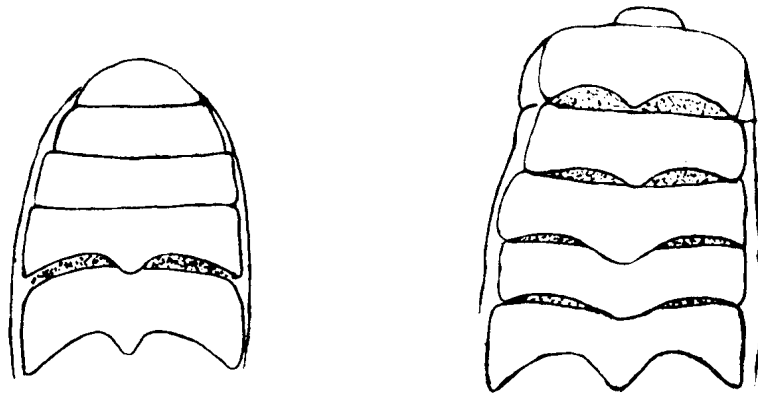


Alan C. Bartlett, Geneticist
Boll Weevil Research Laboratory
USDA ARS Ent. Res. Div.
Box 1518
State College, Missi 39762

TRIBOLIUM

Information Bulletin

7



March 1964

MATERIAL CONTRIBUTED BY WORKERS ON TRIBOLIUM
AND OTHER COLEOPTERA

DEPARTMENT OF GENETICS, UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

TRIBOLIUM INFORMATION BULLETIN

Number 7

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FOREWORD

As TIB goes to press, Triboliumists may be pleased to know that a Tribolium Stock Center is being established at the Department of Genetics, University of California, Berkeley, California. An announcement that such a center is in operation is included elsewhere in this issue of TIB. It is hoped that this Center will be of use to Tribolium investigators as a ready source of material they may need, and as a repository for new mutants. In connection with the operation of the Stock Center it has also been possible to reissue TIB 2 and 3. It was hoped that these numbers, which have long been out of print, would be rerun in the Department of Genetics, but the secretaries were overworked. Finally, however, funds which were made available by the National Science Foundation enabled us to reissue those exhausted numbers. To those who requested TIB 2 and 3 and waited so patiently, a heartfelt thanks from the writer.

The issue of this number of TIB was greatly facilitated by the cooperation of the contributors, and by the patient assistance of Miss Jean Jamison, who typed the original, and of Mrs. Louise Flynn Overton, who helped to proofread it.

The publication of TIB-7 was made possible by Public Health Service Grant RG-08942.

A. SOKOLOFF

Berkeley, California
March, 1964

EDITOR'S RANDOM NOTES

Dr. Harvey A. Bender, University of Notre Dame, writes: ". . . there is available at our computing center (free of charge) a program (Fortran IV) for utilization in a simple genetics laboratory experiment (selection against a recessive factor) which may be of use and value to some of your readers. I developed this in conjunction with my elementary course this past semester and it ran rather well. The students very much enjoyed the computer experience. The experiment was run with Drosophila but it could be adapted for Tribolium studies."

* * * *

The section on mutant and wild type stocks from Berkeley has been revised completely for two reasons: (1) a fairly large number of stocks was made available to us chiefly by the Pest Infestation Laboratory, Slough, England, which made it necessary to renumber our stocks; (2) the list was brought up to date to make it available for distribution to those who may be interested in obtaining stocks from the Tribolium Stock Center. It is hoped that those who write for stocks will use the identifying numbers included in this issue of TIB.

* * * *

As it should become evident by glancing at the New Mutant section in this issue, the number of spontaneously occurring mutants has become too great for one laboratory to handle. For the sake of advancing knowledge of the basic genetics of Tribolium species, we are willing to supply some of this material to those individuals who are having difficulty in finding mutants, or to teachers of advanced courses in Genetics who may be interested in giving their students laboratory exercises with more than one organism. It is true that some of the unmapped mutants available are somewhat difficult to work with because of poor penetrance or variable expression. But this in itself should be of interest to the student, in part because the material is new, and in part because the student will realize that not all characters (such as those perhaps deliberately chosen in Drosophila for genetics exercises), have uniform expression. Thus, the students will have a chance to use special methods for analysis of linkage data, and the Triboliumist will gain from their work, since linkage relationships will be established in an accelerated rate.

The material will be available with no restrictions other than that the linkage studies be performed within a reasonable period of time, say a semester or two, and that linkage (or lack of it) information be supplied to the writer before a manuscript describing such linkage relationships is

submitted for publication to some journal. See announcement regarding distribution of these mutants elsewhere in TIB-7.

* * * *

I am frankly puzzled by the fact that some investigators who have found a number of mutants in *Tribolium* fail to make them available, once they are reported officially to other investigators. It seems to me that the usefulness of *Tribolium* as a tool of research has been established beyond doubt by ecologists. Those who are using it in genetic research were handicapped in the beginning because of the scarcity of mutants. But the fact that in the last six years the number of mutants in *Tribolium* has increased by about 20- to 40-fold would seem to indicate that holding on to one's mutants for three or four years beyond the time of their discovery without making them available to others (when the reverse is not true) is an unreasonable practice. Perhaps the fact that some of us are making unworked mutants available to others may influence others to release theirs.

It is also hoped that at a future meeting of *Tribolium* biologists these matters will be discussed.

* * * *

The front cover drawings represent two mutants having their abdominal segments affected. That on the left is the "pointed abdominal segments" (pas) in *T. confusum*. The pas mutant is a sex-linked recessive having lethal effects. The few surviving males are sterile. It is interesting to note that pas affects all the abdominal segments, each becoming pointed like the first apparent abdominal segment. The one on the right is the ppas (partially pointed abdominal segments) autosomal recessive mutant in *T. castaneum*. In this mutant only the apparent second abdominal segment becomes pointed like the apparent first abdominal segment. The stippled areas in both mutants are unsclerotized.

Sokoloff, A. Establishment of a Tribolium Stock Center.

A grant from NSF will make it possible to operate a center for the preservation of mutants in Tribolium and related forms at the Department of Genetics, University of California, Berkeley, California. The mutants, as well as information on their culture, will be distributed free of charge to those interested in using them in laboratory exercises in genetics. Investigators finding useful mutants in tenebrionids (or in beetles of other beetle families easily maintained in the laboratory) are urged to forward descriptions of their mutants to the Editor, Tribolium Information Bulletin, and to send samples to the writer for inclusion in the stock center as soon as the mutants are ready to be released unconditionally. Address inquiries regarding available Tribolium stocks to the writer.

Sokoloff, A. Availability of Early Issues of Tribolium Information Bulletin.

A grant from the National Science Foundation has made it possible to reissue TIB 1-3. They are being mailed to those who have requested them simultaneously with TIB-7. If for some reason your name has been overlooked or you have a graduate student who may be interested in these early issues, we will be glad to supply you or him with a copy.

Sokoloff, A. Request for Permission to Quote from TIB-7.

It has been noted that material included in the section on new mutants has not been asterisked in previous issues of TIB. The editor would appreciate it if those who have included descriptions of mutants in the past would send him authorizations to quote from this section (to be included in TIB-8). In the future it is hoped that describers of new mutants will add "reference authorized" at the end of their descriptions. This will obviate a lot of unnecessary correspondence in the future.

Sokoloff, A. Availability of New Mutants in Tribolium for Linkage Studies.

The number of spontaneous mutants in Tribolium found at the Berkeley, California laboratory has grown to the extent that it would keep at least a couple of technicians busy making the necessary crosses to establish linkage, and perhaps one or two persons analyzing the data, which would interfere with other work currently in progress.

In the interest of advancing our knowledge of the basic genetics of Tribolium, we are ready to release some of these mutants, together with tester stocks to establish linkage relationships to geneticists who may be having trouble in finding mutants, or to teachers who may be interested in trying Tribolium in their genetics courses.

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It is true that some of the material available may not be the best for linkage tests because of poor penetrance, variable expression or lowered viability, but it would seem that the student should be given this type of material as well as mutants which give clean-cut results. The writer will be glad to supply whatever information he has available on the behavior of these mutants.

The mutants will be released unconditionally, with freedom of publication of any results the investigator may obtain. The only stipulation is that the writer be informed within a reasonable length of time (say, a semester or two), of the results (either in analyzed or raw form), and if possible in advance of submitting the manuscript for publication.

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NEW MUTANTS

REPORT OF A. C. BARTLETT

Anthonomus grandis

yellow (y). Bartlett, 1963. Autosomal recessive, spontaneous in A & M stock. Complete penetrance with expressivity changing as weevils age. Yellow is characterized by a lightening of the normal eye color of the boll weevil. The trait is detectable in late pupae. Good viability and fertility. No other mutants available for linkage tests.

*REPORT OF HAROLD EDDLEMAN

Cordovan (b^{cd}). A preliminary report of this mutant (TIB 5, page 13), listed this mutant as cordovan (cd). However, the mutant appears to be an allele of black; b/+ and b^{cd}/b^{cd} yield phenotypes of comparable color; b/b^{cd} individuals are darker than b^{cd}/b^{cd} individuals but are lighter in color than homozygous black (b/b) individuals. The cross: b^{cd}/b : b^{cd}/b^{cd} gave 125 b/b^{cd} and 131 b^{cd}/b^{cd} progeny. The cross: b^{cd}/b : b^{cd}/b gave 23 b^{cd}/b^{cd} : 26 b/b : b/b^{cd}. No wild types were observed in either cross.

*REPORT OF A. E. BELL

1. black (b^D). Dewees. Spontaneous, found in a sample collected from a food storage sack at Southern Illinois University, Carbondale, Illinois. Autosomal semidominant, the heterozygote being a distinguishable bronze color and the homozygote being black. Allelic to McGill black (b).
2. bowleg (bl). Reynolds. Spontaneous autosomal recessive found in GPL population. The expression is variable with about 70-75% penetrance. The possibility of modifying genes affecting the expression is suggested. The legs are deformed with the tibia appearing twisted or bowed. The last pair of legs are usually affected but occasionally other pairs of legs may be affected. Viability is fair to good. Linkage tests are being made.
3. dented (d). Ganguly. Autosomal recessive found in an X-rayed population. Uniform expressivity with about 60% penetrance possibly being due to modifying genes. Characterized by a hemispherical depression on the mid-ventral line of the metasternum. Similar to Eddleman's dent (dt) reported in TIB-5, but tests for allelism have not been made. Fair viability, but linkage relationships have not been established.

4. light ocular diaphragm (lod^D). Dewees. Spontaneous in ruby eye beetles (see below) from same population as b^D. Autosomal recessive of good penetrance and fair expressivity. The ocular diaphragm, normally black, appears reddish-yellow when viewed through the ommatidia of ruby eye mutants. When lod^D is present with pearl, the whole eye takes on pearl color. Allelic to light ocular diaphragm (lod) mutation described by Sokoloff (TIB-5, p. 15).
5. peach (ph). Dewees. Autosomal recessive, found in same population as b^D. Good penetrance and expressivity. Bicolored eye with center facets having a reddish-pink color. Not allelic with available autosomal eye mutants - p, c, rb, w, m, and i. No linkage data available.
6. rose (rs). Reynolds. Spontaneous sex-linked recessive eye color mutation. Phenotype resembles red (r) as pupa and young imago. Phenotype in old adults darken toward the wild type. Complete penetrance, good expressivity, and excellent viability. Located about 15 recombination units to the left of pygmy (py).
7. ruby (rb). Dewees. Autosomal recessive found in same population as b^D. Good penetrance and expressivity. Bicolored eye slightly lighter than chestnut. Darkens some with age, but can easily be distinguished from wild type. Not allelic with available autosomal eye mutants - p, c, w, m, i, and ph. No linkage data available. (For further information on b^D, lod^D, ph, and rb, see note by Dewees in Research Note section.)
8. urogumphiless (u). Roark. Spontaneous autosomal recessive mutation found in Capetown inbred at 7th generation of inbreeding. Observed in pupal stage with one or both of terminal urogumphii missing. Also, genital lobes may be deleted. Variable expression and reduced penetrance (65%) more than likely being due to modifying genes. Linkage relationship not established and viability is poor.

*REPORT OF P. S. DAWSON AND A. SOKOLOFF.

Latheticus oryzae

1. brown body (bwb). Sokoloff, 1963. (Formerly reported by C. E. Dyte as "dark form." See TIB-6, p. 19). Spontaneous in wild strain of unknown origin, maintained at Pest Infestation Laboratory since before 1947. Autosomal recessive of good expressivity but a 30-40 per cent reduction in viability. The color in fully aged, imagoes which in normal compares with zinc orange, ochraceous orange or ochraceous buff in Ridgway's (1912) Color Standards, is modified by bwb to a color comparing with yellow ocher, ochraceous-tawny, buckthorn brown, russet and Mikado brown in the same reference. (The variation in identification probably being due to the fact that a structural color is being compared with a surface color).

bwb is located in the p (II) linkage group but so far away from p that it gives results resembling independent segregation. But it is about 36 units away from cas (q.v) which is linked with p.

2. creased abdominal sternites (cas). Sokoloff, 1963. Spontaneous in the course of determining whether bwb and p are linked. Autosomal recessive of good viability, incomplete penetrance and variable expressivity. Resembles mutant of same name in T. castaneum and T. confusum in the possession of diagonal grooves running from the lateral edge of the sternite somewhat diagonally toward the mid-point of the posterior edge of the sternite. The grooves may be present at least in apparent abdominal segments 1-3. cas is linked with p the recombination fraction being 35.85 ± 2.93 units.
3. red-1 (r-1). Sokoloff, 1963. Spontaneous in the F_1 of bwb × P₁L wild type ♀ crosses. (Three out of the eight females producing F_1 were +/r.) Allelic with the red described by Sokoloff, 1960. It may be pointed out that the first red was found in a wild type stock derived in 1955 from a strain kept in T. Park's lab. He obtained his sample from L. M. Roth, at that time with the Pioneering Research Lab, Army Quartermaster Corps, Natick, Mass., who in turn had received a sample from P₁L (about 1953?). When the writer received p and + he found the latter contaminated with p. By appropriate tests he finally established a pure stock of +/+, and these he used to determine the inheritance of p. The r gene was found in the F_1 of +♀ × p♂ matings. The wild type strain has since failed to produce r males. Hence, it is believed that, despite the obvious relationship of these 2 wild strains (one being derived from the other) the two reds must be independent occurrences. It would be of interest to determine the frequency of r in the P₁L wild type stock.

T. anaphe

1. sternites incomplete (sti). Sokoloff, 1964. Spontaneous in stock maintained at Pest Infestation Laboratory, Slough, Bucks, England. Autosomal recessive of good penetrance but variable expressivity, resembling the sti mutant in Tribolium castaneum (q.v).

T. castaneum

1. antennae and tarsi fused (atf). Sokoloff, 1961. Spontaneous. Autosomal recessive of variable expression and incomplete penetrance found in the course of determining linkage relationships between aa and c^S. There is a fusion of the funicular segments (not always the same ones) and a fusion of the tarsi. But both organs must be examined for fusions, for absence of fusions on the antennae does not automatically make the beetle normal. In general, however, when the antennae are affected, so are the tarsi. It is not known whether the expression is modified with a decrease in temperature. (The present experiments were carried out at 32°C.)

