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Foreword i

Announcement ii

Poem-Tribolium Supreme Ora Lee Matthews McCoy Watts iii

Notation on Stock Lists iv

Stock Lists 1

New Mutants 86

Notes - Research 87-147

Survival rate at different stages of development in

Tribolium castaneum. Pran P. Bhat 87

Estimation of genetic variation and heritability of

Ovariole number in Tribolium castaneum.

Pran P. Bhat and N. Puskhar 90

The map position of Charcoal. Eric Brown and

Alexander Sokoloff 95

A case of pseudoallelism involving body color genes in

Tribolium castaneum. Eric Brown and Alexander Sokoloff 96

Pattern of sorbic acid sensitivity in Tribolium confusum

Embryos. F. Dunkel, E. De Las Casas and P.K. Harein 98

Comparison of natural selection for three deleterious

Genes in Tribolium castaneum. DuWayne C. Englert, Wehr

Englert and Nancy L. Englert 102

Size as a factor in the infection of Tribolium spp and

Eleodes sp by Hymenolepis diminuta. Sarojam K. Mankau 110

Natural selection on adult body weight in Tribolium castaneum.

David E. McCauley 114

New strategy to maximize the egg-laying of virgin female in

Tribolium castaneum with reciprocal recurrent selection.

G. Palomares 116

Ultrastructural characterization of a single gene effet on

The egg surface of Tribolium castaneum.

Clara V. Riddle and Russel A. Riddle 117

Variability for dispersal behavior in a wild population of

Tribolium castaneum. Uzi Ritte and Zvia Agur 122

Stage and syndrome of lethality in Tribolium castaneum

Homozygous for Fta. K. Sander and I. Kuld 132

Subpopulations of Tribolium castaneum (Hbst.) resulting

From routine culture maintenance. H. W. Smith 134

Observations on a natural population of Tribolium brevicornis

LeC. A. Sokoloff, D. Faustini, M.A. Sokoloff and

E.A. Sokoloff 135

The effect of culturing together on adult longevity of

Tribolium audax. M. Hani Soliman 139

Rate of egg production in fecundated and virgin females

Of Tribolium castaneum. V.K. Taneja 141

Clustering of nuclear pores to “nuclear sieves” in some

Oedionchina flea beetles. N. Virkki 143

The effect of propagule sex ratio upon the number and sex

Ratio of the adults produced in a 37 day interval by

Tribolium castaneum. Michael J. Wade 144

Tribolium confusum brei in vitro. V.F. Wright, E. De Las Casas,

S. Olson and P.K. Harein 146

Notes - Technical 148 – 154

Automated cultivation of a chain of polymiktic

Generations of flour beetles. Wolfgang Briegleb 148

Some modifications to culturing techniques for

Tribolium. R.E. Goodman and J. R. Humphrey 149

A simple technique to immobilize Tribolium adults.

R.G. Ruano 151

A new dye for use in coloring Tribolium eggs

Steven B. Teleky 153

Bibliography

1 Anatomy, Histology and Morphology 155

2 Behavior 157

3 Insect Tissue Culture and Embryology 159

4 Cytology ad Electron Microscopy 161

NEW MUTANTS

Report of M. Hani Soliman

Tribolium audax

1. Brown (br). Discovered in 1974 in a population obtained from Dr.A. Sokoloff. The ‘br’ vene was found to be semi-dominant. The heterozygote “br/+” produces a chestnut body colour. The homozygote recessive |br/br” resembles the normal colour in T. castaneum, while the wild type is black.

The heterozygote has a slightly faster developmental rate than the homozygote recessive which is as fast as the wild type. Although the wild type showed the highest viability and the homozygote recessive the lowest, the difference was not significant.

**NOTES -RESEARCH**

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\*Survival Rate at Different Stages of Development in Tribolium castaneum.

An experiment was conducted to study rate of survival at different stages of development in Tribolium castaneum. Material and culture methods used in this experiment were the same as reported by (Bhat and Bhat Tribolium Inform. Bull. Vol 17 : 82-87).

The eggs were collected from PAU-I foundation strain, 36 females, on 7th, 8th, 9th, 10th and 11th day of life. These eggs were counted and kept for hatching and subsequent adult survival. Table 1 details the various parameters and adult survival. The hatchability was 95.27%. Sokoloff and Ho (1962) have given an average hatchability of eggs as 80.23% and percentage of eggs hatching as adult as 77.0%. The percentage of larvae forming adults was around 90%. Indicating a 10% mortality after the larvae are formed. There was no significant difference in hatchability between days. Survival of larvae based on the total number of eggs set was 88.57% and based on the number hatched was 92.95%. Percentage survival of pupae was 84.70 based on eggs set. The percentage calculated on the basis of number of eggs hatched was 89.16% and based on survived larvae was 95.91%. There was no significant drop in rate of survival from larvae to pupae, there was a significant drop in survival by 15.30 from egg to pupa. Percentage survival of adults based on the number of eggs set was 80.40%, a significant drop by 19.60%, Based on the number of eggs hatched was 84.39%, a drop in survival by 10.82% and based on number of survived larvae was 90.77%, a drop in survival by 9.23%, based on surviving adult pupa was 94.64%, a small drop by 5.36%. It is obvious that the most sensitive period of development was from egg to pupa especially from hatched egg to larva. Sokoloff et al. (1966) also showed that the most critical period in the life cycle was from hatching to larval development.

