. scalloped prothorax (scp)--Hoy, 1965. In a stock of sta-like were found some pupae (as shown in the accompanying figure (a)) which exhibit an indentation on the anterior margin of the prothorax. This condition persists in the adult (c). Expression of scp is variable. It is probably a phenodeviant since it is present in small numbers in the F_2, but the frequency increases upon inbreeding.



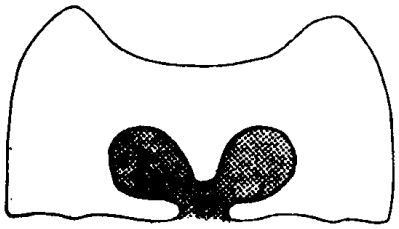
a. scp pupa

b. normal pupa

c. scp adult

9. short, split spinasternum (sss)--Hoy, 1965. A number of beetles with short, slightly split spinasterna was found in a stock containing black and nonpunctate prothorax (Npp). A stock having very strong expression was established (see the following figure). The sss gene is inherited as an autosomal recessive with variable expression, and incomplete penetrance in some genetic backgrounds. In extreme expression the spinasternum is shortened (often to the anterior margins of the epimera) and sometimes it has a medial split on its tip. In addition, the anterior ventral margin of the

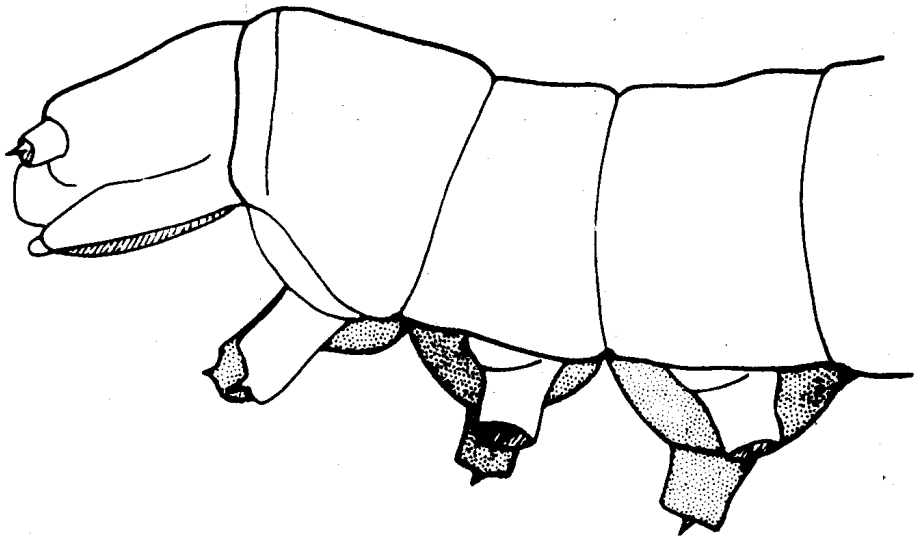
prothorax may have an indentation in a region directly anterior to the line of the spinasternum. The epimera are usually quite normal in length and conformation.



10. stumpy (stu)--Hoy, 1965. Fifty-three immature beetles, including larvae and pupae, and one adult were found in a stock containing fas^D, tet, eta. All larvae had reduced legs and antennae. All pupae had greatly reduced legs, antennae, and mouthparts. The legs of the adult beetles were reduced so that only deformed coxae plus reduced and deformed larval claws remained (see picture of an adult on cover and accompanying figure of an stu larva). Adult antennae were represented by one basal segment and a single terminal segment. Adults appear to have subtly abnormal shaped heads and prothoraxes. A split elytral condition is also common. Pupae have slightly reduced urogomphi. Male pupae appear to have normal genital lobes; female pupae, however, have greatly reduced genital lobes, making it difficult to sex them without males nearby for comparison. All of the 53 stu immatures died either during the larval stage, the pupal stadium or as very young adults. None reproduced. Normal sibs of the stu beetles were mated in hopes of obtaining more stu. It was found that stu is due to an autosomal recessive lethal gene. Of eight successful single pair matings of normal virgin beetles from a stock that produced stu the following ratios were found:

1-3.	F_1 progeny all normal		
4.	144 +	:	38 stu
5.	118 +	:	27 stu
6.	49 +	:	4 stu
7.	106 +	:	25 stu
8.	69 +	:	20 stu

The sex ratios of the above five creamers were normal. Stocks are maintained by selecting + (2/3 of which are +/stu) males and females from single pair crosses that yield stu individuals.



stu larva

*REPORT OF DORIS M. SHIDELER

1. denotched (d)--Shideler. Autosomal recessive appearing spontaneously in Selection Line 2, Replication II derived from Purdue Foundation p. The notch of the eye is completely lost resulting in the exposure of all ommatida. The scape and pedicel of the antennae are fused to yield 10 instead of 11 segments. Antennae are held in a vertical rather than the normal manner. Good viability and penetrance.

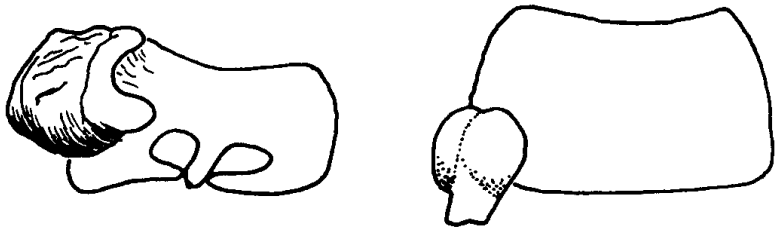
2. short antenna (sa_3)--Shideler. Autosomal recessive appearing spontaneously in Purdue "wine" stock. Allelic to Sa and similar to Sokoloff description of sa.

G. Tribolium confusum

REPORT OF MARJORIE A. HOY, BARBARA B. DALY AND A. SOKOLOFF

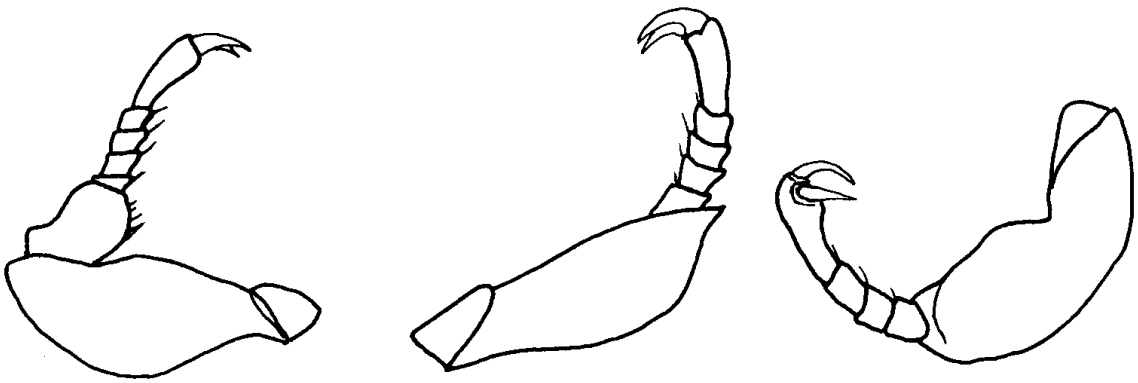
1. alate prothorax (apt)--Hoy, 1965. Thirty-five adults and pupae were found in a "pockets" (poc) stock which in every way resemble the alate prothorax condition in T. castaneum (see TIB-8, p. 44):

Adults have prothoraces with lateral bulges or holes appear on the prothorax after these prothoracic extensions break off from the lateral aspect of the prothorax. The phenotypic expression of apt is quite variable. Two examples of the expression in the adult are shown below. In extreme expression very well formed elytra appear on the prothorax of the pupa. In milder expression, however, only knob-like processes appear on the prothoracic segment. A stock of apt has been established with difficulty owing to the semilethal effect of this gene.