2. ballooned (bal). Dawson, 1963. Spontaneous. Autosomal recessive. In the pupa the elytra may lie flat over the legs resembling the normal condition, or they (along with the membranous wings which are closely applied) are lifted gradually away from the abdominal sternites exposing the tarsi and tibiae of the hind legs. Found in a full and half-sib mating design using synthetic stock. Not allelic with:
- pointed elytra
 - split
 - aa
 - short

In the extremely expressed adult the elytra are widely split beginning at the scutellum. The proximal end of the split is about as wide as the widest part of the scutellum. The distal parts of the elytra diverge by about as much as the body width. The elytra appear shorter, by at least the equivalent of one abdominal segment, and they appear narrower than normal. The membranous wings exhibit a huge blister in teneral adults. In older imagoes the fluid is withdrawn into the body, and the tips of the membranous wings remain extended (and they curl upward) beyond the tips of the elytra. Information on viability and degree of penetrance of the gene is being collected.

3. bent tibia (bt). Dawson, 1961. Origin obscure, probably CS synthetic stock. Possibly a phenodeviant - present at low frequency in CS synthetic strain. Matings of bt × wild type give a small proportion of bt progeny; thus overlaps wild type. Mass matings of bt × bt give bt : + ratios of 1:1 to 2:1. Viability reduced. Many fail to shed pupal skin from deformed legs. Bend usually occurs just below point of attachment of tibia to femur. Femur often curved and/or shortened. Tarsi not affected. Often accompanied by an elbow in the antennae. Legs often lost at point of bend.
4. bent tibia (btt). Sokoloff, 1963. Spontaneous autosomal recessive of poor penetrance and variable expression. In extreme expression the tibia of the hind legs has a double bend located approximately one-seventh of the distance between the femur-tibia and tibia-tarsus joints (closer to the former) resulting in a hind leg apparently much shorter than normal, but over all the whole tibia is probably there. Detectable in the pupa, and probably also in the larva, but this stage has not been checked.
5. black (b^S-1). Sokoloff, 1964. Autosomal semidominant. Found as a single heterozygous female among beetles shipped to me by Howard Erdman from the G. E. laboratories, Richland, Washington. Personal communication from Erdman indicates he has not kept any mutants; hence, this must be a recurrence of black. The stock is being maintained, since it has a different (Brazil) background from that in the Chicago and McGill black strains, as well as the black reported by Sokoloff in 1963.

6. bowed femur (bf). Sokoloff, 1963. Spontaneous in linkage studies between antennapedia and squint. Autosomal recessive of variable expression. The femur on the third legs is curved to a variable degree, the curvature of the legs in a semiflexed position paralleling the curvature of the abdomen. Usually midway between the joints there appears a line running from the medial surface of the femur to a point about half way to the lateral edge of the femur. (Shadowing of the femur helps to identify this line.) Sometimes this line appears closer to the trochanter, but still on the femur. Linkage relationships have not been worked out.
7. creased abdominal sternites (cas). Sokoloff, 1963. Autosomal recessive of good viability, incomplete penetrance, variable expressivity. Characterized by the possession of a groove which runs from the lateral edge of the sternite diagonally toward the midpoint of the posterior edge of the sternite. In extreme expression all the segments possess such a groove. Not linked with genes serving as markers for chromosomes IV or VIII. (By analogy with results for T. confusum and L. oryzae, may be linked with pearl.)
8. curly elytra (cye). Sokoloff, 1964. Spontaneous in $F_1 \times F_1$ matings of ca +/+ ble to obtain a double homozygous stock. In the adult the elytra are only about two-thirds as long as the normal elytra. They may appear curved upward, split-like, or she-like. In the pupa a certain proportion of the mutants will have curly elytra, with the tips separated from and exposing the membranous wings which remain in their normal condition, i.e., closely applied to the ventral part of the body of the pupa. Not allelic with aa, she or pe. No linkage data available.
9. elongated juvenile urogomphi (eju). Sokoloff, 1962. Spontaneous in the Berkeley synthetic population marked with sooty. Autosomal recessive of variable expression. The appearance of the "urogomphi" is more blunt, and they may appear fleshy rather than sclerotized. The location seems to be the same as that of ju, namely, next to the anal opening. Not allelic with ju or rju.
10. disjoined (dj). Dawson, 1962. Found in suspended selection line. Autosomal recessive. Not allelic with split, short, rsp. No significant reduction in viability. The tips of the elytra are divergent (resembling the dve condition in T. castaneum) with the split beginning sometimes at, but mostly one-third to one-fourth of the elytral length behind, the scutellum and gradually tapering towards the sides, the posterior edge of the elytron producing a flattened sigmoid curve. The elytra appear shorter, by almost the length of the terminal abdominal segment. Identifiable in the pupa by the exposure of the tarsus of the hind legs which in normal pupae do not extend beyond the tarsal tips.

11. elytra and tarsi affected (eta). Sokoloff, 1963. Spontaneous autosomal recessive found in some crosses of fas^D and mc. Whether two separate, closely linked genes are involved or whether the gene has pleiotropic effects is not known. The expression involves a divergence of the tips of the elytra, often all the way to the scutellum. The tarsi, particularly those of the posterior legs, are fused, resulting in a hind tarsus with only three segments. Fusion may be partial and exhibited only by one of the pair of hind legs. In a fairly large number of tarsi-affected beetles the elytra appeared normal.
12. glass (gl). Sokoloff, 1963. Spontaneous in crosses involving red and a red modifier. Autosomal recessive of good penetrance and expressivity but, lethal to semilethal in various crosses. The eye appears like squint, but the ocular foramen is not reduced in size. Ommatidial cells present, but the corneal lenses are completely absent. The ommatidia acquire the pigment they are supposed to, but the whole eye is smooth and shiny. Recognizable in middle-aged pupae by a peculiar line appearing parallel with the gena.
13. incomplete metathoracic projections (imp). Sokoloff, 1963. Spontaneous while attempting to map the position of the Mf gene. Autosomal recessive of variable expression but good penetrance. The posterior processes of the metathorax (which normally form an inverted V where the anterior projection of the apparent first segment of the abdomen fits) are missing or displaced laterally. In addition, the medial groove is deflected to one side, giving the metathorax a humped appearance, somewhat resembling the ventral hump in antennapedia.
14. reduced juvenile urogomphi (rju). Sokoloff, 1963. Spontaneous, in crosses attempting to establish heritability of sta (spikes on trochanters and antennae, q. v.). Autosomal, essentially recessive. The spine-like growths which appear next to the anal opening are much smaller than the cerci-like growths in ju. Not identifiable in the larva or pupa. Tests of allelism between rju and ju indicate the two are not allelic. Linkage tests are on the way.
15. miniature appendaged-1 (ma-1). Sokoloff, 1963. Found among the progeny of females derived from the Berkeley synthetic stock marked with sooty in the homo- and hetero-specific mating experiment. Two males were produced in different broods of one of several coexisting females, about two weeks apart. Males failed to survive to reproduction, but the appearance of these beetles leaves little doubt that they represent a recurrence of ma (Sokoloff, 1959).

16. partially pointed abdominal sternites (ppas). Sokoloff, 1963. Spontaneous in crosses involving curved appendages (now sa, short antenna) and blistered elytra, and about the same time in an unrelated population experiment involving mc. (Possibility of contamination is excluded.) Autosomal recessive of complete penetrance but somewhat variable expression, and possibly reduction in viability, judging from the high mortality of ppas beetles in various initial crosses. The second apparent abdominal segment has a median sternal projection (almost as pronounced as the median sternal projection of the first apparent abdominal segment) which projects anteriorly, the tip lying just anterior to the posterior margin of the first apparent sternite. (See sketch.) The anterior margin of the second sternite fails to meet the posterior margin of the first, and a broad unsclerotized groove may be visible. The variation in expression lies in the size of the medial sternal projection and the extent of intersegmental surface failing to sclerotize.
17. pokey (pok). Dawson, 1962. Sex-linked recessive semilethal. Elytra fail to cover entire dorsal surface of abdomen, leaving one-fifth to one-third of abdomen exposed. Elytra may diverge to varying degrees. Antennae sometimes fused and legs often appear shorter and thicker than normal. May be identified in pupal stage. Mutant individuals extremely delayed in development. Linkage data given elsewhere in this bulletin.
18. prothoraxless (pt1^D). Dawson, 1963. Identical in phenotypic expression to mutant described by Lasley and Sokoloff, 1960, TIB3:22. Found in mating of original sa-2 ♀ × wild type ♂ (synthetic stock) as a single female. Viability quite reduced. Probably allelic, but would be difficult to prove except by linkage studies since both mutants have variable expression and incomplete penetrance.
19. raised split (rsp). Dawson, 1962. Found in suspended selection line. Autosomal recessive. Not allelic to split, short, dj, warped elytra; some reduction in viability. The elytra are divergent, exposing considerable unsclerotized portion of the abdominal tergites behind the scutellum. In most cases the elytra diverge, the posterior tips of the elytron ending one on each side of the last abdominal segment, the latter extending well beyond both elytra. In a few cases the elytra are widely separated but do not diverge at the tips. The membranous wings also appear shorter, and the tips fail to fold under. Elytra often appear to be raised slightly from the abdomen (hence the name).

20. red (r^D). Dawson, 1963. Spontaneous in a competition experiment. Allelic to red (r). Differs from previously described alleles of red in that expression appears to be sex-influenced. In males, easily distinguished from wild type - light red though not as light as other alleles. In females very dark, difficult to distinguish from + except in pupae and young imagoes.
21. Short antenna-3 (Sa-3). Sokoloff, 1961. Found in a irradiated population of Be s/++ and originally designated Cua in our laboratory. Autosomal dominant with recessive lethal effects. It appears similar to Sa, Sa-1 and Sa-2 in the expression of the antenna, becoming curved as a result of fusion of funicular segments 2-8 in strongly expressed beetles. It resembles Sa in that the tibia is affected but seldom, becoming short and thick but not gnarled like in the Sa-1 or Sa-2 alleles.
22. Short antenna-4 (Sa-4). Sokoloff, 1963. Found as a single female in a stock heterozygous for p and pg. Autosomal dominant with recessive lethal effects. It resembles Sa, Sa-1 and Sa-2 in the expression of the antenna, with a fusion of segments 2-8 in strongly expressed beetles. It resembles Sa in that the legs are seldom affected.
23. short antenna (sa). Sokoloff, 1960. Formerly curved appendages (ca). This mutant has proved to be allelic with the Sa, Sa-1 and Sa-2 alleles; hence, it is being renamed. Re-examination of its mode of inheritance reveals that this is not as recessive as previously reported, but overlaps in dominance with wild type; hence, it should be considered a semidominant. A fair proportion of the heterozygotes will appear normal in regard to the legs, but adjacent segments 3-4, 4-5, 5-6, and on up to 7-8 of at least one antenna may appear fused. The fusion sometimes is very difficult to see, since the segments are normal but the annuli fail to separate completely. For testing allelism at the sa locus, there are now two other sa alleles, sa-1 and sa-2 (q. v.).
24. short antenna-1 (sa-1). Sokoloff, 1961. (Designated originally IW in our laboratory to avoid confusing it with ca--see previous description.) Like curved appendages, this mutant resembles the expression of Sa, Sa-1 and Sa-2 in the antennal phenotype, but it differs from these alleles in that the femurs become short and thick, and sometimes the tibiae of all legs appear variously curved or twisted. Should be considered as a semidominant, since a larger proportion of the F₁ appear with feeble fusions of the antenna than when ca is mated with wild type. sa-1 is, like ca, a mutant of good viability, but it has variable expression both in regard to the antennae and the legs.

25. short antenna-2 (sa-2). Dawson, 1963. Found in linkage tests between p, s and ap^D as a single female. See description in Dawson and Sokoloff, 1964, American Naturalist.
26. serrate (ser). Dawson, 1963. Found in a reverse selection line as a single male. Sex-linked recessive. Preliminary data indicate not allelic to pd. Distinguished by fusion of antennal segments--much variation in affected segments, those most often affected are 3-4, 5-6, 8-9 and 10-11. Often all fusions are on one side of the antenna giving this appendage a serrated appearance. Usually fusion is asymmetrical, occasionally one antenna not affected.
27. spikes on trochanters and antennae (sta). Spontaneous. Autosomal recessive with variable expression, incomplete penetrance and lethal in effect. In extreme manifestation all the trochanters have peculiar (unsegmented) growths directed cephalad or ventrally. The pedicel also has a similar growth. These beetles have difficulty in shedding the pupal skin and die in it. In less extreme cases the "spikes" are spine-like and confined to the antennae or to the trochanters only. No linkage information available.
28. sternites incomplete (sti). Sokoloff, 1963. Autosomal recessive of good viability and variable expressivity. In extreme expression the abdominal sternites fail to overlap, and a window-like unsclerotized area extending between the pleurites and adjacent sternites forms in the shape of a long and narrow slit. In less extreme cases only small unconnected unsclerotized areas develop between successive sternites. Appears to be located in linkage group IV, but position on this chromosome has not been determined.
29. thumbed (thu). Dawson, 1963. Found in a CF inbred line. Autosomal recessive. Not allelic with split, short, dj. A mutant with elytra shorter almost by one abdominal segment. Expression varies, but generally the posterior edges of the elytra meet at the mid-line, and yet the posterior part of the elytra appears raised off the abdomen, giving these structures a vaulted appearance, on the whole as if the wrong size elytra had been put on a much larger beetle. From the antero-lateral angles of the elytra, to a point about one-fifth to one-sixth of the elytral length behind the scutellum, is noticed a crease as if the elytra had been pushed down at this point. In some beetles there is a weak split at the tips of the elytra. In most cases the membranous wings also appear shorter than normal, the tips failing to fold under.

T. confusum

1. bent tibia (btt). Sokoloff, 1962. Spontaneous. Found in matings designed to determine the map position of lethal-1 (l_1). The proximal end of the hind tibia has a double sharp angle in extremely expressed beetles resulting in apparently shorter legs. More weakly expressed beetles have a tibia which is gently curved. Autosomal recessive of poor penetrance and variable expressivity.
2. creased abdominal sternites (cas). Sokoloff, 1963. Autosomal recessive of good viability, incomplete penetrance, variable expressivity. Discovered in crosses attempting to establish allelism between pearl (p) and dirty pearl eye (dpe). Mutant has a groove on the lateral fourth of the abdominal sternite, often the groove being rather shallow and hidden by the hind legs. The groove runs diagonally from (almost) the lateral edge of the sternite (near the pleurite) towards, but never reaching, the midpoint of the posterior edge of the sternite. In extreme expression all of the abdominal sternites show such a groove, although that of the apparent first and second abdominal segments is much longer, the remaining three sternites showing only a short groove. More often the groove is restricted to the apparent first abdominal segment, and may show only on one side. Reflection of the light on the sternite helps in identifying cas in poorly expressed beetles. Examination of our stocks for cas reveals that most of them, including the wild type stocks have cas. cas is linked with pearl, with an estimate of recombination of about 37.62 ± 4.82 per cent.
3. dirty pearl eye (dpe). Sokoloff, 1963. Spontaneous in experiments determining the genetic load in inbred strains. Autosomal recessive of good penetrance but variable expressivity (a function of age). In late pupae and teneral adults the eye appears pearl-like, but the ommatidia over the ocular foramen appear "dirty." On aging, the imago's eye becomes chestnut-like but resembling rus (q. v.). Not allelic with pearl, rus or ruby (q. v.). Recent linkage tests place dpe in the second linkage group, about 5 units away from pearl.
4. elongated elytra (ele). Sokoloff, 1963. Spontaneous. Found in experiments attempting to establish the position of rae, with respect to es and lp. A pair mating of two normal sibs produced a number of beetles with elytra extending the equivalent of a whole abdominal segment beyond the tip of the abdomen (in extreme expression of the gene). Autosomal recessive of variable expressivity. It may be confused with normal in mildly expressed condition. Viability of strongly expressed imagoes appears to be lowered. No linkage information.