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\*Estimation of Genetic Variation and Heritability of Ovariole number in tribolium castaneum

Tribolium castaeum has been extensively used by a large number of workers to study the quantitative genetic theory. Genetic variation and heritability has been investigated for growth and larva weight (Hardin 1962; Yamada and Bell 1964) for pupal weight (Bray et al., 1962, Bhat, 1965). Ovariole number though strongly connected with fitness has not been studied so far.

Material and culture methods used in this experiment were the same as reported by (Bhat and Bhat in Tribolium Inform. Bull. Vol. 17: 82-87).

A large population of pupae from the PAU-I foundation strain was sexed and 300 females and 100 males were drawn at random. The females were kept in three culture bottles each containing 100 pupae when they were 9 days old, in one group left antenna was cut, in the second group. right antenna was cut, while in the 3rd group both left and right were cut for identification. Three females were allotted to each male at random, in this way thirty sire families were established in one replicate and similarly another replicate was made up with thirty sire families. Each family was kept in a small vial with standard medium. Individuals were allowed to mate for 10 days. On the 10th day the females were separated in single vials, each vial containing properly labeled single female. 48 hour egg collection of each female was taken. The pupae were sexed and separated. The female pupae were watched for emergence. The adult females were dissected and ovariole number per ovary and per individual was recorded. For genetic analysis a statistical model involving sires, dams within sires and progeny within dams   
and sires was used.

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The mean ovariole number per ovary was 5.86 + 0.59, with a coefficient of variation of 10%. The mean ovariole number per female was 11.51 + 0.76, with a coefficient of variation of 6.5%.

Replicate differences were not significant. Table I details the analysis of variance of ovariole number. The sire and dam component were both negative, consequently the estimate of heritability from sire variance was small and negative (h2 = - 0.073).

Estimate of genetic variance by the intrasire regression of daughter on dams was  
 o2A = 0.04, and heritability was 0.14. The results are detailed in Table II.

A large number of experiments have been reported with Tribolium castaneum to study the genetic variation and heritability for growth and larval weight (Hardin, 1962, Yamada and Bell 1964) for population time (Englert 1964) for pupal weight (Bray, 1962, Bhat 1965). All these workers have reported varying estimates of genetic components for these traits.

So far there has been no report on the genetic variation of ovariole number in Tribolium castaneum.

Robertson (1957) reported a mean ovariole number of 21.6 with a coefficient of variation of 13.3 in Drosophila melanogaster. A range of 16.4 to 24.5 was observed in various stocks. He reported the cumulative heritability of ovariole number as 0.46 after 10 generations of two way selection. Starting with a mean ovariole number of 44 in the first generation in 10 generations the ovariole number increased to 62. An appreciable genetic reduction in ovariole number reduced egg production. The estimate of genetic variance in our case was small and negative by half sib analysis and small by intra sire, regression. Dickerson (1959) suggested that this situation could arise from two possible reasons. Firstly the estimates of variance components could be negative from sampling, a necessary and sufficient condition for that to hold would be that size should have been small. In this case the sample size per sire was fairly large, about 7.81 progeny per male were scored. Second possibility is that the genetic variation with respect to this trait was really small and not different from zero. This observation is supported by the fact that the range between two extreme in this trait is as small as

4 ovarioles.

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TABLE - I : ANALYSIS OF VARIANCE FOR OVARIOLE NUMBER IN

TRIBOLIUM CASTANEUM

Sorce of variation D.F. S.S. M.S.

Between sires 54 2.9204 0.064  
Between dames within sires 90 7.7360 0.086

Between progeny within

dams within sires 286 107.7800 0.377

Kl = 2.97, K2 = 2.95, K3= 7.81  
 62e = 0.377, 62d = -0.097, 62s = -0.005,

h2 = -0.073

TABLE II : ANALYSIS OF VARIANCE OF INTRA SIRE REGRESSION

OF DAUGHTER ON DAM

Source of variation Dams Variation Dam Daughter covariane

D.F. S.S. M.S. S. S. M. S.

Between sires 50 39.7800 0.7956 3.2733 0.0655

Within sires 84 6.9240 0.0824 1.1450 0.0136

Dam variance Dam daughter covariance

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\*The map position of Charcoal

Charcoal (Chr), an autosomal dominant with recessive lethal effects, is a body color mutation resembling, but distinct from black. Previously, Sokoloff (1977) determined that black (b), aureate (au) and light ocular diaphragm (lod) are linked, and the frequency of recombination frequencies are : b – au = 39.79 + 1.78, au – lod = 27.67 + 1.62 and b – lod = 13.79% when the heterozygotes are males and b- au = 37.43 + 1.21 and b – lod = 21.05 + 1.51% when the heterozygotes are females.