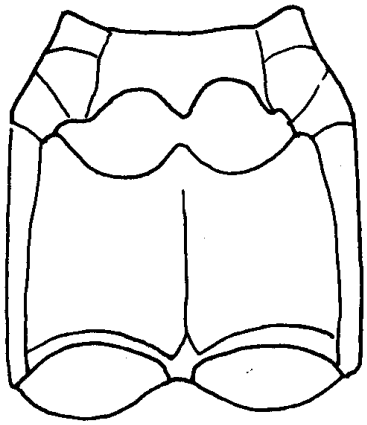


ventral view dorsal view

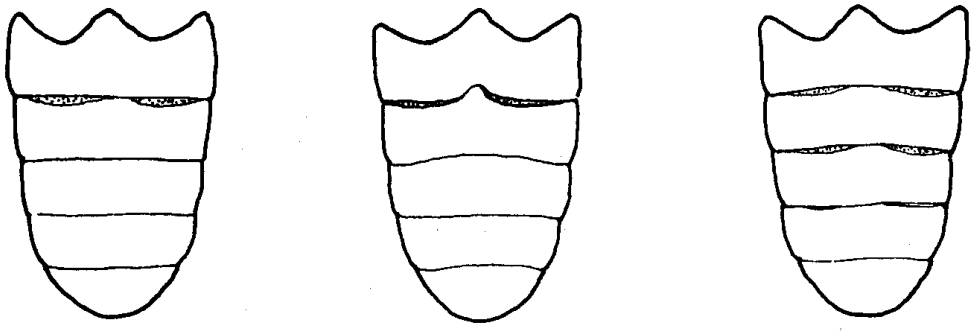
2. deformed legs (dfl)--Hoy, 1965. Six male and female pupae were found with abnormal legs. The effect of dfl is identical to that produced by dfl in T. castaneum: the tibia is reduced or eliminated, and sometimes the femur is deformed (see figure below). Progeny of dfl ♂ × dfl virgin ♀ produced a few beetles with apparently normal legs indicating incomplete penetrance at 32°C. A previous mutant described as deformed legs (dl) was no longer available for tests of allelism. If they are allelic, the expression of dfl is stronger than that reported for dl.



3. incomplete meso-meta-thoracic suture (ims)--Hoy, 1965. Two ims males were found in a stock containing b, rus, and spl. This gene in every way resembles the gene ims in T. castaneum. It appears to behave as an autosomal recessive with incomplete penetrance. A stock has been started. No linkage data are available.



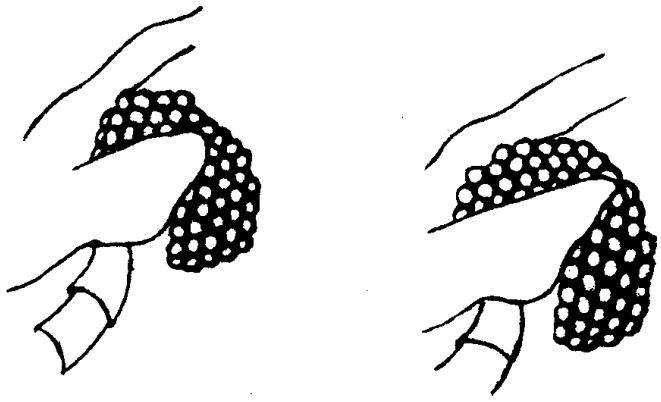
4. pockets (poc)--Hoy, 1965. Found as a single male in a population containing rsp that had an abnormal abdomen somewhat resembling ppas in T. castaneum. A second male was found in a second population containing rsp. In the F_2 10 poc : 112 + were found; and 40 poc : 131 +. The backcross produced 60 poc and 113 +. The gene is thus an autosomal recessive with reduced viability or incomplete penetrance. Expression is variable. Expression most frequently is manifested as pockets or cavities along the anterior margin of the second apparent abdominal segment. The pockets are located lateral to the midline, with an incomplete "point" in the midline (Fig. 1-a). Sometimes there is a complete point on the midline (Fig. 1-b). Occasionally, the pockets are quite weak and resemble extreme cases of the gene sti. With selection, beetles show up with "pockets" on the margin between apparent segments II and III (Fig. 1-c). No linkage data are available.



a b c

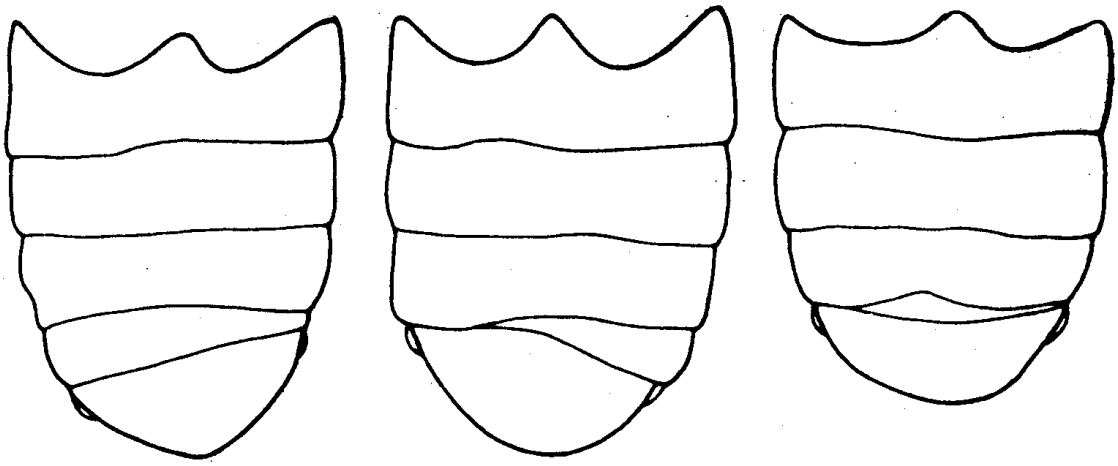
Fig. 1

5. Reduced eye (Re)--Hoy, 1965. A single ♂ was found in a population containing rsp. The eyes were reduced in size to approximately 2/3 their normal size (see figure below). The shape of the head was entirely normal--in contrast to eye mutants such as Microopthalmic and strong expressions of Be in T. castaneum. The ♂ was mated to three synthetic +/+ ♀♀ virgins. In the F_1 progeny 50 Re and 59 + beetles were scored, with a normal sex ratio for both categories. Re beetles are readily scored by examination of the lateral aspect of the head, where it is seen that there are fewer facets than normal. An abnormal outline of the eye is also evident. As an added aid to scoring, most of the Re beetles (both ♂♂ and ♀♀) are noticeably smaller in overall body size than their + sibs. However, on outcrossing, body size cannot be relied upon to classify Re. Crosses of + F_1 × + F_1 yielded no Re progeny. A cross of Re × Re yielded 32 Re: 17+. These crosses indicate that Re is a dominant gene with recessive lethal effects. Linkage tests are under way.