5. fused antennal segments-2 (fas-2). Spontaneous. Autosomal recessive of good expressivity and good viability. The mutant can be easily recognized even with low (10 x) magnification by the shorter antennae resulting from fusion of segments 3-4 and 5-6. The basal segments and the 5 club segments appear not to be affected.
6. incomplete metathoracic projections (imp). Sokoloff and Hoy, 1964. Spontaneous in a stock bearing p and possibly umb, dent and ems, as a single male. Like in T. castaneum the posterior processes of the metathorax (which normally produces an inverted V where the anterior projection of the apparent first segment of the abdomen fits) are missing. In addition, the medial metathoracic groove may be deflected, usually to the right.
7. light eyespot (es^{lt}). Sokoloff, 1963. Spontaneous. Discovered in linkage tests between eyespot and labiopedia. In eyespot the central facets appear blackish with only a small reddish spot (which moves if the beetle is rotated) identifiable under a strong beam of light. In es^{lt} the eye is uniformly red. A recessive allele of es.
8. pointed abdominal sternites (pas). Sokoloff and Ho, 1963. Found as a single male in experiments designed to determine genetic load. The male died, but two of eleven sibs proved to be heterozygous for pas. Sex-linked recessive with lethal to semilethal effects. Males reaching the adult stage may live for at least two months but appear to be sterile. Normally only the first apparent abdominal segment appears pointed. In pas all the abdominal segments are pointed. Lateral to the projection (which is directed cephalad) the sternites are connected to each other by an unsclerotized membrane, forming a pit, extending laterally to the pleurites, which may accumulate flour. The last apparent abdominal segment appears truncated, sometimes telescoping into the preceding one.
9. ruby (rby). Sokoloff, 1962. Spontaneous in inbred material being tested to determine the genetic load in T. confusum. Autosomal recessive of good penetrance but variable expression as a function of age. Resembles es, rus or dpe in that a small area of the eye appears pigmented red, the rest black. It differs from rus in that the pigment is more intensely dark red approaching Burgundy red. Identifiable in the fully colored imago with difficulty, but with ease in the late pupa or teneral adult. Not allelic with rus, dpe or p.
10. sternites incomplete (sti). Sokoloff, 1963. Autosomal recessive of good viability but variable expressivity. Adjacent sternites of abdominal segments fail to overlap, and there is no intersegmental

sclerotization, with the result that, in extreme expression, the unsclerotized area may be almost one-half abdominal-segment wide, and extend from one side of the abdomen to the other. In less extreme expression the unsclerotized area consists of very small, bilaterally placed areas located approximately equidistant between the midline and the pleurite. (Flour may accumulate in these areas.) Spontaneous in studies on genetic load in reconstituted populations.

11. thickened elytral tips (tet). Sokoloff, 1963. Spontaneous in crosses attempting to establish position of St, es and lp. Sex-linked recessive of good viability but variable expressivity, the tet phenotype being somewhat difficult to work with. The tips of the elytra appear somewhat less rounded and appear thicker. In extreme expression a tiny blister develops at the elytral tip. Preliminary crosses indicate the gene is located about 53 units away from es.

T. destructor

1. creased abdominal sternites (cas). Sokoloff, 1964. Spontaneous in a sample derived (1963) from a stock maintained at Pest Infestation Laboratory, Slough, Bucks, England. Like in other species of *Tribolium*, the grooves appearing in the abdominal sternites (mostly on the apparent first segment) have variable expression. The mutant has incomplete penetrance.
2. bent tibia (btt). Sokoloff, 1964. Spontaneous in stock derived from a stock maintained at Pest Infestation Laboratory, Slough, Bucks, England. Resembles the mutant described elsewhere for T. castaneum. Mostly the hind legs are affected, appearing shorter because of a double bend of the tibia. Autosomal recessive of poor penetrance.

T. madens

1. split (spl). Sokoloff, 1963. Spontaneous in progeny of a sample derived from Pest Infestation Laboratory, Slough. Autosomal recessive of variable expression, the extreme mutants having the elytra split all the way to the scutellum, weakly expressed ones having only a feeble split condition at the tip of the abdomen. Resembles the split (spl) in the fifth linkage group of T. castaneum.

2. bent tibia (btt). Sokoloff, 1964. Spontaneous in crosses attempting to establish mode of inheritance of split (spl, q. v.). Autosomal recessive of poor penetrance and variable expression. The hind tibiae are bent at a double sharp (roughly straight) angle, resulting in a much shorter leg in strongly expressed mutants. In more weakly expressed ones the tibiae are curved.
3. fused antennal segments-1 (fas-1). Sokoloff, 1963. Spontaneous in sample derived from stock maintained at the Pest Infestation Laboratory, Slough. Autosomal recessive of variable expression and incomplete penetrance. Resembles fas-1 in T. castaneum in that the funicular and/or club segments are fused.
4. creased abdominal sternites (cas). Sokoloff, 1963. Spontaneous in wild type stock derived from a sample obtained at the Pest Infestation Laboratory, Slough. Autosomal recessive of variable expression and incomplete penetrance. Resembles cas in T. castaneum and T. confusum.

TESTS OF ALLELISM

T. castaneum

1. black (b^S-1) found in the Brazil strain as a single heterozygote is allelic with black.

T. confusum

1. McGill black is allelic with the semidominant black listed by McDonald as derived from a stock at Sault Ste. Marie.
2. A mutation found in some competition vials involving CFI 11b proved to be allelic with eyespot.
3. A mutation listed as Ruby (Ru) apparently was an allele of eyespot.
4. A light red eye color mutation found in the course of trying to map lp in relation with es and St proved to be allelic with and recessive to es (see description in New Mutant section).
5. St. Paul black is allelic with McGill black. Also the black strain maintained by McDonald at Dickinson College is allelic with McGill black.

NOTES - RESEARCH

BELL, A. E. and DORIS M. SHIDELER
 Population Genetics Institute
 Purdue University
 Lafayette, Indiana

*Tests for allelism of the scar (sc) and
 the engraved metasternum (ems) mutations
 in Tribolium castaneum.

The scar (sc) mutation was described by Eddleman in TIB-4, page 14 as engraved transverse groove on the metasternum just anterior to the coxa with one or both sides being affected. Later studies (see Bell, Shideler and Eddleman, TIB-7) revealed sc to be autosomal and partially dominant with incomplete penetrance and a variable expression. Also, both penetrance and degree of dominance were found to be temperature dependent. Scar is an interesting mutation, but clearly of dubious value for linkage studies.

In December, 1962, Dr. Sokoloff forwarded us a sample of a new mutation (named engraved metasternum, ems, and described in TIB-6, page 24) to be checked against scar. The phenotype of the two mutations were found to be identical and crosses were made to check for allelism. The results, as summarized in Table 1, were suggestive but not conclusive.

Table 1

Frequency of Mutant Phenotype from Various Crosses of
sc and ems as found in Trials 1 and 2

Mating Code	Mating		% Mutant Phenotype	
	♂	♀	Trial 1	Trial 2
1	sc/sc	sc/sc	85 (133)*	73 (92)*
2	ems/ems	ems/ems	67 (3)	78 (27)
3	sc/sc	ems/ems	37 (147)	90 (77)
4	sc/ems	sc/sc	--	50 (183)
5	sc/sc	+/+	7 (46)	--
6	ems/ems	+/+	--	6 (92)

* Total number of individuals observed

It is evident from matings 1 and 2 that sc and ems show incomplete penetrance of roughly the same magnitude (70-85%). Also, matings 5 and 6 reveal both sc and ems to be incompletely dominant to wild type under our conditions (33° C. and 70% R. H.). Matings 3 and 4 did not clarify the allelic relationship between sc and ems to our satisfaction.

In order to specify more accurately the degree of dominance and penetrance for both mutations plus their segregation ratios, a third and more extensive trial was made. The progenies from all matings were cultured simultaneously under a common environment of 33° C. and 70% R. H. The results are summarized in Table 2.

Table 2
Frequency of Mutant Phenotype in Trial 3

Mating Code	Mating		Number of Offspring		% Mutant Phenotype
	♂	♀	Mutant	+	
1	sc/sc	sc/sc	99	59	63
2	ems/ems	ems/ems	110	43	72
3	+/+	+/+	0	705	0
4	sc/sc	+/+	22	282	7
5	ems/ems	+/+	4	266	2
6	+/+	ems/ems	6	158	4
7	sc/ems	+/+	43	827	5
8	sc/sc	ems/ems	63	155	29
9	sc/ems	sc/sc	85	117	42
10	sc/sc	sc/ems	53	64	45
11	sc/ems	ems/ems	118	130	48
12	sc/ems	sc/ems	129	278	32

In Trial 3 penetrance in the two homozygous matings (1 and 2) remained in the neighborhood of 70%. Mating 3 was made to demonstrate that the scar mutation was not present in the wild type population used in these matings. The fact that both sc and ems are partially dominant to the wild type allele was confirmed by matings 4-7. The low frequency

of the mutant phenotype in mating 8 was not expected if sc and ems are the same mutation, yet the frequency is higher in this trial as well as in the other two trials than one would expect if sc and ems were nonallelic. Likewise, the results from matings 9-12 are not in agreement with a simple hypothesis of allelic genes. However, the earlier history on the isolation of the scar mutation suggested the presence of background genes modifying both expressivity and penetrance. If different sets of modifying genes had been selected in the two mutant stocks, then the lower frequency of the mutant phenotype in the later matings in comparison with matings 1 and 2 would be expected.

In summary, we can say that sc and ems have similar phenotypes, both reveal incomplete penetrance (70-85%) and are partially dominant to the wild type allele. Under standard culturing conditions (33° C., 70% R.H.) the direction of dominance is toward the wild type. The F₁, F₂ and Back-Cross results suggest that sc and ems are allelic genes, but different sets of modifying genes are present in the two stocks.

BELL, A. E., DORIS M. SHIDELER and H. L. EDDLEMAN
Population Genetics Institute
Purdue University
Lafayette, Indiana

*Dominance and penetrance of the scar
(sc) mutant in Tribolium castaneum as
influenced by temperature.

The scar (sc) mutation was isolated in 1960 by H. L. Eddleman and students at Salem-Washington Township High School, Salem, Indiana. The mutant was described in TIB-4, p. 14 as an engraved transverse groove on the metasternum just anterior to the coxa with one or both sides being affected. Genetic studies revealed scar to have incomplete penetrance (70-80% under standard conditions of 33° C. and 70% R.H.), a variable expression and good viability. Preliminary matings suggested that scar was inherited as an autosomal recessive; however, a more careful classification revealed sc to be partially dominant to its wild type allele.

Krause's finding (TIB-6, p. 44) of increased penetrance for the ti mutant when the culturing temperature was increased from 24.4° C. to 32.8° C. suggested that the scar phenotype might be temperature dependent. In five different trials progenies from various scar matings were cultured at 33° C., and at 35° C. Relative humidity in both chambers was maintained at 70%. When the results from these five trials were summarized as below we were surprised to find that in addition to scar's increased penetrance at the higher temperature, its dominance relationship to the wild type allele was changed.

In Trial I only homozygous scar matings were cultured at both temperatures, but we observed a significant increase in the penetrance of the scar mutation. For Trials II and III we cultured simultaneously pure line, F_1 and test-cross progenies at both temperatures. Again we observed an increase in the penetrance of sc at the higher temperature. The effect of temperature on the dominance of sc is clearly revealed in the $sc/sc \times +/+$ matings. Results from reciprocal matings have been pooled since they showed no evidence of sex-linkage. Under these environmental conditions we must conclude that sc is partially dominant rather than recessive. It is equally clear from these matings as well as from the $sc/+ \times sc/sc$ and the $sc/+ \times +/+$ matings that the degree of dominance is temperature dependent. At the higher temperature the direction of dominance is toward the sc allele, while at the lower temperature the direction is toward the wild type (+) allele.

CUTLER, J. R.

Department of Agriculture and Fisheries for Scotland
East Craigs, Edinburgh, Scotland

Use of *T. confusum* at Department of Agriculture
and Fisheries for Scotland.

T. confusum is the species normally used for investigational work. Its main use has been as an indicator of the success or failure of fumigations of flour and provender mills and of various other bagged commodities in stack.

DAWSON, PETER S.

Department of Genetics
University of California
Berkeley, California

*Facilitation and developmental
rate of *Tribolium castaneum* and *T. confusum*.

Lewontin (1955) and others have shown that survival of larvae of *Drosophila melanogaster* is dependent not only on the density but also on the genetic composition of the competing population. Similarly, the selective values of various chromosome arrangements in *D. pseudoobscura* have been found to depend on the frequency and type of other arrangements present in population cages (e.g., Levene, et al., 1958).

Similar results, which have been obtained for developmental rate from pilot experiments using T. castaneum (CS) and T. confusum (CF), form the basis of this brief note.

The experimental material was composed of five series of vials, each containing 40 pairs of parents of comparable age. The five sets of parents were the CS synthetic stock; the CF synthetic stock; a CS strain selected for fast development; CS-stock plus CF-stock; and CS-fast plus CF-stock. In the two competition sets, 20 pairs of each species were used. The synthetic stocks are standard outbred strains which have been maintained in this laboratory as closed populations since their inception (see Lerner and Ho, 1961).

From each series, four vials each containing one day's egg production in about 12 grams of flour were collected by transferring the parents to fresh flour at the same time each day. When pupae started to appear, the vials were removed from the incubator daily (again at the same time) and the pupae were removed, counted and discarded. The character scored was thus the number of days from egg to pupa. Cultures were randomly arranged in an incubator maintained at 29° C., 70 per cent relative humidity.

Owing to the fact that constant numbers of parents rather than constant numbers of eggs were used for all series, there were variations in the number of offspring obtained from each experimental vial. The differences were, however, quite small, and corrections for density effects did not alter the basic conclusions.

Developmental times (in days from egg to pupa) are listed in Table 1. There appears to be a trend in the direction of shorter duration of developmental time in CS when CF is also present, whereas the results with CF are inconclusive. Mann-Whitney rank tests for differences between paired series within species showed that the only significant difference was the faster development of the CS stock when CF was present.

Under the same conditions of temperature and humidity utilized in these studies and in the absence of sporozoan parasites, the CS synthetic strain invariably eliminates the CF synthetic strain in competition experiments initiated with 10 pairs of adults of each species (Lerner and Dempster, 1962). The present results indicate that at least one component of competitive ability of the CS strain is facilitated by the presence of CF in the same culture.

Measurements of developmental time and other population parameters have previously been made primarily on the level of the individual species. The data presented here suggest that interactions between individuals of the same and of competing species should be studied more intensely since they are ultimately of great interest in our attempts to better understand the phenomenon of competition.

Table 1

Developmental Time (days from egg to pupa)
Under Conditions of Intra- and Interspecific Competition

Competitors		Developmental Time	
CS	CF	CS	CF
stock	--	23.66	--
stock	stock	22.87*	23.70
--	stock	--	23.67
fast	--	21.11	--
fast	stock	21.01	23.52

*Significantly different from CS-stock at .001 level by Mann-Whitney rank test.