We now report that Charcoal (Chr) is in the vicinity of b. This is suggested by the recombination frequencies (Chr – au = 44.11 + .50; Chr – lod = 17.67 + .38; and

au – lod = 28.08% for heterozygous males and Chr – au = 41.28 + .49; Chr – lod =

20.03 + .40 and au – lod = 22.19 + .42% for heterozygous females) and by the tests of allelism : Chr/+ X b/b give black and bronze F 1, and backcrosses of Chr+/+b

X +b/+b produced 8/11,853 = 0.07% recombinant bronze beetles and the reciprocal cross gave 2/14,583 or 0.014% recombinants. It is suggested that the order of these pseudoalleles is Chr – b – lod – au.

This investigation was supported by U.S. Army Research Office grant DRXRO-CB-14447-L.

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Sokoloff, A. 1977. Sex and crossing over in linkage group III of Tribolium castaneum.

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\*A case of pseudoallelism involving body color genes in Tribolium castaneum

The third linkage group of Tribolium castaneum is identified with black, an autosomal semidominant gene which was probably the second mutant discovered in this flour beetle. A recent study (Sokoloff, 1977) has established that black (b) and the autosomal recessive mutants aureate (au) and light ocular diaphragm (lod) are linked. The distances between b and au = 39.79 + 1.78 units; au – lod = 27.67 + 1.62 units and b – lod = 13.97 units in the males, and b – au = 37.43 + 1.27 units; au – lod = 18.32 + 1.21 units and b – lod = 21.05 + 1.51 units in the females. The order of the three genes is the same in the two sexes : b – lod – au.

A more recent study with Charcoal (Chr), (an autosomal dominant with recessive lethal effects) has determined that it too is linked with au and lod. The recombination values are: Chr - au = 44.11 + .50 units; Chr – lod = 17.67 + .38 units; and au – lod = 28.08 + .45 in the males, and Chr – au = 41.28 + .49 units; Chr – lod = 20.03 + .40 units; and   
au – lod = 22.19 + .42 units in the females. The order of these genes is, therefore,   
Chr – lod – au in the two sexes, the differences in recombination values being attributed to differences in genetic background.

These linkage data suggest that Chr and b are either alleles of ech other, or pseudoalleles. We have carried out experiments which suggest that b and Chr are pseudoalleles.

Crosses between Chr/+ and b/b produce two types of beetles: one group is phenotypically as dark as the black strain, and indistinguishable from b/b beetles. The other group is genetically +/b and phenotypically these beetles are bronze, a color characteristic of the heterozygotes +/b which is expected.

Using every precaution to prevent contamination, we crossed Chr+/+b females back to black males. We obtained 8/11,853 bronze beetles giving a recombination value of 0.07 per cent. While in the reciprocal cross, Chr+/+b X b/b we obtained 2/14,583 bronze beetles, or a recombination value of 0.014%.

Hence the two genes, b and Chr cannot be considered alleles (occurring in the same locus) but they must be considered pseudo-alleles.

We do not have three point crosses to give the exact order of these four genes, but the linkage data so far obtained suggests that the order of the genes is probably Chr – b – lod – au, with Chr and b very close to each other.

This investigation was supported by U.S. Army Research Office grant DRXRO-CB-14447-L

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\*Pattern of sorbic acid sensitivity in Tribolium confusum embryos.

Sorbic acid is a short chain di-unsaturated fatty acid which produces no significant chronic or acute toxic effects when taken orally by small mammals (Tanase, 1974, Gaunt, et al., 1975). It is used as a broad spectrum fungistat in many foodstuffs including cereal products and is inexpensively synthesized. Sorbic acid is also ovicidal for stored product Coleoptera at levels approved for human consumption (Boush et al., 1968, Burkholder et al., 1973, Baker and Mabie, 1973). In our studies to determine the insecticidal mode of action of this food additive, it was found that the Tribolium Confusum embryo is sensitive to sorbic acid only during the first 24 hours after oviposition. Further information on the pattern of its sensitivity during this period was desired.

Oxygen consumption measurements were obtained with an 8 flask recording Gilson differential respirometer. Eggs were obtained within a 2 hour period from surface sterilized adults and maintained in the respirometer at 30 degree C and 93% R.H. Under these conditions, the period of embryogenesis is 115-120 hours. Sorbic acid was added to the autoclaved whole wheat flour containing 5% yeast. Sample size was   
200 eggs/flask with 2 replications at each concentration.

At concentrations of 0.25% and 0.50% sorbic acid, mortality was not observed unless eggs were laid directly in the treated flour (Fig. 1). Only then did significant (P .001) mortality occur after an 18 hour exposure with 0.50% and after a 24 hour exposure with 0.25%. There was no difference in the pattern of sensitivity with 4.60% and 8.00%. These higher concentrations caused 100% mortality when eggs were exposed at (1) oviposition and for only 2 hours thereafter and (2) 18 hours after oviposition for the remainder of embryogenesis.