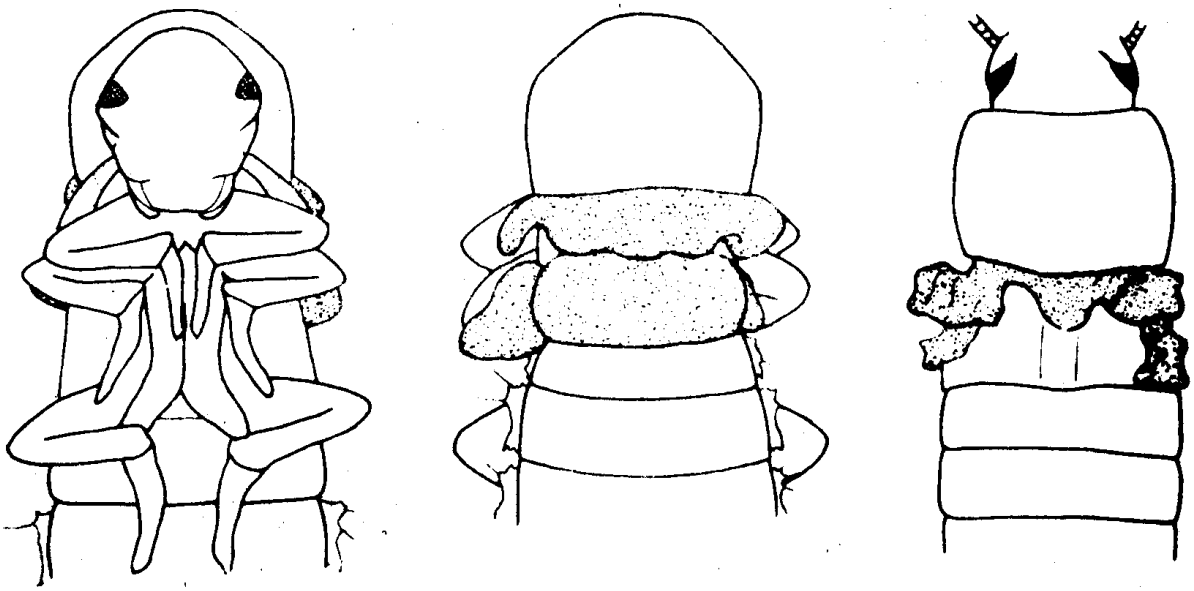


Re normal

6. twisted abdomen (twa)--Hoy, 1965. From time to time beetles exhibiting abnormalities similar to those shown in the following figure have been found. When crossed, however, the condition fails to appear in subsequent generations, or the crosses fail. In single pair crosses designed to test for lethals two twa non-virgins were found which, when crossed with their sibs produced 11 + and 8 twa ♂♂ and 14 + and 1 twa ♀. The twa condition is detectable in larvae, pupae and adults. In larvae and pupae the effect is observed both on the dorsal and ventral aspects but in the adults only the ventral sclerites can be used to detect twa. Penetrance and viability information not available.



7. wingless (wgl)--Hoy, 1965. A single male with vestigial elytra and wings was isolated from a stock containing b; es lp. Female pupae were also found. Crosses of wgl × +/+ yielded 8 wgl progeny in the F_2 (7 ♂♂ and 1 ♀) from progeny of three crosses. The normal F_2 were mated together and produced a total of 28 F_3 wgl pupae. The wgl pupae have variable expression. Some have only tiny rudiments of elytra and wings. These tiny buds often are deflected from the body of the pupa. Some wgl pupae have elytra and wings that are shortened by only one-half. These beetles have very strong elytral splits. All wgl beetles have difficulty in molting. All of the above 28 F_3 wgl beetles died during the pupal stadium or while molting. Stocks of "normal" beetles occasionally still yield wgl pupae but no pure stock has been established. The wgl gene is an autosomal recessive lethal that has strong effects only upon elytra and wings.



NOTES - RESEARCH

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Genetics of pearl eye in Carpophilus dimidiatus F.

The genetics of the pearl eye mutation in C. dimidiatus has been determined. Breeding experiments using a stock of pearl eyed beetles obtained from the Pest Infestation Laboratory, Slough, Bucks, indicated that the inheritance of the pearl eye is governed by a single recessive, autosomal gene for which the symbol pp is proposed (see Amer. Nat., 1965, XCIX, No. 9, p. 421-423).

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*Response of Tribolium castaneum to adult feeding of n-methyl-n-nitroso-n-nitroguanidine

In testing the mutagenic effects of chemicals in adult Drosophila the usual practice has been to inject the chemical into the abdominal cavity. This technique should be applicable to Tribolium with little modification. However, the unusually high mutagenicity of diethyl sulphate without producing apparent chromosomal aberrations when fed adult Drosophila males (Pelecanos and Alderson, D.I.S. 37:116) interested us in searching for a chemical mutagen on which Tribolium would feed. The feeding technique Pelecanos and Alderson used for Drosophila was attempted for diethyl sulphate at concentrations of 0, 0.5 and 1.0%. While adult T. castaneum would eat moist medium without diethyl sulphate, they would not consume the medium when as little as 0.5% of the chemical was added. Efforts to feed the chemical mutagen ethyl methane sulphonate were equally unsuccessful.

Recent reports that n-methyl-n-nitroso-n-nitroguanidine was mutagenic for E. coli (Adelberg et al., 1965, Biochem. Biophysical Research Communication, Vol. 18, page 788) and for Arabadopsis (Muller and Gichner, 1964, Nature 201:1149) interested us in testing the effects of this chemical when

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fed Tribolium castaneum. In preliminary trials the chemical was mixed with standard medium (95% wheat flour plus 5% dried brewers' yeast) in concentrations of 0, 0.01, 0.1, 1 and 5%. It was observed that fertility was sharply reduced in adult beetles when fed only 24 hours on concentrations as low as 0.1% of nitroguanidine. The reduction in fertility was greater at higher concentrations and was observed to be affected for as long as 96 hours following the treatment. No increase in mortality was observed for those beetles fed the higher concentrations of the chemical; however, those on levels of 1% and higher appeared stupefied.

The following study was made in order to quantify the effects of nitroguanidine on reproduction and to make a limited test of its mutagenicity:

Adult T. castaneum males (24-72 hours following emergence) of the Purdue Foundation Strain were starved for 24 hours before being fed for 48 hours on one of five levels of n-methyl-n-nitroso-n-nitroguanidine (0, 0.1, 0.5, 1.0 and 5.0%) in standard medium. Fifteen males from each treatment group were individually mated immediately following the treatment to untreated females of the same strain approximately one week of age. To assure the virginity of these females they had been isolated into individual creamers as pupae. The average number of offspring per mating for each successive 48-hour egg collection following the treatment of the males is reported in Table 1. One sees a sharp reduction in fertility for the first 48-hour collection from those males which had fed on the higher concentrations of the chemical. The numbers of offspring were still depressed in the second 48-hour collection from males receiving the two higher concentrations. By the third collection all males had recovered to an apparently normal level of fertility. No effort was made in this study to relate the reduced number of offspring to dominant lethal mutations, copulation rate, or sperm inactivation.

Table 1

Average number of offspring per mating in successive 48-hour egg collections following the feeding of Tribolium males on various levels of nitroguanidine.

Treatment	Mean number of offspring by 48-hr. collections				
levels, %	1st*	2nd	3rd	4th	5th
0	24.7	41.8	43.2	43.0	50.5
0.1	33.5	52.0	53.2	50.6	55.0
0.5	12.5	40.1	46.2	48.8	46.8
1.0	5.9	22.4	44.8	49.8	53.6
5.0	2.8	24.2	46.8	50.2	54.2

* 15 single pair matings per treatment level for 1st collection. Only 5 matings continue for each successive collection.