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DAWSON, PETER S.
Department of Genetics
University of California
Berkeley, California

*An interesting behavioral phenomenon
in *Tribolium confusum*.

Recent studies involving a number of inbred lines of *T. confusum* have uncovered a behavioral character which appears to possess a genetic element of control. In this report some preliminary observations from analysis of the inbred lines will be presented.

The inbred strains had been maintained for 30 to 35 generations of sib mating before the present studies were initiated. Each line was originally started with duplicate matings from the progeny of a single pair of adults. Whenever, in the course of the inbreeding process, one of the duplicate sublimes was lost, a new one was initiated from the remaining subline. Thus some of the duplicates had been kept in isolation for the entire 30 to 35 generations.

Each inbred strain was assayed for developmental rate and productivity by transferring a maximum of 30 pairs of adults of comparable age to large shell vials (25 × 95 mm) containing about 12 grams of flour for three consecutive days. It was noticed at the time when adults of one particular inbred line were transferred that a large number of beetles were present on the surface of the flour. The number of offspring recovered from the 30 females of this strain was very low (a total of seven in three days).

Later, when the duplicate subline to the one just described was assayed, the same phenomenon of adults remaining on the surface of the flour was observed. This led to the preliminary studies to be outlined in the remainder of this report.

Thirty pairs of adults of four inbred lines were transferred on ten consecutive days to fresh flour. For the first five days the beetles were placed on the surface of the flour. For the next five days the adults were introduced into vials containing about six grams of flour and another six grams was then added to the vials. Each day the number of beetles on the surface of the flour was crudely measured either by counting or, when large numbers were on the surface, by carefully dumping them into a dish without removing any of the flour from the vial.

The results, listed in Table 1, indicate that definite differences exist between lines with regard to the position of the beetles in the vial at the time when they were observed. These differences are, at least in the case of line A, reflected in the number of offspring produced by the adults used. Sonleitner (1961) has previously demonstrated that productivity is decreased when females are confined to the surface of flour.

Probably the most interesting observation from these pilot studies is that inbred line A and its duplicate culture, which have been separately maintained for some 30 generations of sib mating, are the only lines for which very high proportions of beetles remain on (or migrate to) the surface of the flour. In addition to the four lines listed in Table 1, a total of seven other lines (including duplicate sublimes for all but one) were included in the original assays. None exhibited such an extreme pattern of surface numbers.

It is of further interest that, when left undisturbed in a culture, adults of the aberrant strain also tunnel down into the flour. The observed behavioral differences are apparently of short duration.

A number of possible explanations for this abnormal behavior pattern could be advanced. Among them are: increased sensitivity to quinones; increased oxygen requirements; changes in chemical composition of quinones; decreased tunneling ability; or differences in the time and rhythm of daily vertical migrations in the cultures observed by Pimentel (1958). Whatever the explanation, these preliminary results indicate that there may be a genetic basis. Further studies are currently in progress.

Table 1

Numbers of adults of four T. confusum inbred lines found on surface of flour in ten consecutive daily transfers. For the first five days (Sec. I) 30 pairs of adults placed on top of flour; for the next five days (Sec. II.) the same adults placed in the middle of a vial of flour. Productivities are total number of pupae recovered in the first three vials of Sec. A.

	A	B	C	D
I.				
1	43	13	1	0
2	57	17	3	0
3	55	17	0	0
4	59	16	1	0
5	58	18	2	0
II.				
1	58	14	1	0
2	58	25	0	3
3	58	29	1	0
4	55	7	3	0
5	58	13	0	0
<u>Productivity</u>	<u>50</u>	<u>263</u>	<u>250</u>	<u>387</u>

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DAWSON, PETER S.
 Department of Genetics
 University of California
 Berkeley, California

The location of pokey (pok) on the X chromosome in Tribolium castaneum.

The purpose of this note is to present the results of linkage studies carried out with pokey (pok), a sex-linked semilethal recessive gene. A description of the morphological abnormalities associated with pok is given in the section on new mutants.

The mutant was discovered in matings designed to determine the location of l₂, a sex-linked lethal, but was not detected at first due to a superficial resemblance to dve which was also present in the crosses. The females used in these matings were derived from a cross between dve py r males and a female possibly heterozygous for l₂. Thus their genotypic constitution must have been + dve py r/pok + +.

During classification of progeny from these crosses, abnormal "dve-like" beetles were observed and noted on the data sheets. It was particularly noticed that these abnormal types occurred predominantly among later-emerging imagoes. After the data had all been collected, an exceedingly high frequency of phenotypic classes where dve was present led to a re-examination of the material. The male progeny from those matings where aberrant "dve" beetles (actually pok) had been noted are summarized in section A of Table 1. Since these males were not scored for pok with one exception the data cannot be used to estimate map distances. This exception will be discussed later.

Phenotypically wild type females from the above matings were again backcrossed to dve py r males and their offspring scored for all 16 possible phenotypes. Data from male progeny are given in section B in Table 1, from which it may be concluded that the order of these genes in pok-dve-py-r. "Corrected" map distances (computed by ignoring the pok classes) are given in section B, Table 2. It is seen that pok is apparently quite close and to the left of dve. It might also be noted that from 1075 females classified in these matings, distances of 11.07 and 13.95 units for dve-py and py-r respectively were obtained. These estimates are in close agreement with previously reported distances of 11 and 14 units.

Returning now to the original data (section A, Table 1), another estimate of the distance between pok and dve may be obtained. Ignoring the pok gene, the X-dve span is computed by dividing the sum of the phenotypic classes +, py r, r and py by the total number of non-pok individuals. Dividing by the total number of males (since the number of pok males is unknown) will lead to an underestimate of the pok-dve distance, but since the per cent emergence of pok males is low, the resulting distance will not be too distorted. This estimate, 1.89 units, is given in section A of Table 2.

Allelism tests between dve and pok were carried out by mating homozygous dve females to pok males and scoring the female offspring. Since all female progeny were phenotypically wild type, the two genes are not allelic.

Data from further matings involving females heterozygous for pok and py and for pok, py and r are given in sections C and D of Tables 1 and 2. In general, the results from all of the described sets of matings are quite consistent, considering the relatively small number of males scored in section D. The pok gene can be considered to be located to the left of dve and about two units away.

Table 1

Phenotypes of male offspring from four sets of matings.
Genotypes of female parents are given for each set.

Phenotype	<u>+ dve py r</u>	<u>+ dve py r</u>	<u>+ py</u>	<u>+ py +</u>
	<u>pok + + +</u>	<u>pok + + +</u>	<u>pok +</u>	<u>pok + r</u>
+	30	4	75	0
<u>dve</u>	467	39	--	--
<u>py</u>	0	0	629	103
<u>r</u>	1	1	--	24
<u>dve py</u>	223	68	--	--
<u>dve r</u>	53	0	--	--
<u>py r</u>	3	0	--	13
<u>dve py r</u>	1020	344	--	--
<u>pok</u>	--	124	294	0
<u>pok dve</u>	--	X	--	--
<u>pok py</u>	--	1	30	0
<u>pok r</u>	--	21	--	47
<u>pok dve py</u>	--	X	--	--
<u>pok dve r</u>	--	X	--	--
<u>pok py r</u>	--	21	--	0
<u>pok dve py r</u>	--	X	--	--
Total	1797	655	1028	187
No. matings	46	22	38	8

-- indicates that a particular class was not scored (Sec. A) or not possible (Sec. C,D) in the mating.

X indicates that a class containing pok and dve could not be distinguished from the corresponding class where dve was absent (see text).

Table 2

Map distances between various genes
computed from the data of Table 1

	A	B	C	D
<u>pok-dve</u>	1.89*	1.02		
<u>pok-py</u>		9.02	10.65	17.14
<u>pok-r</u>		22.75		26.43
<u>dve-py</u>		7.99		
<u>py-r</u>		14.14		9.29
<u>dve-r</u>		21.93		

* underestimate (see text)

DEWEES, A. A.
Population Genetics Institute
Purdue University
Lafayette, Indiana

Variation in a "wild" population
of *Tribolium castaneum*.

Ho and Sokoloff (TIB-5, p. 29), after finding *Tribolium* mutants in populations obtained from flour mills and feed storage bins, suggested that estimations of gene frequencies in these seminatural populations would be of interest. A study was conducted to ascertain occurrence and gene frequency of mutants in a "wild" population of *Tribolium castaneum* (M. A. Thesis, Southern Illinois University). Gravid females were collected from a feed storage sack and isolated. From each of 17 parental females, 16 F₁ sib-crosses were made and the resulting F₂ beetles were analyzed for visible mutations. Gene frequencies of the eye color mutants were estimated by calculating the frequency of F₁ sib-crosses producing F₂ mutant beetles. Since the main purpose of this study was to reveal any mutants that might be present, the best method for achieving this was to sib-cross the F₁ beetles. The gene frequencies, estimated without correcting for this inbreeding effect, are probably over-estimations.

Three eye color mutants and a black body color mutant were found during the study (see New Mutants, report of A. E. Bell). The black body color mutant proved to be a recurrence of McGill black. Since only three distinguishable bronze heterozygotes produced by one of the F_1 sib-crosses were observed, there is the possibility that they resulted from mutations in the F_1 beetles, therefore no gene frequency was calculated.

A second mutant, an autosomal recessive affecting pigmentation of the ocular diaphragm, was found at an estimated gene frequency of .22. The ocular diaphragm, normally pigmented black, appears colorless to reddish-yellow when viewed through the peripheral ommatidia of a bicolored eye mutant. This mutant, allelic with and indistinguishable from Sokoloff's lod, has been designated as lod^D. Penetrance is good and expressivity is fair.

Two new bicolored eye mutants, ruby (rb) and peach (ph), were found at estimated gene frequencies of .26 and .03 respectively. Both are autosomal recessives with good penetrance and expressivity. Ruby is similar to chestnut in color, but does not darken as much with age as chestnut. Peach is dark pink in color. Ruby and peach are nonallelic with respect to each other and to c, i, m, p and w. Linkage studies involving rb and ph will be undertaken soon.

DUCOFF, H. S. and GAYLE C. BOSMA
Department of Physiology and Biophysics
University of Illinois

Effect of some inhibitors on survival
and fertility of *T. confusum**

As part of a program to assess the role of metabolic activities in the recovery of *Tribolium* from X-ray and other injury, we have examined the response of *T. confusum* to a number of antimetabolites and inhibitors. Three types of agent have yielded particularly interesting preliminary results.

1. Aminopterin, which interferes with the folic-folinic pathway and therefore, eventually, with DNA synthesis, proved toxic to larvae. At a level of 0.2% in flour + 4% yeast (FY) medium, growth was suppressed, and all larvae were dead within 2 weeks. At a level of 0.1% aminopterin, older larvae developed and survived to eclosion, but younger larvae died before pupation. Two-tenths per cent aminopterin was slightly toxic to adults.
2. N-Ethyl maleimide (NEM), a sulfhydryl inactivator, was toxic to larvae and to adults. In 1% or in 0.5% NEM, all larvae were dead within a few days. In 0.25% NEM, large larvae reached

* Supported in part by Grant GM 10208, USPHS; Miss Dorothy Batterton participated in portions of this work.

pupation and eclosion normally, but about 50% of young larvae died within 3 weeks. The specificity of NEM action is demonstrated by the fact that toxicity of 1% NEM was overcome if the -SH- containing amino acid cysteine was also incorporated into the medium at the 1% level. Furthermore, N-phenyl maleimide (1% in FY) killed all exposed larvae or adults within 2 weeks at 30° C.

3. Triethylenethiophosphoramidate (THIO-TEPA), an alkylating agent, at levels of 0.05%, was virtually nontoxic both to larvae and to adults, but exposures for a few days in THIO-TEPA-FY rendered adults temporarily sterile and markedly reduced subsequent fertility.

ERDMAN, HOWARD E.
Biology Laboratory, Hanford Laboratories
General Electric Company
Richland, Washington

Fast neutron effects on flour beetles*

Day old Tribolium castaneum Herbst (Brazil CI strain) were given 830 rads of 2.5 to 5.0 Mev (mean 4.6 Mev) fast neutrons from a Van de Graff accelerator. Ten mating combinations, neither-exposed, male-exposed, female-exposed and both-exposed, were kept at 25, 29, and 32 C and at 65-70% relative humidity for two weeks. Each day for two weeks the pairs were given fresh food and that from the previous day was reincubated until F₁ adults emerged.

Onset of reproduction and the average number of adult progeny were influenced by temperature and the sex exposed to fast neutrons. More advanced sexual maturity of males compared to that of females made the males more radiation resistant, thus allowing earlier reproductive onset. This is consistent radiobiologically in that radioresistance of a tissue increases with differentiation of cells and with less actively dividing cells.

During the second week of reproduction, male and female germ cells were adversely affected to the same extent as indicated by scoring the number of adult F₁. The effects appeared additive when both members of a pair were exposed. Increased cell proliferation with increased temperature could account for increased productivity. Mating activity and preferential use of sperm (Schlager, 1960) might explain the productivity trend of male-exposed groups.

* Work performed under Contract No. AT(45-1)-1350 between the Atomic Energy Commission and the General Electric Company.

From these data the relative biological efficiency (RBE) of fast neutron irradiation on flour beetle productivity will be obtained. Neutron irradiation effects are of concern with problems associated with space travel. Temperature, physiological age, and biology in general altered productivity and were important factors to help interpret radiation induced effects.

ERDMAN, HOWARD E.
Biology Laboratory, Hanford Laboratories
General Electric Company
Richland, Washington

Age and temperature effects on
reproductive onset and productivity
of flour beetles, *Tribolium castaneum* Herbst*

Virgin beetles of one day (young) and eleven days (old) post eclosion were paired in various mating combinations at 29 C. Only young x young pairs were run at 25 and 32 C. At all temperatures the relative humidity was 65-70%. Table 1 gives the day of reproductive onset and the mean numbers of adult F_1 + standard errors. Young females were not productive until day 4 at 29 and 32 C (supports similar data of Dawson - Annual Ento. Soc. Am., 57: 1, 1964); whereas 25 C extended the onset to day 6. Productivity measured as the mean numbers of adult F_1 progressively increased from 25 to 32 C. Age did not influence total productivity at 29 C.

Age and temperature determine the time at which productivity begins. Temperature, but not age, determined the total productivity, at least during the first two weeks of reproduction. Effects due to these variables would be important to radiobiological studies of populations and to cytological or histochemical studies on development and differentiation of the reproductive tissues.

* Work performed under Contract No. AT(45-1)-1350 between the Atomic Energy Commission and the General Electric Company.

Table 1

Influence of Age and Temperature on the Day of Reproductive Onset and Total Productivity for Two Weeks of Flour Beetles, Tribolium castaneum Herbst (Brazil CI Strain). Young = 1 day old adult; Old = 11 day old adults.

<u>Mating</u>	<u>Mean day reproduction began</u>	<u>Total productivity mean no. adult F₁ per female + std. error</u>
$\sigma \times \text{♀}$		
<u>25°C</u>		
young × young	6	74 + 5
<u>29°C</u>		
young × young	4	181 + 12
old × young	4	194 + 18
young × old	2	180 + 11
old × old	1	172 + 29
<u>32°C</u>		
young × young	4	246 + 9

HO, FRANK
 Department of Genetics
 University of California
 Berkeley, California

*Identification of mature larvae of Tribolium confusum and T. castaneum.