At levels of sorbic acid which produce 100% mortality, no metabolic activity was detected in embryos. At levels of sorbic acid which produce 50-75% mortality, embryos began to respire at a rate similar to controls, but a significant difference (P .001) was detected at 84 hours postoviposition (Fig. 2). At sub-lethal exposures there was no detectable difference between the test and control patterns of O consumption.

These results suggest that 4.6% sorbic acid is acutely toxic to T. confusum embryos on contact. The toxic effect of 0.25% is detectable when monitored by O consumption late in embryogenesis. However, in both mortality patterns, exposure must occur in the first 24 hours after oviposition and the most critical time during this period is the 2 hours immediately after (and including) oviposition. Events occurring during this period that may be interrupted by sorbic acid are synthesis of the vitelline membrane which confers water impermeability, closing of the micropyle, blastoderm formation and drying (polymerization) of the exochorion.

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\*Comparison of natural selection for three deleterious genes in Tribolium castaneum

Three autosomal dominant mutations in Tribolium castaneum,

Three autosomal dominant mutations in Tribolium castaneum, each lethal when homozygous, were examined to determine their rate of elimination from laboratory populations under extreme competitive conditions. The three mutations used were short antenna (Sa), Deformed (Df), and bar eye (Be). For detailed descriptions of the three mutations, consult Sokoloff (1966).

Four populations were initiated for each of the mutant strains, using 10 pairs of mating adults possessing the desired heterozygous genotype. The four populations were examined as follows:

Population 1 – Censused at 40-day intervals for 240 days, with all contents of the   
 population box being replaced after censusing.

Population 2 – replication of Population 1.

Population 3 – Censused at 120-day intervals for 240 days; all contents replaced  
 after censusing.

Population 4 – Censused at 40-day intervals for 80 days; all contents wild-type adults   
 being replaced after censusing.

All populations except Population 4 were run concurrently. Population 4 was introduced toward the end of the study. Each population was reared in a plastic population box (13x17.5x 6cm) containing 100 g of the stand culturing medium (95% whole wheat flour, 5% dried brewer’s yeast). Culturing conditions of 33 degree + 2 degree C and 65 + 5% R. H. were maintained in darkened Labline Incubators (Model 844B). New culture medium was introduced only after the 200-day census, when it became obvious that severe starvation would terminate the study prematurely. The contents of each population box was censused for the number and genotype of live and dead adults and the number of pupae. Larvae and eggs were censused. After the census was completed, all contents were returned to the box. In Population 4, all contents except the living wild-type adults were returned to the box.

Dawson (1970), in a study also using the Sa gene, observed a decline in gene frequency which did parallel that expected of a homozygous recessive lethal in populations with discrete generations except for populations which had a recessive lethal coupled to Sa. His study, however, was performed under conditions in which fresh medium was introduced into each new generation. The conditions of the present study would provide the maximum competitive environment necessary for the genotypes in question. Since conditioning of the culture medium had been shown to have an effect upon fecundity, survivorship of eggs and developmental rate in some genotypes (Karten, 1965), it is highly likely that the Sa/+ individuals may have been better able to adapt and respond to the changing medium conditions during the last   
40 days of the study, thus, the increase observed in gene frequencies.

While sex ratios of the surviving adults had not been taken, another possible answer for the increase of Sa following addition of the new medium might be that the majority of the Sa/+ beetles present were females. It has been shown both by Krause et al. reduced in mating success, which would incresee selection against Sa initially, but when coupled with an adaptation to medium conditioning could enhance its retention on a short term basis.

The rapid elimination of the Be gene from all populations, including the one subjected to artificial selection, is the consequence of a combination of a serious decrease in viability of the Be/+ genotype and the inability of the male to successfully mate as observed by us. This latter has been demonstrated to be a key factor in the rapid elimination of mutant genes in Drosophila melanogaster populations (Merrell, 1965). While there is no direct evidence, this is probably also true of the Df/+ males although when artificial selection is practiced, the population size appears unaffected (Table 3).

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\*Size as a Factor in the Infection of Tribolium spp and Eleodes sp by Hymenolepis diminuta

Introduction

Tribolium spp have been established as a successful host for the rat tapeworm,  
Hymenolepis diminuta. The tapeworm ova are passed out of the rat host with the fecal matter. When they are ingested by a suitable species of beetle, the eggs hatch in the intestine. The onchospheres then penetrate the intestinal wall and enter the haemocoel where they develop into cysticercoids. Factors affecting the incidence of infection in beetles has been investigated. Role of sex of the beetles in infection has been studied by Mankau et al 1971, where they reported a significantly higher rate of infection in the female beetles compared to the males. A comparative study of the two sexes in three different species of Tribolium showed that in T. confusum and T. castaneum   
a significantly greater percentage of female beetles became infected compared to the males and that the females of those two species harboured significantly greater numbers of cysticercoids per beetle than the males. T. brevicornis showed no significant difference between the sexes in the percentage of infected beetles or in the number of cysticercoids harboured by each beetle (Mankau 1977). The role of age of beetles on the incidence of Hymenolepis infection in T. confusum beetles had been reported by Kelly et al (1967) where they observed that old females had   
a significantly smaller burden of cysticercoids when compared to young or middle-aged females, whereas middle-aged males generally had a higher incidence only where compared with young or old males, concluding that age resistance to H. diminuta cysticercoids in T. confusum occurs only in the females.