The viable offspring from the above treatments should be unaffected other than possible genetic changes transmitted by the treated males. Female offspring would receive one treated X chromosome from their sire and one untreated X chromosome from their dam while male offspring would receive only an untreated X chromosome from their dam. Therefore, sex-linked recessive lethal or visible mutations would be observed only in male offspring of the second generation.

A number of single pair matings were made within each treatment level among offspring of the first 48-hour collection given in Table 1. The number of matings, average number of offspring produced per mating, sex ratio, male adult mortality, and number of viable mutations observed in the second generation are reported in Table 2.

Table 2

Observations on second generation offspring from Tribolium males fed various levels of nitroguanidine.

Treatment levels, %	Number of mating	Number of offspring per mating	Sex ratio ♂/♀	% ♂ dead pupa-adult classification	Number of viable mutations
0	31	64.1	0.98	4.3	1
0.1	29	72.3	1.07	2.1	1
0.5	30	66.5	1.05	3.8	3
1.0	32	70.8	0.99	4.6	3
5.0	25	53.2	1.03	3.2	1

While the data in Table 2 were not analyzed for statistical significance, the reduced number of offspring for matings from the 5% treatment was fairly consistent for all matings. If this reduction were due to an increased frequency of sex-linked lethal mutations, the sex ratios reported in the fourth column of Table 2 would confirm this fact. Such is not the case. Not a single sex-linked lethal was found in any treatment group. Furthermore, all treatment levels had males and females in approximately equal frequencies. Therefore, the reduced number of offspring for the 5% treatment level must be (1) associated with genetic sampling of fertility differences among initial parents assigned to the various treatment levels (not supported by the data from 3rd, 4th, and 5th collections found in Table 1) or (2) manifestation of incompletely recessive deleterious mutations which could be acting to reduce fertility of the parents or viability of offspring.

Mortality from pupa to adult classification of males at about 2 weeks of age was equally observed for all treatment levels. None of the viable mutations observed were sex-linked. Their low frequencies suggest the possibility of either low penetrance or environmental origin.

These limited data reveal that n-methyl-n-nitroso-n-nitroguanidine fed at levels as low as 1/2% adversely affects fertility in Tribolium. However,

the mutagenic effect, if any, certainly is not of the magnitude reported for diethyl sulphate in Drosophila.

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Simplified bioassay method with adults of T. confusum Duv.

The distribution and persistence of fumigants in grain, flour and other cereal products involved measurement of trace amounts of fumigant by polarography (1, 2, 3, 7) and gas chromatography (4). To evaluate the insecticidal effectiveness and concentration-time relationships of small amounts of fumigant in air, Strand flasks and similar paraphernalia have been used in this laboratory for the past 20 years. Such test equipment require a relatively large amount of space and, because of their geometry, do not permit a close inspection of the effects on test insects during the course of their exposure to the fumigant gases.

A simplified bioassay method used by the author for the past five years for screening and validation tests is a modification of the micro fumigation chamber previously described (5). It consists of a 100-cc. all-glass syringe capped with a rubber septum. With the syringe piston removed, a hypodermic needle (22-gage) to act as an air vent is inserted through the cap center to extend about 1 inch past the bottom of the syringe. After 25 Tribolium adults are transferred into the chamber through a long-stemmed powder funnel, the syringe piston is inserted and advanced slowly to the 10-cc. mark, and the venting needle is removed. Various amounts of test gas may then be introduced through the cap by means of a second syringe. Filtered air is then drawn in through a needle to a standard volume of 100 cc. Twenty or more of such syringes may readily be placed in a constant temperature oven, or refrigerator, for incubation at a desired temperature.

For field use, septum-capped polyethylene sampling lines, placed beforehand at desired locations in grain bins, are flushed with a suction source (small pump at 200-250 cc./min., or a rubber hand bulb) for a period long enough to clear the lines of static, residual air. The insect-loaded syringes are then used to draw in 90 cc. of air from the lines, using a needle at the syringe end, or preferably by fitting a needle attachment to the sampling line.

No mortality was observed among the insects in the control (untreated) syringes during a 13-day period at 23°C. The percent mortality of the insects exposed to traces of fumigant varied with the gas concentration, period of exposure, and test temperature, among other factors. Differentiation of the dead from moribund insects is readily achieved. Furthermore, the amount of gas sorbed by the insects can be determined by gas chromatography (6).

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Use of Tribolium species to assess fungistatic and insecticidal vapors generated by wheat in hermetic storage

Investigation of the relationships between individual atmospheric gases and biocidal effectiveness of nine fumigants was undertaken. Closed (hermetic) storage conditions were selected as a starting point. Changes in the CO_2-O_2-N_2 patterns of wheats of 12.5 - 22.5% moisture content stored hermetically in glass milk bottles at 22° C. (72° F.) were monitored by gas chromatography. Effects on wheat quality and germination were also determined. Biocidal properties of the air of the stored wheats were assessed with six species of stored products insects and 10 pure cultures of molds. The test insect species included adults of T. confusum Duv. and T. castaneum (Herbst.).

Wheat of 15.0 - 22.5% moisture content developed a "silage" odor, in association with a high CO_2 and low O_2 content. It was found that the supernatant air of the bottled grain, without addition of fumigants, was insecticidal and fungistatic. This was not entirely due to the high CO_2 content of the air, as was ascertained in simulated tests, in which wheat was

absent. Insecticidal and fungistatic behavior were enhanced by and correlated with presence of new peaks in the gas chromatograms. The peaks are not yet identified, nor have we determined whether the physiology of the test molds was changed as a result of the treatment. The test molds and those naturally present in or on the wheat gradually assumed apparently normal growth patterns after they were transferred from hermetic to room air. Nitrogen levels were distinctive and showed a marked drop in the 22.5% moisture wheat.

(This note pertains to part of a collaborative investigation by B. Berck, H. A. H. Wallace, V. M. Bendelow and E. Robertson).

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An unsuccessful attempt to implicate infection as a contributing factor in the death of irradiated adult Tribolium

When rodents are exposed to X-ray doses in the low lethal or midlethal dose-range, i.e., 500-1500 R, virtually all deaths attributable to the radiation occur within the limited time period of 7 to 21 days following exposure. This and similar findings led to the concept of specific modes of death. Subsequent investigations demonstrated the critical role of damage to hemopoietic tissues, and suggested that infection plays a prominent part in the events leading to the two-week death. This suggestion was based on the following observations:

1. Bacteremia occurs in lethally irradiated mice a day or two before death. The bacteria found are of the normal enteric flora.

2. Heavy bacterial contamination of feed and/or cages increases the incidence of mortality in mice exposed to low X-ray doses.

3. Sublethally-irradiated mammals die rapidly after parenteral administration of small amounts of non-pathogenic bacteria.

4. Mortality is decreased if antibiotics are administered after irradiation.

5. Crowding increases mortality following a given dose in the midlethal range, whereas isolating irradiated rodents one to a cage significantly enhances survival.

6. If a large number of animals are exposed to an approximate LD_{50} and housed several/cage, the proportion of cages with no deaths

and the proportion with 100% dead greatly exceed predictions based on a random distribution.