Tribolium confusum closely resembles T. castaneum in all stages. To distinguish between the species, methods have been worked out by Good (1936) and Hinton (1945) for the adult stage, Ho (1960) and Mertz (1961) for the pupal stage. Since the two species are often used in ecological and population studies, identification of the larvae in cultures is of considerable importance.

The writer has found that the larvae of the two species can be identified readily by an examination of the setal map of the thoracic and abdominal segments under high magnification ($\times 60$).

In general the T. confusum larva has more setae than T. castaneum. In both species a few additional setae may be found as the larval instar increases. Also, in the same larval instar, fewer setae may be counted in some individuals. This may be due either to the fact that the setae have not developed, or that the setae have been lost. The following

description emphasizes only the important parts of the setal map of the mature larvae, especially of the abdominal segments. No distinct fold or line can be found between the tergites and the pleurites, but according to the arrangement of the setae and the darker area of the middle part of the tergites, the spiracular line area should be included in the tergites. Eight pairs of abdominal spiracles, similar to the mesothoracic spiracles are located near the anterior third of each segment (Figs. 1 and 2).

Abdominal tergites -- In T. confusum, segments 1 to 8 each has a transverse anterior row of 8 setae and a transverse posterior row of 10 setae and a single seta between the two rows on each of the spiracular lines (Figs. 1 and 3). The length of the anterior setae is rather uniform, but 4 of the posterior setae are much shorter than the others; in fact, sometimes only the setal tip can be seen. In T. castaneum, segments 1 to 8 have an anterior row of 4 setae and posterior row of 6 setae each, the anterior setae are slightly shorter than the posterior ones (Figs. 2 and 4). In the ninth tergite the setae on the urogomphi are generally shorter in T. castaneum than in T. confusum.

Abdominal pleurites -- In T. confusum, segments 1 to 8 all have one anterior, one middle, and one posterior seta; the posterior seta is the longest (Figs. 1 and 5). In T. castaneum, both anterior and posterior setae are almost equal in length or the posterior seta is slightly longer. In addition to this difference, T. castaneum either lacks the middle seta or only a setal tip can be seen (Figs. 2 and 6).

Abdominal sternites -- In both T. confusum and T. castaneum, segments 1 to 8 each has an anterior and posterior row of 4 setae, the inner pair of anterior setae near the ventral line are distinctly shorter than the others. The only difference is that the setae are comparatively shorter in T. castaneum than in T. confusum (Figs. 5 and 6).

Tergites of the thorax -- In T. confusum, the thoracic tergites are setose in a manner similar to those of the abdomen, but with a few additional setae at the sides of prothorax. In T. castaneum, each thoracic tergite also has more setae than the abdominal tergite, but less than in T. confusum. In T. confusum, each tergite of the meso- and metathorax has an anterior row of 8 setae and the posterior row of 10 setae (Fig. 7), but in T. castaneum, only 6 setae are found in each row (Fig. 8).

Key to Mature Larvae of T. confusum and T. castaneum

T. confusum

1. Tergites of the mesothorax and metathorax, each with an anterior row of 8 setae and posterior row of 10 setae.
2. Abdominal tergites 1 to 8, each with three setae near the spiracular line.

3. Abdominal pleurites 1 to 8 each with three setae, the posterior seta being the longest.

T. castaneum

1. Tergite of mesothorax and metathorax, each with an anterior row and posterior row of 6 setae.

2. Abdominal tergites 1 to 8, each with 2 setae near spiracular line.

3. Abdominal pleurites 1 to 8, each with 2 setae, almost equal in length or the posterior seta being slightly longer.

Captions of the Following Figures

Fig. 1 -- Left lateral view of mature larva of Tribolium confusum.

Fig. 2 -- Left lateral view of mature larva of T. castaneum.

Both figures show the arrangement of the setae of the left half of the body, especially on the tergites of the thorax, and the tergites, pleurites and sternites of the abdomen.

Fig. 3 -- Third and fourth abdominal tergites of mature larva of T. confusum.

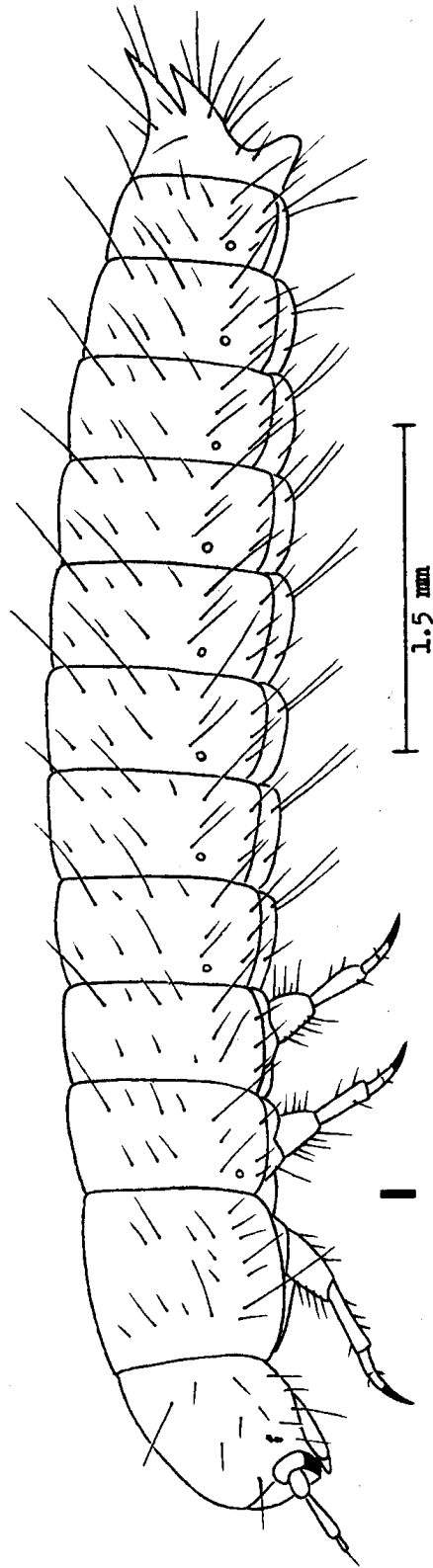
Fig. 4 -- Third and fourth abdominal tergites of mature larva of T. castaneum.

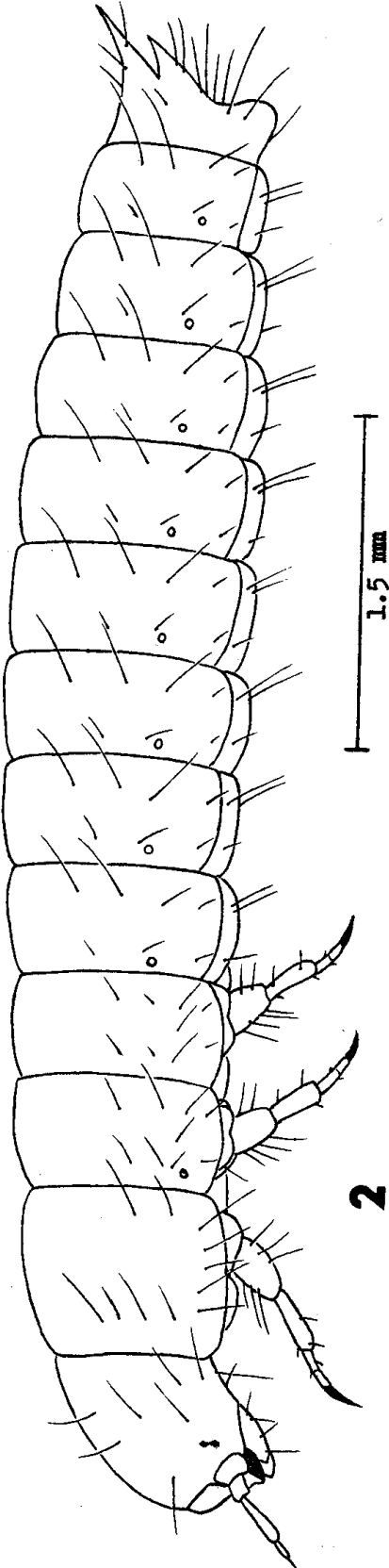
Fig. 5 -- Third and fourth abdominal pleurites and sternites of mature larva of T. confusum.

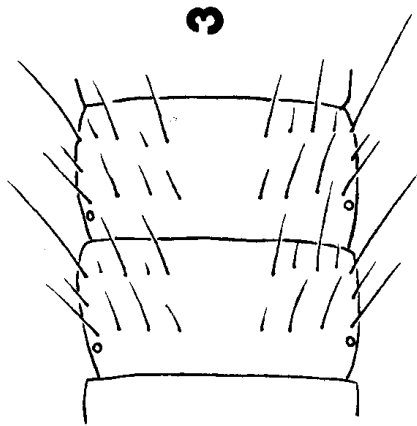
Fig. 6 -- Third and fourth abdominal pleurites and sternites of mature larva of T. castaneum.

Fig. 7 -- Thoracic tergites of T. confusum.

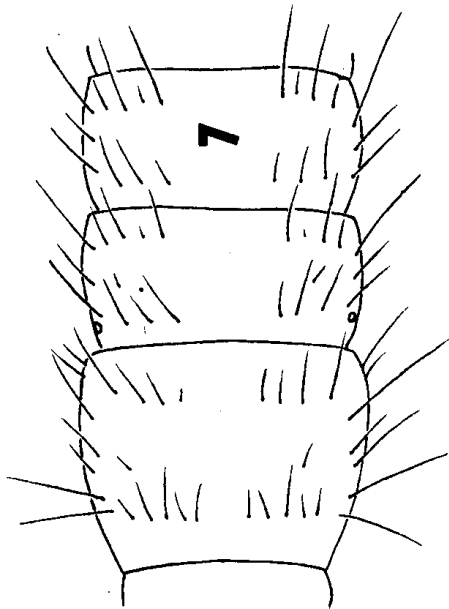
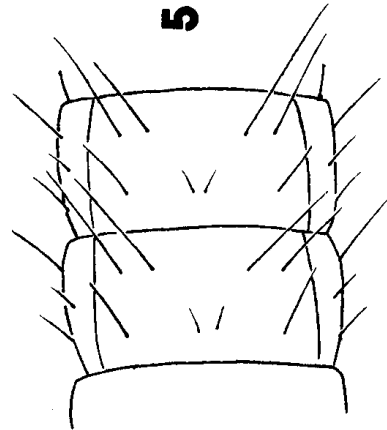
Fig. 8 -- Thoracic tergites of T. castaneum.



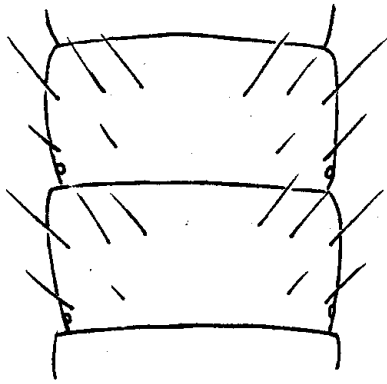




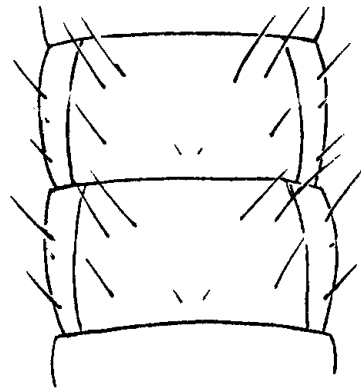
.5mm



4

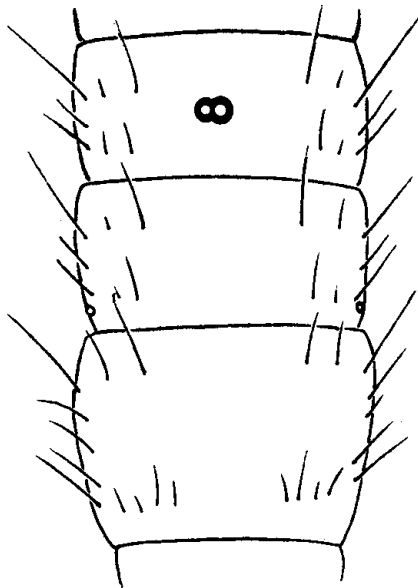


6



.5 mm

8



LECLERCQ, J.
Zoologie Générale, Institut Agronomique de l'Etat
Gembloux, Belgium

The F and G strains of *Tenebrio molitor*

Both strains have been used recently for investigations on the protidic nutrition of *Tenebrio molitor*. So far every attempt to replace purified casein in their diet by a mixture of amino acids have failed and recent data suggest that *Tenebrio* larvae need some amino acids already linked in dipeptides. Other investigations are now in progress with the view of using *Tenebrio* larvae for comparing the real biological value of various natural proteins and foodstuffs. There seems to be little hope that the cytogenetics of our strains will ever be studied in this department, so we would be glad to supply the necessary material to anybody who would become interested in that promising direction.

LEFKOVITCH, L. P.
Pest Infestation Laboratory
Slough, Bucks, England

A re-evaluation of Reynolds' study of the inheritance of food effects in *Tribolium destructor*

Reynolds (1944, Proc. Roy. Soc. (B) 132: 438-451) studied the effect of flours of different extraction rates when fed to larvae and the resulting adults of *Tribolium destructor* upon the rate of development of the first filial larvae. He claimed to have found evidence that the parents' food affected the rate of development and mortality of offspring. Even though this observation can be explained on non-Lamarckian grounds, it is remarkable in itself. If such effects truly occur, this pattern of response will require modifications to be made to the generally accepted ideas on population growth. Obviously, Reynolds' results should be subjected to re-examination and, if they stand scrutiny, an attempt be made to repeat his observations.

Reynolds' statistical methods are open to criticism. He gives in his Table 1 the mean and standard deviation of the post-oval development on 12 different food combinations and, he states, "Fisher's t test [sic] has been used to compare these results." In Table 1 are given the 11 results from which he drew his conclusions.

It is now impossible to examine Reynolds' original experimental records. In any case, there are so many gaps in the table as not to

make an analysis of variance worthwhile. However, it seems that for his conclusions to have been justified, his evidence should support the relationship

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3$$

where the b_i are constants, x_i the composition of the foods as in Table 1 and y is the developmental periods of the filial generation.

The estimates of the regression coefficients and the analysis of variance associated with them are given in Table 2. It can be inferred that this regression relationship could occur frequently by chance if y was independent of the x_i . Of the regression coefficients, only one seems to be different from zero; it is that which relates the developmental period of the larvae with the food that their parents had eaten as adults. It seemed worthwhile, therefore, to study the relationship of these two factors alone, without making any allowance either for the food the parents eat as larvae or for the food of the filial larvae. This relationship is thus of the form

$$y = a + bx_2$$

where a is a constant. The estimate of b is given in Table 3 together with an analysis of variance; a chance relationship of this kind would occur about once in thirty times if there were no relationship between the two variables.

Reynolds data suggest that the developmental period of larvae of T. destructor is independent of their food but is determined primarily by the food of their parents as adults. This is a biological paradox; it is now inferred that the regression analysis in Table 3 has recognized no more than a spurious relationship. It is concluded that Reynolds' inferences were unjustified on his data; if they are insufficient to establish that larval development was directly affected by the nutritional quality of their own food, how can they be taken to support other than a chance relationship between larval development and parental food?