The purpose of the present study was to determine whether or not the differences in size and weight between a pygmy mutant strain and the normal strain of T. castaneum has a significant effect on the number of H. diminuta cysticercoids harboured by the beetles. Also whether Eleodes, a commonly found beetle in So. California could serve as an intermediate host for H. diminuta and if so, whether its relatively huge size would harbour a proportionate number of cysticercoids per gram of body weight.

Methods and Materials

60 beetles of normal strain and 60 of the mutant pygmy strain of Tribolium castaneum species were obtained from Dr. Alex Sokoloff, Director, Tribolium Stock Center, California State College at San Bernardino. 30 Eleodes dentipes sp beetles were collected from the Riverside-San Bernardino area. The beetles were denied food for 3 days prior to the infection. They were grouped in petri dishes, each containing   
10 Tribolium beetles/dish. Eleodes were separated in groups of five.

Hymenolepis diminuta inoculums was obtained by removing tapeworms from   
an infected white rat (Carolina Biological Supply, Inc.). Gravid proglottids were placed in mammalian ringer’s solution and teased apart to liberate the ova. Ova concentration was determined by direct count under a dissecting microscope. A suspension of approximately 200-240 ova per drop was used to infect the beetles by placing a drop of the inoculums on an oatmeal flake 3-4 mm in diameter. The inoculums was absorbed into the oatmeal flake and 5 such flakes were placed in each of the petri dishes containing the beetles, providing approximately 100-1,200 ova per dish. The petri dishes were covered and taped to prevent dessication of the ova. Most of the oatmeal was consumed in the first two days and subsequently the beetles were fed their regular diet of 95% unbleached wheat flour and 5% brewer’s yeast. After 7 weeks all the beetles were dissected under a dissecting scope and the cysticercoids counted.

Results  
  
Thirteen of the initial 135 beetles died during the experiment. 63% of the remaining 119 beetles were infected. The survivors consisted of 58 T. castaneum (normal),   
52 T. castaneum (pygmy) and 9 Eleodes, showing 71, 64 and 10 percent infection respectively.

Incidence of H. diminuta cysticercoids per infected beetle was highest in T.castaneum (N) with 3.4 per beetle, followed by T. castaneum (P) with 2.9 per beetle, with   
Eleodes harbouring 2 systicercoids, both found in the single infected beetle.

Table 1 : Rate of infection and incidence of H.diminuta cysticercoids in experimentally infected T.castaneum (N), T.castaneum (py) and Eleodes.

---------------------------------------------------------------------------------------------------------------------  
Beetle Morta- Survi- Infe- % infec- Total cysti- Cysticercoids  
 lity vors cted tion cercoids per beetle per gm of bo./wt.  
---------------------------------------------------------------------------------------------------------------------

T.castaneum ( R) 2 58 41 71 138 3.4 1.36  
T.castaneum (py) 8 52 33 64 95 2.9 2.37  
\*Eleodes 3 9 1 10 2 2.0  
---------------------------------------------------------------------------------------------------------------------  
Total 13 119 75 63 235 3.1  
---------------------------------------------------------------------------------------------------------------------  
\*3 were missing within the first week.

Discussion  
  
The average number of cysticercoids harboured per infected T. castaneum (N) beetle (3.4) appear not to be significantly greater than those found in the mutant,   
T.castaneum (P) (2.9). This is remarkable, since the pygmy mutant weighs only half as much as the normal T.castaneum beetle. This therefore indicates that the difference in size and weight between T. castaneum (N) and T.castaneum (P), has no significant effect on the incidence of H. diminuta cysticercoids.

In a similar study (Mankau 1977) using 3 different species of Tribolium beetles, namely T.castaneum, T.confusum and T. brevicornis, it was found that the average number of cysticercoids harboured by T. castaneum was greater than in T. brevicornis even though the latter species weighed 8-10 times more than T. castaneum.

The number of cysticercoids per gram of body weight of the beetles was significantly higher in the pygmy mutant (2.37) compared to the T. castaneum (N) (1.36).

Although only one out of the 30 Eleodes sp beetles used in the experiment became infected, it nevertheless is significant information. This beetle is commonly found in the fields and backyards of southwestern United States and no doubt serves as a source of good for wild rats and mice. The cysticenoids recovered from these beetles were normal in appearance and development and therefore it is evident that they can serve as effective intermediate hosts for Hymenolepis diminuta.