If young T. confusum adults are X-irradiated, the time course of mortality of decedents is similar over a wide range of dose, from 10,000 R, which kills only a small proportion, to 75,000 R, about 5 times the dose necessary to kill almost all exposed beetles. At 30°, none of the irradiated beetles die during the first week, most deaths occur during the next two weeks, with occasional deaths during the fourth week; irradiated beetles surviving this period generally go on for many months, so that deaths after the first four weeks cannot be attributed to the radiation. Thus, it appears that there is a specific mode of death after X-irradiation of adult flour beetles. That infection might be an important contributing factor was suggested not only by analogy with the mammalian situation, but by the interesting reports of Jafri (1963, 1965) that irradiated Tribolium harboring certain latent infections were much more radiosensitive, and that irradiated adults exhibited both damage to the gut wall and tissue invasion by bacteria found in the gut. Most of these findings were based on post-mortem examination, and could have resulted from agonal invasion; accordingly we decided to test whether the survival of irradiated Tribolium might be influenced by factors which appear to affect the course of infection and death in irradiated mice.

1. Either one week before irradiation or one day after irradiation, adult beetles were transferred to flour-yeast moistened with sterile nutrient broth, or with a heavy culture of Escherichia coli or of Serratia marcescens. None of these procedures had any significant effect on either the dose-survival response or the mean survival time of the irradiated beetles.

2. One hundred T. confusum of each sex were placed in flour-yeast, and another 100 of each in flour-yeast supplemented (0.2%) with streptomycin. Each of these sets was divided into three groups, and 10 days later, each group received 0, 12, or 14 kilorads. Each group was then subdivided into two subgroups, one of which was incubated with no further handling for the standard five-week observation period, and the other was sieved and counted twice weekly for the five weeks. Surviving fractions are shown in Table 1. Neither the streptomycin nor the frequency of handling influenced survival.

Table 1

Five-week survival of irradiated adults

	Examined	0 krad	12 krad	14 krad
w/strept.	10/5 wk.	29/32	2/33	0/33
	1/5 wk.	31/32	1/32	1/32
0 strept.	10/5 wk.	32/33	1/34	0/34
	1/5 wk.	33/33	4/34	0/34

3. Adult beetles were placed in flour-yeast medium, distributed either 40/vial or 5/vial, and X-irradiated. At the end of five weeks, 18 of the 160 irradiated beetles placed 40/vial still survived, as did seven of the 80 placed 5/vial. Furthermore, the seven survivors in the groups-of-five were found in six vials, only one vial having as many as two survivors. From the per cent surviving and from the distribution of survivors, it is apparent that there is no "cage-effect" in irradiated Tribolium.

No one of the foregoing experiments constitutes strong evidence against infection contributing to the acute lethal syndrome in irradiated flour beetles. The bacterial species tested may not survive long under our conditions, or may not remain viable once taken in by the beetles; the normal enteric flora of T. confusum may not be streptomycin-sensitive; the well-known bactericidal properties of Tribolium quinones, secreted in particularly large amounts under conditions of crowding, may more than compensate for any beneficial effects of low populations per vial after irradiation. Nevertheless, taken together, they suggest that infection of the usual sort, passed from one irradiated animal to another, is probably of little or no importance in Tribolium lethality, and that further effort should be concentrated on elucidating the possible role of activation of latent or endogenous infection.

(This work was supported by a grant, GM 10208, from U.S.P.H.S.)

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Fitness modifications in populations of strains and species of flour beetles stressed with X-radiation and/or the insecticide, DDT

Frequently genetic adaptations of arthropod populations under stress from adverse environmental conditions, such as insecticides, have evolved to man's disadvantage. Radiations increase variability and induce stresses in organisms upon which selection can work. Studies were initiated to determine the effects of an insecticide and X-radiation, singly and in combination, on fitness of populations of flour beetle strains and species.

Pupae of flour beetles, Tribolium confusum Duval ("Chicago Standard") and T. castaneum Herbst ("Brazil cI" and "sooty"), were sexed and kept separately. Four weeks after eclosion the beetles were X-rayed (250 kvp, 30 ma, 0.25 mm Cu + 1.0 mm Al filtration, 0.86 mm Cu HVL and 2.5 inches between subject and target) with 0, 1, 2, and 4 kR at a dose rate of 1 kR/min. Five replicate populations consisting of 10 pairs of beetles in 20 g food previously spiked with 0, 5, 10, 20, and 50 ppm DDT were established at 32 C and 70-75 per cent relative humidity. Each two weeks for six weeks

dead beetles were counted and discarded; living beetles were placed on comparable food. Adult F_1 were weighed and the numbers of individuals were counted. Viability values (proportion of controls) were considered genetic recessives and lethality values (1 minus viability value) were considered genetic dominants. These values were used to calculate the expected lethality when populations were doubly stressed. Expected and observed lethality values were compared to determine whether or not lethality could be explained on the basis of dominant lethals.

Briefly, the findings of this experiment were:

Parental mortality was (1) not affected by X-radiation, (2) increased for "Brazil cI" at 50 ppm DDT, and (3) greater for females than males but unrelated to radiation or insecticide.

F_1 Biomass on an individual basis was (1) not altered by DDT concentrations and (2) increased for "Chicago standard" at 4 kR regardless of DDT concentration, probably due to more food because of less individuals and/or decreased competition.

F_1 Biomass on a population basis decreased with increasing X-ray or DDT. This productivity measurement showed that T. confusum was more radiation-sensitive than were T. castaneum strains. However, the T. castaneum strains were more DDT-sensitive than "Chicago standard."

Observed and expected lethality in doubly stressed populations when lethality was assumed to simulate genetic dominants. Proportions of observed and expected lethality agreed for populations of "Chicago standard" and "Brazil cI."

In "sooty" populations, expected values were consistently greater than those observed, indicating that the population performance measured as productivity (adult F_1) was better in DDT-environments after exposure to 1 or 2 kR of X-rays than when no DDT was present. Because of the economic importance of this observation in a radiation environment which might result from a nuclear war, this study will be elaborated.

In summary, differential productivity responses were found and described for species and strains of flour beetle populations which were subjected to X-radiation and/or the insecticide, DDT.

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Failure of a radiation-protection effect of dimethylsulfoxide (DMSO) on female germ cells of flour beetles

Responses of irradiated biological systems can be modified by chemical agents. DMSO was reported (Ashwood-Smith 1961, Internatl. J. Rad. Biol. 3: 41-48) to provide radioprotection in whole-body acutely X-rayed mice. Experiments were conducted to test the ability of DMSO to protect germ cells of treated female flour beetles, Tribolium castaneum Herbst: mutant "sooty," from radiation-induced damage.

Various concentrations of DMSO were applied for different time periods in different ways to "sooty" females which were then given 0 to 4 kR of X-rays (see research note above for description of X-rays). Fecundity (number of eggs/female/day), fertility (mean numbers of adult F_1/female/day), and viability (total numbers of adult F_1/total numbers of eggs) were the productivity parameters measured.

DMSO gave no radiation protection to female germ cells.

Female sterility resulted on food containing 4.6% DMSO because of unpalatability. Comparable histological observations were found for ovarioles of females which were starved or presented with 4.6% DMSO food.

DMSO might prove useful as an insecticide or insect repellent on stored products such as foods, clothing, and tobacco; however, the disagreeable garlic or oyster odor and taste may limit such economic importance.

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Effect of removing or putting males to fecundated or virgin females, respectively, on the egg laying rate during a long period of time, in Tribolium castaneum*

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* Partial and preliminary study included in Grant No. FG-Sp-137 of P.L. 480, contract with the USDA.

Fecundated females of T. castaneum lay eggs at a very high rate as compared with virgin females. Elsewhere in this Bulletin Tagarro and Rico have shown that females mated on the day of their adult emergence lay slightly fewer eggs when the male is removed during the 4-day testing period (days 7 to 11 after emergence), but the difference was not significant.

We were interested in determining the laying response during a long period when virgin females are mated or fecundated females are deprived of their males.