Table 1

Means and Standard Deviations (in days) of the Developmental Periods of Tribolium Destructor as Estimated by Reynolds (1944) on Flour of Three Different Extractions (expressed as a percentage)

Adult Parental Food (x_2)	Filial Food (x_3)	Food of Parent as Larvae (x_1)		
		85%	75%	60%
85%	85%	43.9 ± 1.7		
	75%	44.2 ± 2.0		43.9 ± 2.4
	60%	44.4 ± 2.4		
75%	85%		49.3 ± 4.8	
	75%		52.8 ± 2.9	
	60%		52.8 ± 6.8	
60%	85%			42.8 ± 3.8
	75%	53.4 ± 4.3		54.4 ± 7.8
	60%			52.4 ± 5.3

Table 2

Partial Regression Coefficients, with their Standard Errors in Parentheses, and an Examination of them in Relation to the Null Hypothesis

$$b_1 = -0.019 (0.121); b_2 = -0.271 (0.132); b_3 = 0.104 (0.129)$$

Analysis of Variance

Source	Sums of squares	Degrees of freedom	Mean square	F
Total	185.87	10		
Regression	74.43	3	24.81	1.56
Residual	111.44	7	15.92	

Table 3

Regression Coefficient Relating y with x_2 , with its Standard Error in Parenthesis, and an Examination of it in Relation to the Null Hypothesis

$$b = -0.280 (0.102)$$

Analysis of Variance

<u>Source</u>	<u>Sums of Squares</u>	<u>Degrees of Freedom</u>	<u>Mean Square</u>	<u>F</u>
Total	185.87	10		
Regression	84.27	1	84.27	7.47*
Residual	101.60	9	11.28	

* $P \leq 0.03$

MILANI, R.
Centro di Genetica, Universita Pavia
Pavia, Italy

Use of Tribolium for detecting water soluble vitamins.

At the Centro di Genetica, Tribolium is used for detecting water-soluble vitamins in various substrata, according to the method of Froebrich and Offhaus (1953).

MUSGRAVE, A. J.
Ontario Agricultural College
Guelph, Canada

Mycetomal micro-organisms in weevils.

Further evidence has been produced suggesting mycetomal micro-organisms supply weevils with useful but nonessential nutrient. Wheat, brown rice and white rice have been compared as hosts. (See Musgrave, Ashton & Homan.)

REYNOLDS, ELISABETH M. and N. K. SYLVESTER
Pest Infestation Laboratory
Slough, Bucks, England

The toxicity of methyl bromide to
six populations of *Tribolium castaneum**

The results given below form part of a survey of the susceptibility to fumigants of different populations of the same species. Samples of insects, usually collected in the field from infested commodities, were used to obtain an F₁ generation. The F₂ generation was tested in a direct comparison with the P.I.L. stock. The cultures were reared at 25° C and 70% R.H. and whenever possible were fed on the foodstuff in which the original sample had been found. Details of the stocks are shown in Table 1 below. Those from the Transvaal were from laboratory cultures, all the others were from infestations in stored commodities. The P.I.L. stock was reared under the same conditions on wheat flour.

Tests were made at a concentration of 7 mg/l of methyl bromide at 25° C and 70% R.H. The concentration was established in a 3000 litre empty fumigation chamber. Test insects were inserted and withdrawn through small ports in the chamber. In this way data for an exposure-time mortality regression could be obtained for each of several stocks simultaneously. The P.I.L. stock was included in each of the three tests that were made.

After fumigation the samples were kept at 25° C and 70% R.H. and counts made at intervals over several weeks. No appreciable increase in mortality was found after 8 days.

Several of the stocks gave heterogeneous results and the precision of the comparisons was therefore low. Parallel probit regressions with a slope of 19.19 were fitted and an estimate of log LD₅₀ obtained for each stock. These gave the estimates of relative susceptibility shown below, in Table 1. None differs significantly from unity.

From the point of view of fumigation practice the absence of important differences is reassuring though these findings can only be regarded as preliminary. More information is required including data for preadults as well as adults.

Table 1

<u>Stock</u>	<u>Commodity</u>	<u>LD50 relative to P.I.L. stock</u>		
		<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>
Trinidad	Brown rice	1.07	--	--
Nigeria	Cocoa beans *	1.02	--	--
Gambia	Ground nuts	1.14	--	--
S. Rhodesia	Sorghum	--	1.00	--
Transvaal 1	White maize meal + 5% dried brewers' yeast	--	--	1.01
Transvaal 2	White maize meal + 5% dried brewers' yeast	--	--	0.99

* Stocks reared on kibbled wheat.

SLATIS, H. M.
Department of Biological Sciences
Michigan State University
East Lansing, Michigan

*Sterility in the McGill black T. confusum.

Stanely (1961 Nature 191: 934) reported sterile crosses involving females of McGill black which was not evident when McGill black males were used. A check on my records indicates that, of four single pair matings of black females by wild males made on January 19, 1953, one female gave a single pupa, while the other three were sterile. It would appear that sterility of the females of this strain in matings with wild males was almost complete soon after the strain was first isolated. The F₂ black females (of the cross of black males by wild females) were fertile with their wild type brothers.

SOKOLOFF, A.
Department of Genetics
University of California
Berkeley, California

*Revised maps of Tribolium castaneum,
T. confusum and Latheticus oryzae.

The purpose of this note is to bring up to date the linkage information on the linkage relationships of genes found in the above beetles, if nothing else, for the benefit of the writer. No attempts will be made to give exact distances, but where the order is given it will be tacitly understood that three point crosses were performed. Where only 2 point crosses were done, the data will be shown in parenthesis. Since this is intended only for quick reference to plan other linkage tests, no probable errors are given.

I have taken advantage of my editorial position in absorbing into this note data provided by Reynolds for his rose (rs) mutant, and by Dawson for pokey (pok). Both of these are sex-linked (see section on New Mutants and research note by Dawson). I have also added maroon to the fifth linkage group on the basis of a personal communication by Eddleman. It would have been desirable to perform three point crosses between m, mc and spl, but Eddleman did not feel he was ready to release the m mutant. Since the values between j and mc and jE and mc are different, the position of m in reference to spl must be ascertained in a future linkage study.

In regard to the data from the seventh linkage group, it should be pointed out that for any two genes involved in linkage studies, the values on crossing over will differ, depending on whether the male or female was heterozygous, probably because there is a difference in chiasma distribution in the two sexes. Hence, the values obtained when the male was a double heterozygote and when the female was a double heterozygote are given. The map of this chromosome, however, is based on three point crosses.

The construction of the map of the X chromosome of T. confusum unfortunately has not progressed. This is due to the fact that the only "good" genes available (red and eyespot) affect the same character and they produce a similar phenotype, so r es in hemi- or homozygotes is indistinguishable from single gene hemi- or homozygotes. The St gene is not of much help because males are sterile. All the other genes described for this species (aer, pas, lp and l₁) are lethal to semilethal. It is likely that any value obtained with lp in association with es or with r will give distorted results. A new mutant, tet, also located on the X chromosome resembles the normal phenotype, and it is extremely difficult to work with it. The values for the X chromosome given, therefore, are based on two point crosses only, except for St-es-lp, which places these genes in that order.

T. castaneum

I	IV	VII
<u>pd-l₁</u> = 61	<u>Be-s</u> = 25	<u>ble-c</u> ♂ = 36-38
<u>r-M^r</u> = 16	<u>s-ims</u> = 10	<u>ble-c</u> ♀ = 42-45
<u>r-te</u> = 11	<u>Be-ims</u> = 43	<u>ble-Fta</u> ♂ = 6-7
<u>pd-r</u> = 1	<u>ju-ct</u> = 7	<u>ble-Fta</u> ♀ = 2-2.5
<u>pd-ma</u> = 13	<u>s-sti</u> = 41	<u>ble-Sa</u> ♂ = 54
<u>ma-py</u> = 2	<u>Be-ju</u> = 40	<u>ble-Sa</u> ♀ = 4
<u>py-l₃</u> = 5	<u>Be-ct</u> = 30	<u>Fta-c</u> ♂ = 50
<u>py-dve</u> = 11	<u>sti-ims</u> = 35	<u>Fta-c</u> ♀ = 41
<u>py-l₂</u> = 23	<u>s-dfl</u> = 24	<u>Sa-l-c</u> ♀ = 40
<u>py-l₄</u> = 25	<u>dfl-ims</u> = 52	<u>sa-c</u> ♂ = 51
<u>pd-sp</u> = 46	V	<u>sa-c</u> ♀ = 39
<u>py-rs</u> = 15	<u>i-spl</u> = 29	<u>Sa-sa</u> ♀ = 0
<u>pok-dve</u> = 2	<u>j-mc</u> = 25	
	<u>spl-mc</u> = 41	VIII
II	<u>m-j^E</u> = 22*	<u>elb-sh^S</u> = 23
<u>p-pg</u> = 30	<u>m-mc</u> = 40*	<u>sh^S-sq</u> = 28
<u>p-p^{Pk}</u> = 0	<u>j^E-mc</u> = 18*	<u>ap^D-sq</u> = 7
	VI	<u>ap-ap^D</u> = 0
III	<u>Mo</u>	<u>sh^S-sh^{HR&D}</u> = 0
<u>b-lod</u> = 24		

* Data of Harold L. Eddleman

T. confusum

I	II	III	Unassigned
<u>r-lp</u> = 48	<u>p-e₂</u> = 2.5 [†]	<u>lod</u>	<u>b-rus</u> = 41
<u>es-tet</u> = 53-55	<u>p-dpe</u> = 5		<u>b-msg</u> = 42
<u>lp-es</u> = 47	<u>p-cas</u> = 30		
<u>St-es</u> = 38 [*]	<u>dpe-cas</u> = 25		
<u>es-l₁</u> = 40			
<u>es-es^{lt}</u> = 0			
(<u>pas</u>)			
(<u>aer</u>)			

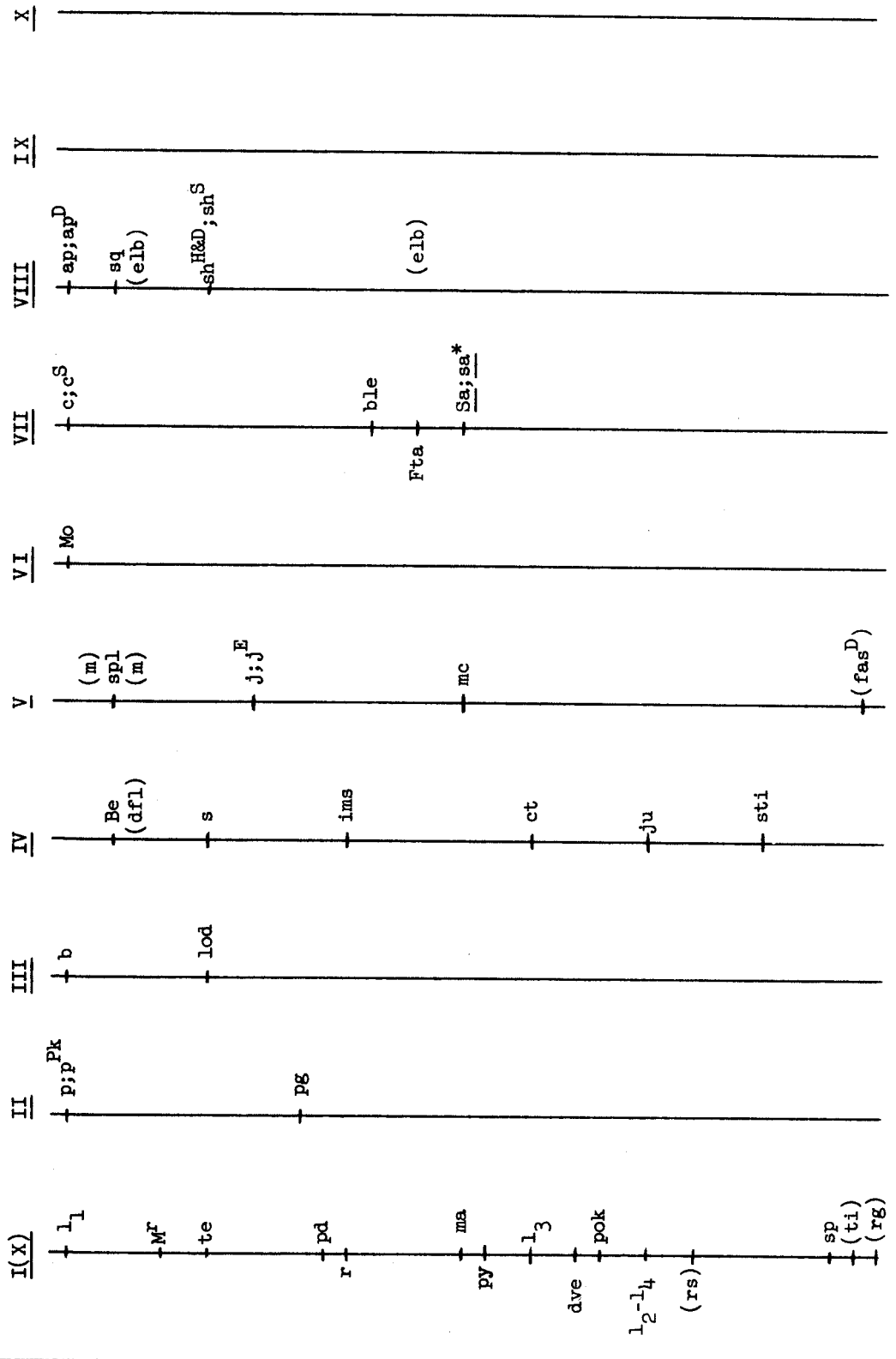
* Data of McDonald

† Data of Dyte and Blackman

Latheticus oryzae

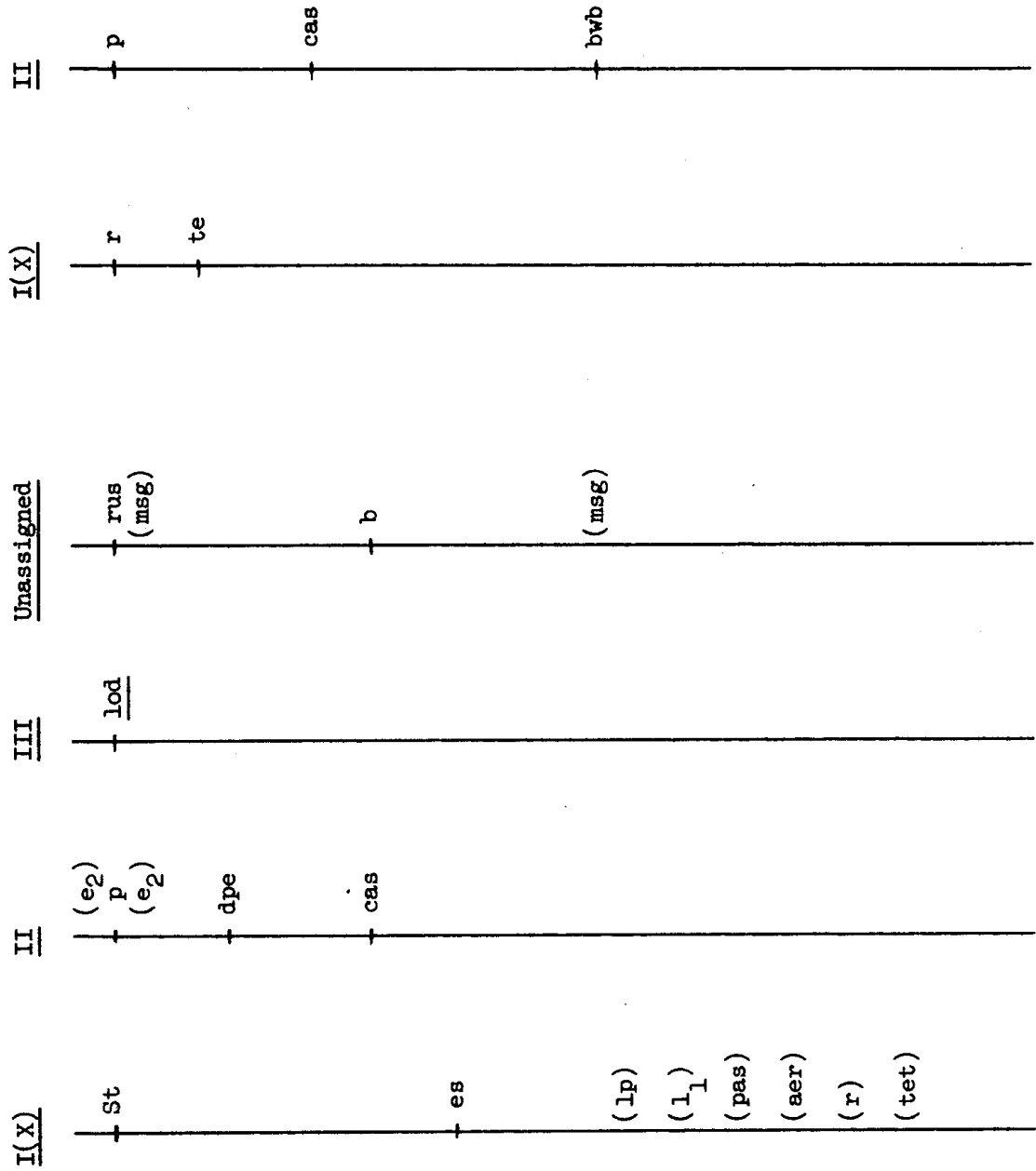
I	II
<u>r-te</u> = 6	<u>p-bwb</u> = 50
	<u>p-cas</u> = 36
	<u>bwb-cas</u> = 36

Linkage maps in T. castaneum



Linkage maps in Latheticus oryzae

Linkage maps in Tribolium confusum



SOKOLOFF, A.
Department of Genetics
University of California
Berkeley, California

*Linkage tests and sex in Tribolium.