It is possible that the extremely large mouthparts in Eleodes sp is related to the very low infection rate. Voge and Berntzen (1961) in their study of “in vitro” hatching of H.diminuta oncospheres have shown that the rupture of the egg shell of the ova is necessary for the successful hatching and continued development of cysticercoids. This rupture is normally caused by the mandibles of the beetle. An interesting case was reported by Mankau and Morse (1973) where in an experiment where T. confusum were infected with H. diminuta ova, and obtained 77% infection with mature infective cysticercoids, one beetle was found to contain approximately 450 ova, with the egg shell intact, but harboured no cysticercoids. When the mouth parts of the beetle was examined it was found to have a defective mandible, affecting its ability to properly puncture the membrane of the onchospheres.

The large mouth parts of the Eleodes beetle could have permitted the passage of the onchospheres without rupture of the membranes thereby preventing subsequent development into cysticercoids.

We hope to test the above hypothesis by removing the cutting edges of the mandibles of Tribolium beetles prior to their infection with H. diminuta cysticercoids to see if it affects proper development of cysticercoids.

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Natural selection on adult body weight in Tribolium castaneum\*

This is a study of changes in adult body weight accompanying seventy generations of   
a husbandry regime in which discrete generations are imposed on populations of the Chicago Black strain of Tribolium castaneum. The regime, described fully in Sokal and Sonleitner (1968), consists of allowing the offspring of three days of adult oviposition to mature and in turn reproduce for three days of adult oviposition to mature and in turn reproduce for three days in fresh medium. To examine the genetic consequences of long term exposure to this regime, adult dry weight was assayed under controlled conditions in populations extracted from ongoing discrete regime lines at generation 70 and in the laboratory stock Chicago Black cultures from which the discrete lines had originated. The stock cultures had been husbanded so as to permit overlapping generations for at least fifteen years. In a separate experiment the effect of population density on adult dry weight was also examined.

Materials and Methods

All experiments were carried out at 29.5 degree C and 70% relative humidity in medium consisting of 95% sifted whole wheat flour and 5% brewer’s yeast. The experiments were performed in 8 g of medium in 8 dram shell vials at a population density prescribed by the particular experimental design. Adults were dried for 24 hours at 100 degree C and mass weighed by replicate. All adults assayed were one generation removed from their particular husbandry regime, the interim generation being at a low density considered optimal for Tribolium development.

Results

Vials were seeded with 20 eggs collected from batches of eiher discrete or stock origin adults that had been mass mated. Five replicates were reun for each group. Forty days after the cultures were initiated (about ten days after adult eclosion) each replicate was mass weighed. The stock regime group, at 1022 ug/adult, was found to be significantly heavier (P 0.01) than the 036 ug/adult weight found I the discrete regime group.

Another experiment was run to determine the effects of population density on the dry weights of the two strains. Replicate vials were each seeded with 50 eggs and compared to those seeded with 400 eggs for both strains. Mean adult weights at the lower density were 972 ug for the stock group and 860 ug for the discrete group. At the higher density they were 903 ug and 796 ug, respectively. A two-way analysis of variance reveals the effects of density and previous husbandry to the highly significant (P 0.001) but not their interaction. In both cases adults raised at high density weigh about 93% as much as those raised at low density.

Conclusion

Lowered adult body weight is a consequence of long term exposure to the discrete generation regime. This is apparently genetic in origin and is probably a result of selective forces generated by this husbandry regime. McCauley (1977) presents a series of assays on other quantitative traits affected by the discrete regime and also presents the demographic consequences of selection on these traits. In that paper   
a model of the selective forces generated by the novel regime is presented.

The lack of an interaction between the effects of husbandry regime and population density indicates that there was no selection on the factors determining the genotype X environment interaction in this character.

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New strategy to maximize the egg-laying of virgin female in Tribolium castaneum with reciprocal recurrent selection.

Starting from a population of the T. castaneum “Consejo” kept in laboratory without selection ad with a minimum of consanguinity, it was designed an experience which consisted in the use of the reciprocal recurrent selection (rrs) and two different pressures of selection – 20% and 2%, respectively – with three replications and standard conditions in the growing and operation of insects; the quantitative character selected was the egg-laying of virgin females between the days 7th to 9th after emergence. On seeing the obtained result after five generations of selection, during which the lines kept at 2% of selection had to be given up on account of the loss of vigour and high mortality and in order to find out a suitable strategy which could  
make compatible the effects of the strong pressure of selection in short time with a graduated adaptation of the studied populations to the environment, new lines were extracted derived from the lines kept until that moment at 20% of selection, advancing with all those until the 9th generation.

The respective analysis of variance for mean on generations of reciprocal crosses show a significant advantage of the pressure of selection of the 2% in front of the one of 20% in the first generation of rrs, although in the following generations the reproduction capacity deteriorates slowly. From this, we deduce that these pressures selected   
a priori don’t allow to study which should be the optimum.

Two new experiences which complete the previous information are still in their period of achievement and they consist in two new strategies where, besides the rrs and previous selection pressures, other ways of selection move simple participate at alternative intervals.