A long experiment was performed starting with 140 females. 70 of them, taken at random, were mated on the day of emergence and housed in individual containers, a male and a female per vial (set M). The remaining 70 were also housed in individual containers but they remained virgin (set 0). At day 17 after emergence, 35 females from set M were deprived of males (sub-set M-0) and the other 35 were kept with them (sub-set M-M). At the same time 35 females in set 0 were mated (sub-set 0-M) and the remaining 35 were kept virgin (sub-set 0-0). In any case the selection of females going to a certain sub-set was at random and the new matings were also in individual pairs.

Egg count began on day 5 after the emergence from pupa stage and thereafter successive counts were performed every 48 hours until day 17 when the sub-sets were started. From this day the eggs were counted every 24 hours in order to detect as soon as possible any produced effect. After day 23 the 48-hour count was resumed. From another experiment run in this laboratory (unpublished) it was deduced that the daily egg laying rate (both in virgin and in fecundated females) was exactly the same taking the lay every 24 or every 48 hours, dividing by two the figure obtained in the second case.

Set 0 was finished at day 31 from emergence (laying day 27) because of its clear interpretation. Set M was prolonged until day 53 and two more 48-hour counts were made at days 62 and 69 after emergence, in order to better test the difference between the two sub-sets in it.

The same strain and technical conditions as in the other report in this Bulletin (Ruano and Orozco) were used. Every time that the eggs were separated (by suction) to do a count fresh medium was added so the female could continue to lay.

Table 1 gives for successive days the egg-laying data obtained in every sub-set. The average figures in these sub-sets are on a 24-hour basis. It was possible to give means for every sub-set before day 17, because each female was identified from the beginning. The table also includes sub-set differences with three levels of significance for three kinds of comparisons:

Table 1.--Effect produced by removing the males from fecundated females (set M) or by adding males to virgin females (set 0). Means, differences between sub-sets,[1] standard errors and levels of significance[2] for 24 hours lay.

laying day	day after emergence	set M		set 0		sub-sets differences and standard errors for 3 comparisons		
		sub-set		sub-set				
		M-M	M-0	0-M	0-0	(M-M) - (M-0)	(0-M) - (0-0)	(M-M) - (0-M)
1	5	4.92	4.91	1.81	1.26	0.01 ± 0.51	0.55 ± 0.38	3.11 ± 0.54**
3	7	15.39	16.03	3.09	3.09	-0.64 ± 1.30	0.00 ± 0.64	12.30 ± 1.13**
5	9	15.00	13.94	3.00	3.03	1.06 ± 0.99	-0.03 ± 0.51	12.00 ± 0.91**
7	11	16.17	16.00	4.20	3.98	0.17 ± 0.97	0.22 ± 0.67	11.97 ± 0.91**
9	13	12.63	12.50	3.61	3.13	0.13 ± 0.86	0.48 ± 0.62	9.02 ± 0.77**
11	15	14.86	15.01	3.52	3.50	-0.15 ± 0.86	0.02 ± 0.63	11.34 ± 0.76**
13	17	17.03	16.70	4.29	4.13	0.33 ± 0.93	0.16 ± 0.64	12.74 ± 0.81**
14	18	16.78	17.21	10.22	6.16	-0.43 ± 1.25	4.06 ± 1.09**	6.56 ± 1.10**
15	19	12.78	12.06	11.40	4.19	0.72 ± 1.05	7.21 ± 0.93**	1.38 ± 1.07
16	20	14.09	11.85	14.00	4.10	2.24 ± 1.17†	9.90 ± 1.16**	0.09 ± 1.21
17	21	13.47	13.94	14.56	5.09	-0.47 ± 1.18	9.47 ± 0.84**	-1.09 ± 1.09
18	22	15.36	14.24	13.59	4.93	1.12 ± 1.26	8.66 ± 1.03**	1.77 ± 1.21
19	23	14.76	13.16	13.48	4.54	1.60 ± 0.91†	8.94 ± 1.12**	1.28 ± 1.11
21	25	15.76	15.45	13.80	5.43	0.31 ± 1.27	8.37 ± 1.09**	1.96 ± 1.19
23	27	11.43	12.24	12.46	5.08	-0.81 ± 0.91	7.38 ± 0.93**	-1.03 ± 1.00
25	29	14.65	13.35	13.13	5.51	1.30 ± 1.14	7.62 ± 0.96**	1.52 ± 0.97
27	31	13.44	13.30	13.61	4.77	0.14 ± 1.07	8.84 ± 0.85**	-0.17 ± 0.96
29	33	12.27	12.13	-	-	0.14 ± 1.06	-	-
31	35	11.58	9.97	-	-	1.61 ± 1.04	-	-
33	37	12.40	11.91	-	-	0.49 ± 1.01	-	-
35	39	14.21	12.78	-	-	1.43 ± 1.24	-	-
37	41	13.80	10.94	-	-	2.86 ± 1.14*	-	-
39	43	14.30	11.82	-	-	2.48 ± 1.02*	-	-
41	45	15.24	12.84	-	-	2.40 ± 1.25†	-	-
43	47	13.61	10.15	-	-	3.46 ± 1.51*	-	-
45	49	14.34	11.96	-	-	2.38 ± 1.11*	-	-
47	51	14.13	10.51	-	-	3.62 ± 1.25**	-	-
49	53	15.93	12.19	-	-	3.74 ± 1.15**	-	-
58	62	12.77	9.09	-	-	3.68 ± 1.22**	-	-
65	69	11.11	6.14	-	-	4.97 ± 0.92**	-	-

(2) † significant at 0.10 level
* " " 0.05 "
** " " 0.01 "

(1) Sub-set M-M: before day 17 with male; after with male
" M-0: " " " with " ; " no "
" 0-M: " " " no " ; " with "
" 0-0: " " " no " ; " no "

(M-M) - (M-0): females always with male vs. fecundated females later on deprived of their males.

(0-M) - (0-0): virgin females mated later on vs. females always virgin.

(M-M) - (0-M): females always with male vs. virgin females mated later on.

Figures 1 and 2 plot 24 hours lay for females in sets M and 0 respectively. Also in graphic 1 is the plot for sub-set 0-M in order to better compare its performance with those in set M. Parts between vertical dashed lines include daily counts; the rest are eggs laid in 48 hours but divided by 2.