It was reported last year (TIB-6, New Mutants section) that back-crosses of various genes associated with the seventh linkage group in Tribolium castaneum gave some peculiar results. Crossover values in the male (regardless of whether the genes entered the crosses in coupling or repulsion) were inflated to the point that the parental and recombinant classes were equal. This phenomenon is a real one, and a check on the literature suggests that this situation may arise when chiasmata (which according to Smith are usually one per chromosome arm in beetles) are unequally distributed in the two sexes. At present it is not known whether this phenomenon is a general one for all the autosomes, or whether the phenomenon will also be found to be true for other Tribolium species. But for those who may be contemplating linkage studies: do the crosses both ways. Otherwise linkage relationships may be missed. (More information on this point will appear in Genetics in the near future.)

SOKOLOFF, A.
Department of Genetics
University of California
Berkeley, California

*Three unusual aberrations in Tribolium castaneum.

From time to time teratological specimens having strikingly abnormal structures have been found. A preliminary report was given in TIB-3: 30-33. The purpose of this note is to record the finding of three more aberrations not previously recorded. Whether they have any genetic basis is yet to be determined.

The first aberration is called "spikes on trochanters and antennae" (sta). It was discovered in the course of attempting to determine the frequency of crossing over between ap and sq. In two separate crosses were found some beetles which had a huge process arising from both antennae. Furthermore, when the pupal exuvium was removed, the trochanters also showed this process. Later it was found that the processes may not be too conspicuous, and they may be present on the antennae or legs or both.

Another, somewhat similar, aberration was found in crosses involving sq and Sa-2. The aberrant beetles showed a process arising from the antennae and from the tibia. The latter appears somewhat leathery and not completely sclerotized.

The third aberration was just recently found. It involves a duplication of the aedeagus. In this beetle these structures were side by side, just to one side of the midline. At this writing it is not known whether any of the two aedeagi is functional.

SOKOLOFF, A.
Department of Genetics
University of California
Berkeley, California

*Two abnormalities associated with microcephalic.

During the course of examining the mc stock or the mc progeny in crosses where this gene is involved, it has recently become evident that some abnormalities, not previously recorded (see TIB 4 for original description) are associated with this gene.

Both of the abnormalities involve the eye. The first results in an apparently larger bulging eye, a condition opposite from that first observed. A closer examination, short of counting facets, indicates that, at least the dorsal lobe of the eye may have one or two extra rows of ommatidia, bringing the total of diagonal rows paralleling the gena to about seven or eight (instead of the normal number of five or six). In view of the fact that the cranium in these beetles is reduced, it is not clear whether there is really an increase in ommatidia or whether the number remains the same, but the ommatidia are displaced from their former position resulting in the bulging appearance of the eye.

The other abnormality has been designated "eyegrowth" and it has been given the abbreviation eg only for purposes of description. eg is characterized by a growth apparently emerging from the eye, but it may originate from the gena, which emarginates the eye producing the dorsal and ventral lobes roughly in the area where the eyegrowths have been observed. (The reason for suspecting the gena as the origin of the eyegrowth is that such a growth may appear in areas where the eye fails to form.)

When first discovered, eg was a small, pearshaped, growth emerging from the eye, as if one of the ommatidia had increased in length, rising beyond the level of the other ommatidia. Since then, a large number of quite variable cases of eg has been observed, ranging from the pear-shape to a structure which appears segmented resembling the antenna. So far the eg condition has been observed only on one side of the beetle when present.

YAMADA, Y. and A. E. BELL.
Population Genetics Institute
Purdue University
Lafayette, Indiana

*Selection responses for larval size in
Tribolium under two nutritional levels.

The experiment carried out so as to evaluate the effectiveness of various selection methods under two nutritional levels in the presence of genotype-environment interaction.

A strain of Tribolium castaneum, so-called Purdue Foundation stock, was used as the experimental organism. The two levels of nutrition, designated here as GOOD and POOR rations, which were originally formulated by Dr. R. T. Hardin who was our graduate student, were used as the environments. The primary difference between the two rations depends on the content of brewer's dry yeast. The GOOD contains 10% of dry yeast but none at all in the POOR. The formulation and chemical composition of the rations are given in Table 1 and 2.

The character for selection is the 13-day larval weight. Genetic parameters of the base population for the character were: 0.25 ± 0.03 in heritability under both environments and the genetic correlation between GOOD and POOR performance was 0.62 ± 0.12 .

The experimental populations which were originated from the base population by random sampling are as follows:

- GL = selected for large under GOOD ration each generation,
- PL = selected for large under POOR ration each generation,
- GPL = selected for large on average performance under both levels,
- GPL = selected for large under GOOD and POOR in alternating generations,
- GS = selected for small under GOOD ration every generation,
- PS = selected for small under POOR ration every generation,
- GPS = selected for small on average under both levels,
- GPS = selected for small under GOOD and POOR in alternating generations,
- C = unselected control consisted of 20 pair matings, each contributes one male and one female to next generation.

The experiment was repeated twice but one week apart. Each set of replicated experimental populations was sampled from two different sub-lines of the base population two or three generations back to the onset of selection.

The mating and selection were made in such a way that each pair produces eggs in the creamer which contains standard wheat media for 48 hours, and then the parents are transferred to GOOD ration for 24 hours, POOR ration for 24 hours and additional GOOD or POOR ration for 24 hours, so as to have two creamers of the same ration for selection depending on the population, until the 7th generation. For instance, GL had two GOOD's and

one POOR, while PL had two POOR's and one GOOD. The GPL and GPS had only one GOOD and POOR. For each mating, randomly sampled five larvae for each creaser were weighed and the sum of two creasers were used as the selection criterion. The measurement taken under the opposite environment rather than for selection was used as the measure of correlated trait. Since the 8th generation inclusively, two GOOD's and two POOR's were measured for all populations in the same manner. Once the families for selection were decided, the full sibs raised under the standard media were picked up and sexed for mating to produce the next generation. Therefore, the parental individuals in all strains were never raised under GOOD or POOR level but standard media. The individuals measured for selection were discarded after weighing.

Results are summarized in Table 3.

Table 1

Gradients of Two Rations

GOOD	POOR
10% Vitamin premix	3-1/3% Vitamin premix
17% Soybean oil meal	11-1/3% Soybean oil meal
58% Corn meal	85-1/3% Corn meal
10% Dried brewer's yeast	
5% Corn oil	

Table 2

Chemical Composition of the Rations

	<u>Moisture</u>	<u>Protein</u>	<u>Fat</u>	<u>Ash</u>	<u>Unanalyzed carbohydrates</u>
GOOD	10.4	18.3	8.5	2.8	70.0
POOR	9.9	13.2	4.2	2.2	70.5

Table 3

Average Gain Per Generation Adjusted to Control ($\times 10 \mu\text{g}$)

<u>Ration</u>	<u>Strains</u>	<u>Rep I</u>	<u>Rep II</u>	<u>Average</u>	
GOOD	GL	5.79 \pm 1.24	7.42 \pm 0.49	6.60 \pm 0.38	
	PL	3.71 \pm 0.29	7.00 \pm 0.41	5.36 \pm 0.29	
	GPL	5.07 \pm 1.13	5.45 \pm 1.01	5.26 \pm 0.34	
	GPL	5.00 \pm 0.49	6.84 \pm 0.52	5.92 \pm 0.14	
	GS	-8.77 \pm 0.75	-12.37 \pm 1.09	-10.60 \pm 0.80	
	PS	-9.06 \pm 0.91	-8.50 \pm 1.37	-8.78 \pm 1.05	
	GPS	-9.72 \pm 0.43	-7.27 \pm 0.54	-8.50 \pm 0.59	
	GPS	-9.79 \pm 0.81	-9.19 \pm 1.05	-9.49 \pm 0.87	
	POOR	GL	5.46 \pm 0.97	6.37 \pm 1.23	5.91 \pm 0.94
		PL	9.36 \pm 1.36	10.38 \pm 0.81	9.87 \pm 0.96
GPL		8.82 \pm 1.16	5.69 \pm 0.84	7.25 \pm 0.74	
GPL		9.03 \pm 1.42	7.64 \pm 0.77	8.34 \pm 0.84	
GS		-5.90 \pm 0.40	-4.94 \pm 0.59	-5.32 \pm 0.33	
PS		-8.48 \pm 0.50	-6.58 \pm 0.55	-7.53 \pm 0.47	
GPS		-6.74 \pm 0.65	-5.46 \pm 0.39	-6.10 \pm 0.40	
GPS		-6.57 \pm 0.50	-5.50 \pm 0.45	-6.03 \pm 0.41	

YAMADA, Y. and A. E. BELL
 Population Genetics Institute
 Purdue University
 Lafayette, Indiana

*Relative fitness of selected strains of Tribolium under two nutritional levels.

After termination of selection for 16 generations all 16 selected lines described in the preceding report were subjected to measure fitness (number of adults emerged from randomly sampled 50 eggs) relative to the Purdue Black Foundation stock marked with dominant black.

In order to measure the characteristics, 50 eggs each from a selected strain and from Purdue Black were put into a vial containing 20 grams of media, either GOOD or POOR, and counted the number of wild and Black adults alive on the 35th day. The experiment was replicated six times for each line on each environment.

Repeatability of the character under two nutritional levels were 0.9070 and 0.9160 under GOOD and POOR rations, respectively.

All of large lines showed higher fitness under both environments while all small lines showed lower fitness under both rations relative to unselected Black line. Analyses of variance showed that there are distinct differences in relative fitness among selected lines including large and small lines together and strain by environment interaction was also highly significant. However, further analyses showed that no appreciable difference was observed among large strains and no interaction was detected. On the contrary, strain difference and strain by environment interaction were highly significant for small lines. This suggests that the relative fitness of these selected lines should be measured on various environmental conditions and thus adaptation of some strains selected on a particular environment can be detected.

WELCH, J.
Pest Infestation Laboratory
Slough, Bucks, England

*Culturing Gnathocerus cornutus

Cannibalism is a well known cause of poor yield from cultures of Gnathocerus cornutus and it is often supposed to be associated with water requirements. The need for large numbers of G. cornutus for tests of fumigant toxicity has prompted a study of culturing methods with special reference to water requirements.

Results so far obtained have shown no increase of egg numbers when adults are offered free water by placing them on saturated blotting paper. In fact somewhat larger numbers of eggs were obtained from adults given no water. In these tests the difference was not significant but Howe (1962 Bull. Ent. Res. 53: 301-310) reports finding that the provision of water depressed oviposition of Tribolium castaneum.

SLADEN, BRENDA K.
Department of Pathobiology
Johns Hopkins University
School of Hygiene and Public Health
Baltimore, Maryland

*A Tribolium teaching experiment.

Introduction -

This experiment has been run once a year, for five years, for a class in Public Health at Johns Hopkins University. The 45 members of the class are mostly post-graduate MDs, studying for the Master or Doctor of Public Health degrees. Some of them are preparing for research and administration in human demography, space biology and medicine, and in other biostatistics. The experiment is part of a course designed by Dr. F. B. Bang, called "The Biology of Populations." Dr. T. Park gave advice when the experiment was initiated.

The experiment is run with a simple incubator. For humidity control the populations are kept above a saturated aqueous solution of sodium chloride, at constant temperature. At 34° C, the relative humidity should be constant at somewhere near 70%. (Diagram attached.)

Experiment 2 -- Experimental Demography with Flour-Beetle Populations

Purpose

Populations of Tribolium confusum flour-beetles will be grown in constant environmental conditions, with the food medium regularly renewed. In these conditions, regardless of their starting sizes, the populations all develop and maintain the same fairly constant density. They are regulated mainly by mortality rates, which at some stages of the life-history are very sensitive to the population-density of other stages. The experiment will be a partial demonstration of this population control, and also an exercise in use of demographic data. (The interpretation of the Tribolium model will be simplified.)

Materials

Life-history of Tribolium confusum

At 34° C and relative humidity of 70%, the life cycle is approximately:

Eggs: hatch at 4 days.

5 or 6 larval stages, with a molt between each stage, totaling 17 days. It is not possible to age them nor to distinguish sexes on sight.

Pupa: 5 days. The sexes are distinguishable.

Adult: Average life-span a year or more. Eggs are laid 5 to 8 days after the female emerges as an adult. Fertility and

fecundity remain high for a much longer period than this experiment; and during this time, the egg-laying rate can be taken to be 15 eggs/female/day.

To summarize, the initial females lay eggs throughout the experiment, and 26 days elapse between egg-laying and appearance of the adults from those eggs.

Culture of the beetles

Tribolium beetles are found in stored grain and cereal products, and the laboratory environment will closely match the natural one. The beetles will be cultured in small vials of sifted unadulterated wheat flour, containing 5% dried yeast. Nine different initial population densities will be used, and there will be 10 replicates of this series of 9. All other conditions for the populations will be kept uniform: the starting ages of the adults will be 7-14 days, 8 grams of medium per vial, temperature 34° C and relative humidity constant at about 70%. The sex ratio will be 1:1. The 90 populations will be allowed to grow for 6 weeks, with a renewal of the flour at 3 weeks.

Experimental Procedure

Summary of the experiment

Count of initial adults	21st day.			47th day.
	Count of dead adults (flour renewed)			Count of live adults
*	*	*	*	*
-----	-----	-----	-----	initial adults
.....	----->	
eggs (4 days)	larvae (17 days)	pupae (5 days)	----->	new adults
			----->	
			----->	
				etc.