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\*Ultrastructural Characterization of a Single Gene Effect on the Egg Surface of Tribolium castaneum.

The “weird gene” (wd) of Tribolium castaneum is completely recessive and produces the easily detected phenotype illustrated in Figure 1. Eggs of homozygous females (wd/wd) fail to acquire the usual coating of flour particles. We previously speculated that this reselts from the absence of an outer “sticky” coating (Dawson ad Riddle, 1975). The present report confirms this and describes the precise anatomical basis of the difference between the normal and weird phenotypes as well as some ultrastructural aspects of the egg shell.

Eggs from a pure breeding line of the weird gene and normal eggs from the outbred Oregon Synthetic stock were examined. For scanning electron microscopy (sem), the flour was removed from normal eggs by washing in a dilute solution of zepharin. Washed eggs retain at least some of their stickiness. For transmission electron microscopy (TEM), washed and unwashed eggs were examined. The results confirmed that no egg component was removed by the washing procedure. Stanley and Grundmann (1966) also found the outer “gummy” layer to be resistant to detergent except within the first few hours after oviposition.

All eggs were fixed in 5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.0 for 3 ½ days at 4 degree C. The eggs were then rinsed in 0.2 M cacodylate and postfixed in cacodylate buffered 2% osmium tetroxide for 2 hours. Dehydration was carried out in a graded series of ethanols. For TEM the eggs were stained en bloc with uranyl acetate and embedded in Araldite under vacuum. Thin sections were cut with a diamond knife, serially stained in uranyl acetate and lead citrate, and viewed in an RCA 3H electron microscope. For SEM the eggs were critical point dried, mounted on double-sticky tape, coated with gold-palladium, and viewed with an International Scientific Instruments Mini-SEM.

The three dimensional surface structure of a normal egg is illustrated in Figures 2   
and 4. Even at low magnification (Fig. 2), a textured appearance is discernible with SEM which is not detectable with the light microscope. At a higher magnification, (Figure 4), the topography is resolved into a reticular network, irregular in height and structure. Compare with this the feature-less appearance of eggs produced by homozygous (wd/wd) females (Figures 3, 5).

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Comparison of transmission electron micrographs of transverse thin sections (Fig. 6. 7) show that the textured appearance of normal eggs is due to an outer layer which is absent in weird eggs. Being the most external layer, it is presumably responsible for the sticky quality of normal eggs and will be labeled as the “sticky layer”. Absence of the sticky layer is the only apparent structural defect in the weird egg. The sticky layer varies in height from 0.1 um to 6 um and is vacuolated but does not have a frothy appearance as in some beetle eggs (Sweeny et al., 1968).

The remaining structure of the egg shell is composed of three layers; vitelline membrane, endochorion and exochorion. The most medial, the vitelline membrane, is not shown since the preparative procedure caused its separation from the remaining egg shell. The crystalline endochorion appears variously as striated parallel to the egg surface, normal to the egg surface (most common), and cros hatched, and thu, is similar in appearance to the crystalline layer of T. confusum (Furneaux ad Mackay, 1970). The adjacent margins of both the vitelline membrane and endochorion are differentiated into bands by a differene in electron density in our micrographs. The endochorion margin also appears to lack the regular structure of the crystalline layer. The structural location suggests that these bands could represent the wax layer(s).

The exochorion is heterogeneous and does not exhibit a regular substructure. Often seen in the exocrorion are circular structures which are electron dense with electron lucent cores. Occasionally, the cores of these structures are contiguous with each other and also with electron lucent channels through the crystalline layer.

In normal eggs, but not in weird, the exochorion has an irregular wave pattern in transverse section (Fig. 6). This difference may simply be due to physical forces acting on the egg surface, however. Furneaux and Makay (1970, 1976) do not distinguish between exochorion ad the sticky layer. However, since a single gene substitution is responsible for the complete absence of one and not the other, it seems clear that the two layers are chemically distinct as well as structurally distinct. It should also be noted that the exochorion of Stanley and Grundmann (1966) is equivalent to what we label the sticky layer.

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Ultrastructural Characterization of a Sing Gene Effect on the Egg Surface of Tribolium castaneum

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Legends

Figure 1 : Light micrograph of ormal and weird eggs. Normal eggs are covered with flour.

Figure 2 : SEM of normal egg with flour removed. The bar represents 50 um.

Figure 3 : SEM of weird egg. The bar represents 50 um.

Figure 4 : SEM of normal egg with flour removed. The bar represents 5 um.

Figure 5 : SEM of weird egg. The bar represents 5 um.

Figure 6 : TEM of transverse section of normal egg. SL, sticky layer; EX, exochorion;  
 EN, endochorion; CL, crystalline layer. The bar represets 0.1 um.

Figure 7 : TEM of weird egg. The bar represents 0.1 um.