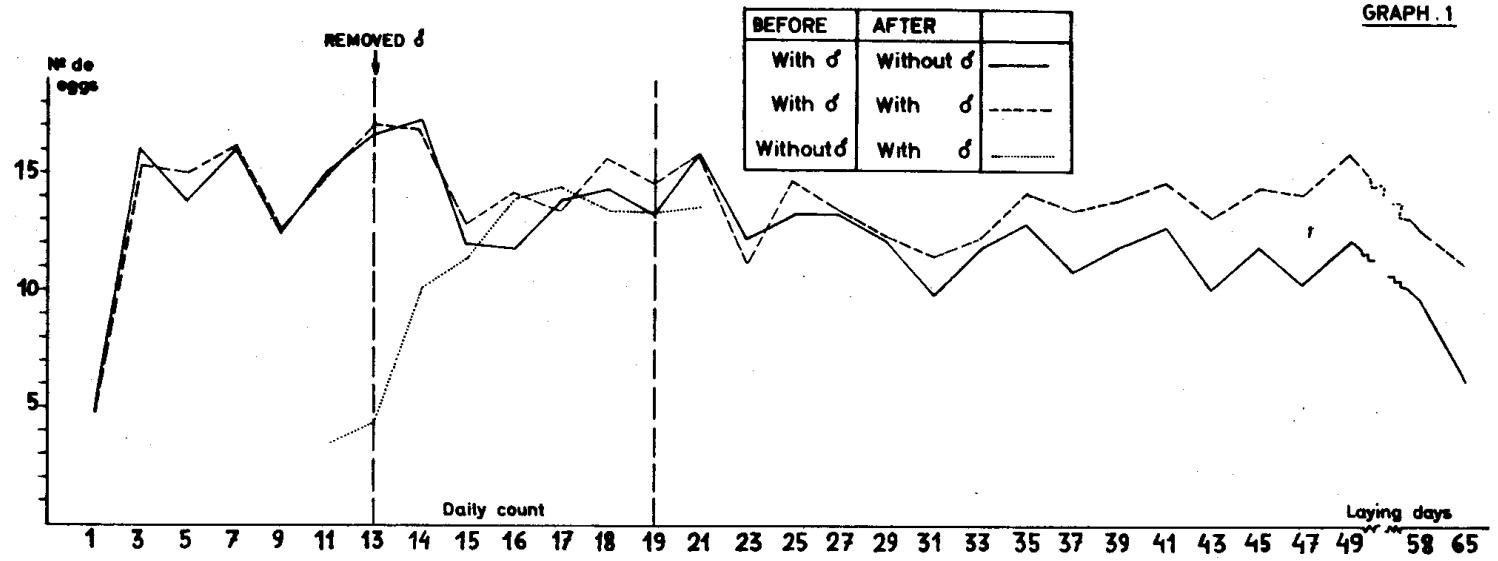
From the data in the table and the figures it is clear that when fecundated females are deprived later on of their males, the egg laying rate is maintained at very high level during a long period of time; the reduction in number is very slow. On the contrary, when virgin females are mated later on, they achieve immediately a very high egg-laying rate, as high as in females mated from the beginning.

In the case of fecundated females deprived of their males there is a tendency to have slightly smaller lay during the first weeks, in accordance with our other report in this Bulletin (Tagarro and Rico), but that tendency is not clear because some days the difference is even negative and only in two cases (days 20 and 23) significant difference at the 0.10 level is obtained. Not until 24 days after removing the male do the differences begin to be significant at the 0.05 level. After 34 days the differences are highly significant (0.01 level).

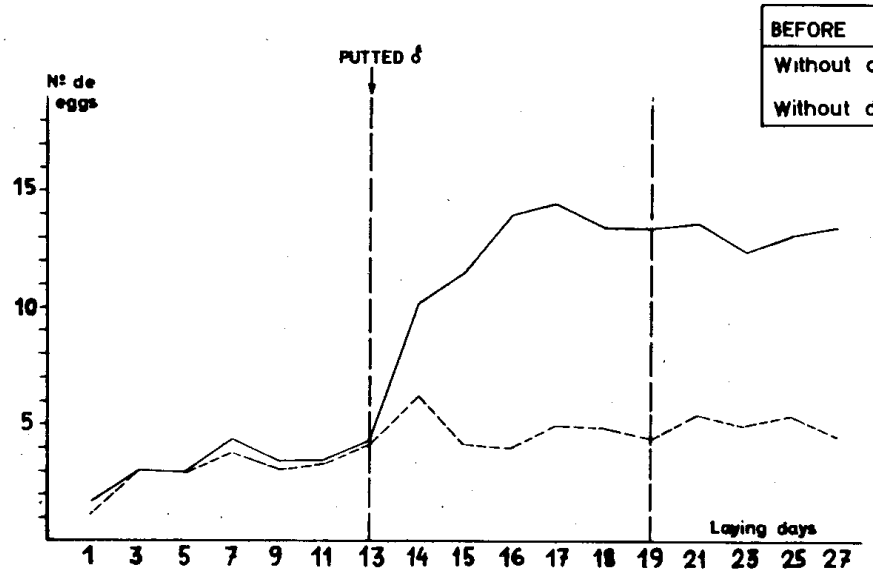
When considering virgin females mated later on it is observed that one day after mating the lay increased greatly compared with virgin females, the difference being highly significant, but still it is quite low compared with that from females fecundated from the beginning. Two days after mating there is no difference at all between them as seen in Fig. 1 and the non-significant difference in the table.

This behavior is easily interpreted if we consider that the stimulus to lay eggs at a high rate is because of fecundation. The fertility of newly mated virgins and the sterility of fecundated females subsequently deprived of a mate follow a similar pattern. We are studying now the correlation between these two phenomena.

GRAPH. 1



GRAPH. 2



EFFECT OF REMOVING OR PUTTING MALES TO FECUNDATED OR VIRGIN FEMALES, ON EGG LAYING RATE.

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*Cytological study of Tribolium confusum inbred line CFI-11a

Four to five days old male pupae of CFI-11a were fixed in Carnoy's fixative (4 chloroform : 3 ethyl alcohol : 1 acetic acid) and the testes were squashed in 1% acetocarmine.

In order to detect the presence of heterozygous paracentric inversions in this line about 1970 primary spermatocytes were examined at anaphase stage. A dicentric chromatid forming a bridge and an acentric fragment in its association were not seen in any cell. This suggested an absence of heterozygous inversions in the genome.

Two different inbred lines, CFI-11a and CFI-1, were crossed and the hybrid pupae were examined for the presence of heterozygous paracentric inversions. Cytological study of this material indicated that inbred line CFI-11a did not carry paracentric inversions in the homozygous state.

It is possible that some deficiency or some small chromosomal rearrangement may be responsible for the aberrant behavior of inbred line CFI-11a in competition experiments reported by Lerner and Ho (1961).

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*Maxillopedia, a homeotic mutant in Tribolium castaneum affecting maxillary and labial palps

A stock of fas-3 yielded 33 males and females with abnormal maxillary palpi. The expression varies from palpi with almost normal appearance except for the addition of a "claw" on the terminal segment, to one in which at least one palpus develops into a fairly well developed leg. These "leg-like" palpi have distinct tarsal claws, tarsi, and almost an entire tibia with tibial spurs. Moderate expression of max produced palpi with tarsal claws, and more segments than normal. Tibial spurs with varying degrees of expression are found. This mutant has been called maxillopedia (max), Hoy, 1965.

Upon inbreeding it was noted that some beetles had modified labial palps. As with the maxillary palpi, the more frequent expression is the addition of tarsal claws and "tibial spurs"; again, one side may be more strongly affected than the other. Occasionally, there is a development of

tarsi and a partial tibial segment by the labial palpi. All beetles having the labial palps affected also have modified maxillary palps, but beetles having leg-like maxillary palps do not, necessarily, have affected labial palps. At present little information is available about the mode of inheritance of max, but the gene appears to have pleiotropic effects (unless two separate genes or alleles are involved).

This is the third homeotic mutant affecting the appendages of the head: antennapedia (ap), an autosomal recessive in T. castaneum causes the antennae to be modified into leg-like appendages, while labiopedia (lp) a sex-linked recessive semilethal gene in T. confusum causes the labial palps to be modified into legs.

Judging from the expression of the labial palps in the max mutant, and the fact that max affects labial and maxillary palps, it is not likely that max and lp of T. confusum are homologous genes.

(My thanks to Dr. A. Sokoloff for suggestions and editorial corrections.)