There will be 10 replicates of each of the following initial population sizes:

2	(1 ♀, 1 ♂)
8	(4 ♀, 4 ♂)
12	(6 ♀, 6 ♂)
18	(9 ♀, 9 ♂)
36	(18 ♀, 18 ♂)
50	(approximately equal sexes)
100	(" " ")
200	(" " ")
300	(" " ")

total of 90 vials

Handling beetles

Treat with care. For counting, live adults can be brushed from a petri dish into a spoon; or it may help to flip them on to their backs. To avoid contact with parasites of cockroaches, keep spoons, needles and paintbrushes on the paper, and do not let beetles run on bench.

1st day:

Each person inoculate two vials with assigned numbers of beetles.

Label vials with replicate number, initial population size and your name.

21st day:

Place all the contents of a vial in a sieve. Sift away the old flour and discard it. Gently blow away shed larval skins.

Remove dead adults and count them.

Transfer from the sieve to a new vial of fresh medium, all eggs, larvae, pupae and live adults. Transfer the label. Check that no eggs have been left behind in vial or sieve.

47th day:

Place the contents of a vial in a sieve and sift away the flour. Count the live adults.

RESULTS

Data will be collected from all the class, means calculated for you, and all mimeographed and redistributed--use these means for your calculations. Please have the calculations done before the class discussion.

Results--continued

1. a. On one graph show changes of all population sizes with time.
b. On another graph show final increment of adults per initial female, against initial adult population size.
2. Calculate two mortality rates for each initial population size
 - a. adult mortality rate during the first three weeks. Plot against initial population density.
 - b. mortality rate during period from egg to emergence of adult (assume the laying rate of 15/female/day). Plot against initial population density.
3. Do any statistical treatments of interest to you.

QUESTIONS

1. Very briefly summarize results, and relate to what is known of the causes.
2. What is the significance in regulation of population numbers, of birth rates and mortality rates that change rapidly with change of population density?
3. Suppose a whole animal community (i.e., of many species--populations) to be damaged by a pesticide. List reasons why some species-populations will recover faster than others.

RESULTS OF TRIBOLIUM EXPERIMENT: Number of Dead Adults at 21 Days.

<u>Replicate #</u>	<u>Initial Population Size</u>								
	<u>2</u>	<u>8</u>	<u>12</u>	<u>18</u>	<u>36</u>	<u>50</u>	<u>100</u>	<u>200</u>	<u>300</u>
I	0	0	1	0	3	1	1	4	35
II	0	0	1	0	1	0	1	9	13
III	0	1	0	0	0	5	2	9	n.d.
IV	0	1	0	0	3	0	4	12	11
V	0	0	0	0	1	1	9	2	20
VI	0	1	1	0	1	6	1	5	7
VII	0	0	1	0	0	1	5	3	8
VIII	0	0	1	0	1	3	7	2	n.d.
IX	0	0	1	1	1	5	0	4	10
X	0	0	1	0	1	4	2	6	n.d.
Total	0	3	7	1	12	26	32	56	104
Mean	0	.3	.7	.1	1.2	2.6	3.2	5.6	14.86
% Adult mortality rate/3 weeks	0	4	6	1	3	5	3	3	5

(n.d. = not done)

RESULTS OF TRIBOLIUM EXPERIMENT: Number of Live Adults at 47 days.

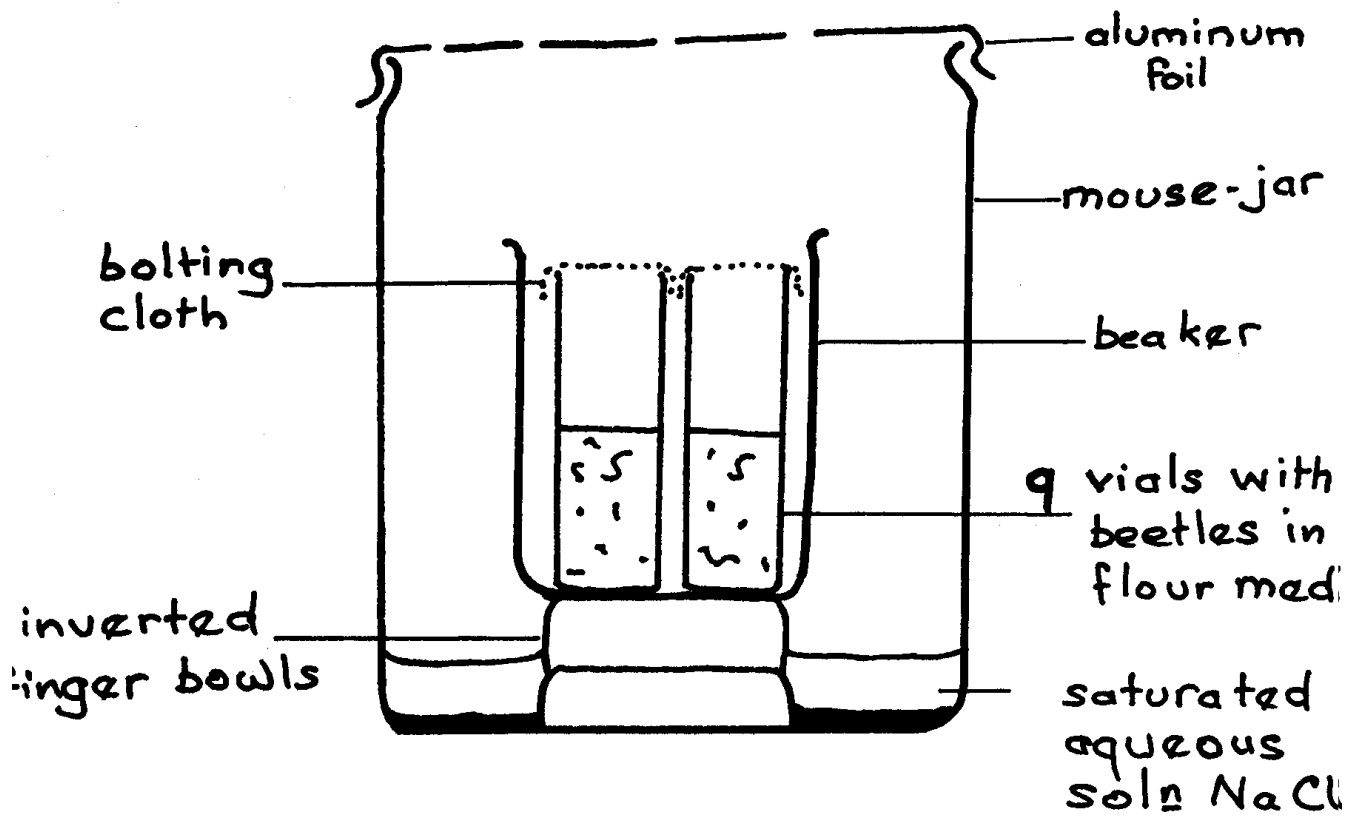
<u>Replicate #</u>	<u>Initial Population Size</u>								
	<u>2</u>	<u>8</u>	<u>12</u>	<u>18</u>	<u>36</u>	<u>50</u>	<u>100</u>	<u>200</u>	<u>300</u>
I	73	247	339	238	230	86	105	189	224
II	104	191	183	254	257	182	104	191	313
III	44	281	223	135	180	130	179	191	283
IV	?	242	260	207	591	78	114	202	n.d.
V	94	218	214	245	342	128	85	206	273
VI	66	317	247	437	222	125	136	201	301
VII	111	248	181	230	264	94	94	208	295
VIII	126	177	268	287	152	186	94	197	n.d.
IX	132	232	288	321	142	70	189	261	283
X	98	249	416	281	205	202	125	182	n.d.
Total	848	2402	2619	2635	2585	1281	1225	2028	1972
Mean	94	240	262	263	258	128	122	203	282

Calculated
% mortality
rate from
laying to
emergence of
adult*

70.8 81.8 86.8 91.3 96.1 99.0 99.9 100.0 100.0

n.d. = not done

* Assuming a constant laying rate of 15/♀/day, and ignoring adult mortality.



10 mouse jars are used, with a series of nine populations in each.

EDDLEMAN, H. L.
Department of Biological Sciences
Purdue University
Lafayette, Indiana

*Some comments on color designations.

Since a large number of eye and body color mutants are known in the *Tribolium* species, it would be useful to compare each new mutant to color samples of a well-known color system and to report the number of the nearest matching color sample as part of the description of the mutant. This practice often releases one from the worrisome task of trying to think of an appropriate name for the mutant.

Color samples may have trivial names such as Four-leaf Clover or Love-in-a-mist. In such cases one may refer to National Bureau of Standards Circular 553 which lists each color name under a general category such as Moderate Yellowish Brown. Circular 553 describes most color systems that are of value to the biologist and offers techniques for comparing microscopic and macroscopic objects, liquids, and powders to color charts.

The light source employed will influence color matching. Light from a north window on a sunny day with blue skies is the universal standard illumination for color matching, but microscopic objects usually force one to use artificial light.

Three currently available color systems are especially suitable to biologists:

Jacobson, E. 1948. Color Harmony Manual. Container Corporation of America, Chicago, 3rd edition, 973 removable solid plastic color chips arranged according to the Ostwald color solid. Approximately \$150.00.

Maerz, A. and M. Rea Paul. A dictionary of color, 2nd edition. McGraw-Hill Book Company, Inc., New York, N. Y. About 1,000 colors printed on paper. Approximately \$20.00.

Munsell Color Company, Munsell Book of Color, current edition. Munsell Color Company, Inc., Baltimore, Maryland. Consists of color chips mounted on paper. This is one of the oldest currently used color systems.

Kelley, K. L. and D. B. Judd. 1955. The ISCC-NBS method of designating colors and a dictionary of color names. National Bureau of Standards (U.S.A.), Circular 553, 158 pp. This reference does not contain any color samples, but lists colors given by other systems under 267 general color categories.

NARDON, P.
Département de Biochimie
Institut National des Sciences Appliquées
Villeurbanne, France

*A technique to obtain virgin Sitophilus.

As Sitophilus oryzae and S. sasakii copulate a short time after they emerge from the grains, virgin insects are very difficult to obtain by the usual rearing technique. However, they can be obtained in the following manner:

Insects are made to lay eggs in plastic boxes or tubes, the bottom of which is closed with wire netting. They are given five grains each per day, thus insuring that only one egg will be laid in each grain.

After the time necessary to produce a sufficient number of progeny, the parents are removed. A few days before the adults begin to emerge, the wheat grains are placed in hatchers. These are lucoflex plates (Polyvinyl chloride made in France by Saint-Gobain) measuring 125 × 125 × 15 millimeters, drilled with 100 holes of 9 × 14 mm. Only one grain is placed in each alveole and the hatcher is covered by a glass plate of 125 × 125 mm held in place by two rubber bands. Thus, insects are isolated from each other at birth. They can easily be observed through the glass. Insects can live a very long time in these alveoles. Furthermore, it is possible to study the complete life cycle and simultaneously to observe the behavior of several insects.

SOKOLOFF, A.
Department of Genetics
University of California
Berkeley, California

*A technique for counting eye facets in Coleoptera.

Owing to the fact that the compound eye in at least tenebrionids is located on the ventral, lateral and dorsal aspects on the head, the eye is pigmented black and the ommatidia may not be very distinct, determination of the facet number in etherized beetles, especially of the small variety, may be difficult, and even when the ommatidia total less than 100, subject to considerable error.

A technique which may prove useful in such work is the following: beetles are etherized and their heads dipped first in ethyl acetate, and then in an acrylic plastic (made thin with ethyl acetate), a sufficient number of times to form a thin film over the head. (It is considered

thick enough when, on drying, the head appears as if enveloped in a pupal skin.) The plastic is then split along the ventral and dorsal midlines of the head with jeweler's forceps, and the two halves mounted, external surface downwards, on a slide. Essentially what is obtained is a negative mold of the eye where ommatidia number is much more accurately determined.

SONLEITNER, F. J.
Department of Entomology
University of Kansas
Lawrence, Kansas

*A note on preparing colored flour.

Colored flour has been used to mark eggs in studies on egg cannibalism.† Various types of vital stains can be used; neutral red being the one most widely used to date. A number of tests have been made indicating that this dye does not affect the egg or its cannibalism by adults. (Sonleitner, 1961 found a higher mortality for methylene blue-marked eggs.) Preparation is simple. 0.2 - 0.5% by weight of the powdered neutral red is mixed with fine-sifted medium. The acquisition of the color by the flour takes some time (about a week or more) and depends on the moisture content of the flour. Thus colored flour should be made well in advance of the time when it is needed. The development of the color can be accelerated by having the flour acclimatized with respect to moisture content before the stain is added. If the new mixture is constantly stirred, by keeping the mixing bottle continually rotating or rolling back and forth, the first appearance of a faint color can be detected usually within a half hour. A stronger color will develop if the mixture is then kept in a humid incubator. The addition of beetles will hasten the appearance of the color. During the first sifting through fine mesh to remove eggs a great many larger lumps of the stain will be removed. Because these powdered dyes are very effective even in minute amounts, care should be taken during the preparation and the earlier sifting operations to prevent the laboratory from turning red by the dispersal of these and finer particles of dye. The color of the flour will continue to deepen and intensify as long as the egg farm is being used. Flour to which originally was added as little as 0.1% neutral red became deep brick red after several months of use.

† See Rich, 1956, Ecology 37:109-120; Polnik, A. 1959, Physiol. Zool. XXXIII:42-57; Sonleitner, 1961, Physiol. Zool., XXXIV:233-255.

WONG, W. C.
Animal Science Department
University of Manitoba
Winnipeg, Canada

*Simplified painting technique for individual identification of beetles

In some experiments individual identity of adult beetles is essential. Painting the dorsal surface of adults with quick drying paint (a different color for each individual in a container) is a common technique. Identification by painting needs to be done with accuracy, speed and without injury to the beetles.

Immobilization by hypothermy, described by Sonleitner (TIB-5), was initially employed here when painting adults to be used in matings in a selection experiment. Several females are placed (mated) with a single male in a creamer jar. Use of the hypothermy technique was not satisfactory for our purposes, primarily because the beetles tended to fold their legs and fall over on their side or back, making painting difficult. In addition, because the beetle was immobilized it could not hang on to anything and tended to stick to the brush when paint was applied.

The method described here is without immobilization. Prepare four petri dishes with blotting paper fitted into the bottom. Place a beetle onto the first petri dish and paint it (we use a No. 2 artist's brush). The beetle may run on the paper, but it is not difficult to touch its back with a brush. As a matter of fact, the beetle will stop running when it is touched. Furthermore, the beetle will not stick to the brush, because it hangs on firmly to the paper. After painting the first beetle, place a second beetle onto the second petri dish and paint it. Do the same for a third and fourth beetle. By the time the fourth beetle is painted the first beetle is dry. The first beetle is then transferred to a mating jar and a new beetle is placed in the first petri dish. The procedure is then continued. The paint color of each individual is decided in advance and recorded. In our case, after all beetles are painted we have a male and four females in a jar, each a different color. About 40 can be painted and transferred to the appropriate mating jar in an hour.

In our experiment we keep the adults about one month, after which they are destroyed. The paint will last this length of time and possibly much longer, although it tends to flake off if applied too thickly. We use a quick drying paint obtained from a hobby shop.