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*"Abnormal abdominal sternites" (aas)--a possible phenodeviant in T. castaneum

Seventeen beetles were found with abnormal abdomens in a stock containing Mo s ims. The beetles' abdomens had several types of abnormalities; some beetles exhibited more than one type of abnormality. A stock was started with the 17 beetles (♂♂ and ♀♀) and their progeny were selected each month for approximately nine months. At the present time, this stock contains 70-75% beetles with one or more type of abnormality detectable. Figure 1 (a-e) illustrates the variety of expression to be found in this stock. Figure 1-a shows the fusion of the posterior three segments as well as a condition resembling a strong expression of sternites incomplete (sti) a very incompletely penetrant gene already discovered in T. castaneum, T. confusum and L. oryzae. Figure 1-b illustrates a more frequent expression of the gene(s) aas in which a "scarring" of the cuticle is evident, especially on the first apparent abdominal segment. The "scarring" usually extends to the anterior margin of this segment. There is frequently a difference in pigmentation of the "scar". Again, a condition resembling sti is often present in conjunction with the "scar".

Figure 1-c illustrates a third condition occasionally found in the stocks. In this case the penultimate apparent abdominal segment is incomplete; the medial area of this segment is membranous. Figure 1-d is a

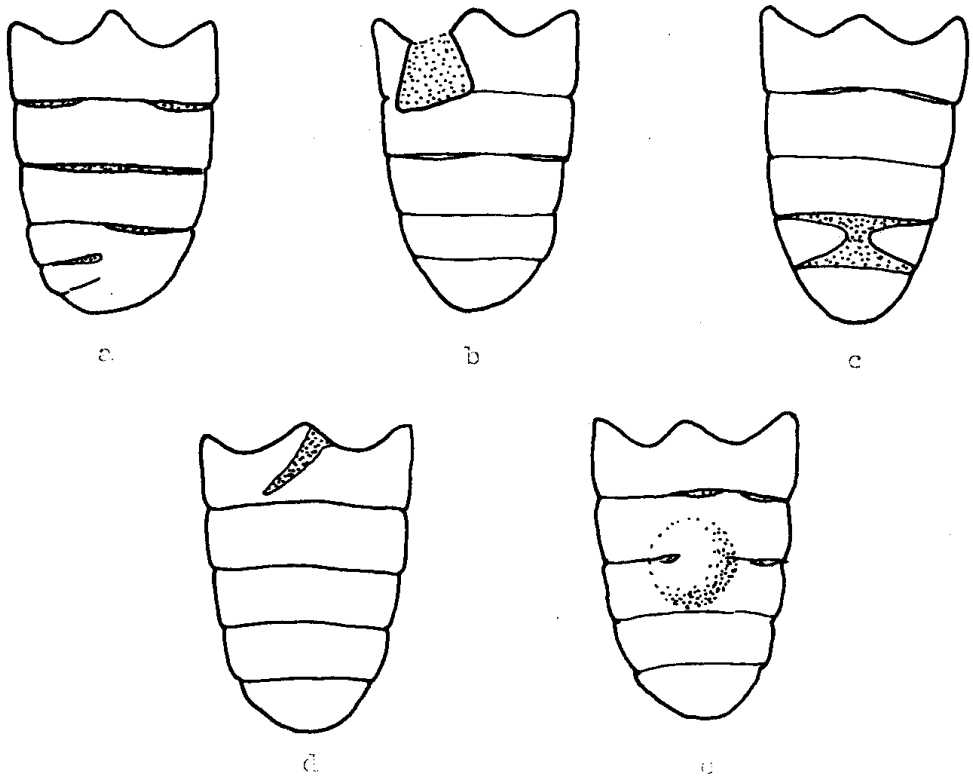

Fig. 1. Variation in expression of the gene(s) aas in T. castaneum, ventral view.

rather frequent example in our stocks. In this case the point of the first apparent abdominal segment is deformed, there being a "scarring" effect, which may or may not include a lack of pigmentation as in Figure 1-b. This area often appears to be "bulged out" in relation to the adjoining area of the sternite.

Finally, Figure 1-e has a distinct bulge in the medial area of apparent segments II and III. The bulge does not lack pigment. Again, the condition resembling sti was present in this specimen.

The presence of the condition resembling strong sti is very frequent in the stocks. On occasion the membranous areas are so large that they resemble the "pockets" of the gene pockets (poc) in T. confusum, reported elsewhere in this TIB. Besides the "sti-like" or "pockets" conditions that occurred with the types described above, individuals are found with combinations of 2, 3 or even 4 different "types" of the expression described for aas.

The question now arises as to whether the conditions comprising aas are due to a number of single genes that happened to be found all at once in the

same stock; whether the conditions are due to a single gene with incomplete penetrance and a wide range of expression; or whether we in fact have an example of a series of genes and/or modifiers contributing to the expression.

To answer these questions a series of matings were made.

1. Three ♂♂ with expression shown in Figure 1-a were mated with virgin synthetic wild type females.

2. Three males with a strong expression of the "pockets" or "sti-like" condition were bred in a similar manner.

3. Additionally, three ♂♂ with expression similar to that exhibited in Figure 1-b and d were mated with normal females. All these males were from the original "parental" group, so no additional "mixing" or "diluting" of genes had occurred through interbreeding.

The F_1 of these crosses were all normal. In the ensuing crosses of the various $F_1 \times F_1$, all F_2 progeny were normal except one cross from the expression illustrated in 1-a. About 1/4 of these F_2 had a weak condition resembling sti. Crosses were made of normal $F_2 \times F_2$ from one type of expression that failed to show up in the F_2. Crosses were also made with normal $F_2 \times$ normal F_2 and with the "sti-like" $F_2 \times$ "sti-like" F_2. These results are summarized below:

		Normal ♂	Normal ♀	Abnormal ♂	Abnormal ♀
3 N F_2 × 3 N F_2 ex "pockets"	(1)	38	31	1	3
	(2)	37	41	5	3
	(3)	41	36	10	6
Abn. F_2 × Abn. F_2 ex "fused segments"	(1)	55	71	7	6
	(2)	16	18	3	4
	(3)	58	21	10	9
N F_2 × N F_2 ex "fused segments"	(1)	44	48	4	6
	(2)	45	58	8	7
	(3)	59	68	5	3

The following matings were subsequently made:

Type: "fused segments"

I	3 pr. each, 3 creamers	abnormal $F_3 \times F_3$
II	" " " " "	normal $F_3 \times F_3$
III	" " " " "	N ♂♂ F_3 × ABN F_3 ♀♀
IV	" " " " "	ABN ♂♂ F_3 × N ♀♀ F_3

Type: "pockets"

V	3 pr. each, 3 creamers	ABN ♂♂ F_3 × ABN ♀♀ F_3
VI	" " " " "	N ♂♂ F_3 × N ♀♀ F_3

Progeny of these crosses were as follows:

	Normal	Abnormal
I	114	13
	92	14
	134	16
II	133	7
	112	6
	68	10
III	115	3
	27	9
	83	8
IV	148	27
	74	9
	156	18
V	124	20
	141	4
	134	7
VI	221	20
	129	5

The progeny in the F_2, F_3 and F_4 were scored for "type" of abnormality visible. Overwhelming predominance of the "sti-like" expression was evident in the F_2, F_3 and F_4. A few isolated cases of other types of aas were found in the F_3 and F_4. Yet, we have stocks in which the expression of all five "types" is frequent and strong. To date, however, no pure stocks have been established. Our best stocks exhibit 70-75% expression of one or more type of aas, as illustrated in Figure 1, a-e. These results indicate that the condition is heritable, but either penetrance is poor, or, since outcrosses reduce the frequence of aas, that this is another phenodeviant. No attempts have been made to rear these beetles at higher temperatures which are known to increase the penetrance of certain genes.