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\*Variability for dispersal behavior in a wild population of tribolium castneum

Introduction

The quantitative study of dispersal behavior in Tribolium was greatly facilitated by   
Prus (1963), who suggested a simple apparatus in which the act of dispersal can easily be separated from other types of movement. Since them it has been demonstrated   
(Prus, 1966; Zyromska-Rudzka, 1966; Ogden, 1970a) that the rate of dispersal depends upon the species, genetic strain, sex, density (of larvae or adults) and degree of conditioning of the flour. The differences between strains (Prus, 1966), and the results of selection experiments (Ogden, 1970b), indicate that the variation in dispersal activity has a genetic component.

So far all of the studies on dispersal behavior were performed on laboratory stocks, in which departure of dispersing individuals is usually not allowed. The present work deals with variability for this trait in a natural population. On the basis of the results some speculations are offered about the role of dispersal in the ecology of Tribolium in nature.

Methods

1. Population. The population of Tribolium castaneum used in this study was derived from beetles that were collected three months prior to the start of the experiment in   
a grain storage silo near Tel Aviv. These individuals were mated freely, and the beetles used in the experiments were collected as pupae from among their progeny.

1. Experimental apparatus. Dispersal was studied in an apparatus based on the one designed by Prus (1963). The beetles to be tested are placed in   
   a measured amount of flour, in a small vial (A) which is connected through its top, by means of a polyvinj tubing, to a small, empty vial (B). A thin thread,   
   30 cm, leaves the surface of the flour in vial A, goes through the tube, ad enters vial B. Beetles found on the bottom of vial B are scored as dispersants. In   
   Prus’ apparatus, the thread ends in vial B with a loop, and does not reach the bottom. In our apparatus, the tip of the thread is connected to a thin metal filament that does reach the bottom. It seems to us that our design is more reliable for scoring dispersing beetles, because, on one hand, the beetles do not have to jump or fall off the thread in order to reach the bottom, while, on the other hand, they cannot climb back once they get down.
2. Experimental design. Tto start the experiment, 19 pairs were chosen at random. From among the progeny of each pair, termed a family, two replicate groups of 30 beetles each (15 males and 15 females) were tested.

In each replicate, dispersal was measured when the beetles were 10, 20, 30 and 48 days old. The beetles were introduced into vial A, with 8 g of fresh flour (95%  
 whole wheat flour, 5% brewer’s yeast), 4 days before each run. All tests were carried out under similar conditions, including non-conditioned flour, absence of larvae or pupae, low but sonstant adult density and after enough time had elapsed to let beetles get used to the new environment.

Males and females were marked differentially, with a dot of quickdrying nitrocellulose paint on the elytra. We found that the marking had no effect on viability, fecundity or the tendency to disperse.

On the day of the experiment, the thread leading to the bottom of vial B was inserted into the flour in vial A. Dispersants were defined as beetles found on the bottom of vial B after 24 hours. They were scored by sex, marked, and returned to vial A. The marking was such that we could know the performance of each beetle in each run. Four days prior to each run, dead beetles were replaced by sibs of the same sex and age.

The experiments were run in a dark incubator with a temperature of 29 degree C and relative humidity 70%. The different families were distributed on the shelves at random, and the two replicates of each family were started several days apart, according to the availability of beetles.

1. Analysis of data. The comparison of dispersal activity according to family, replicate, sex and age was done using a program for Analysis of Variance, Factorial design (BMD 02V), available at the Library of the Computation Center, The Hebrew University. The analysis was carried out on the transformation arcsin m/n, where m = number of dispersants and n = total of beetles in vial A.

Results

1. Differences between families and between ages. The numbers of dispersing beetles, listed according to family, replicate and age are given in Table 1.  
 The analysis of variance (Table 2) shows that while no difference exists between the means of the two replicates, the differences between families and between ages are highly significant.

The dependence of dispersal rate on age is shown in Figure 1. The mean rate of dispersal is highest at the age of 20 days, and its level at 48 days is very low. In order to find out whether it is only the low rate at 48 days which is responsible for the difference between ages, a second analysis of variance was carried out, this time excluding the data of 48 days. The results again show significant differences between families and between ages.

The analysis of variance also shows a significant contribution to the variation in dispersal activity of the interaction Age x family, suggesting that the age of maximum probability to disperse varies from family to family.

Figure 1 can be supplemented by the results of a separate experiment, performed with 5 days old adults from the same population. Dispersal rate of these beetles was 13%. We conclude that on the average the tendency to disperse is low right after eclosion, reaches a maximum at the age of 2 – 3 weeks and declines sharply thereafter.

2. Differences among sibs. The following test was performed in order to find out whether differences exist also between individuals, within families:

If all members of a family (sibs in our case) have the same tendency to disperse, then at age I the probability for dispersal of any individual will equal p , the rate of dispersal of that family at that age. The determination as to whether a particular individual will move to vial B or not will depend only on p . Under such conditions, 60 individuals, which are tested 3 times (at 10, 20, and 30 days), are expected to form the four following groups, according to the number of times each beetle moves to vial B:

If, on the other hand, some individuals in a given family have a higher tendency than others to disperse, the dispersing beetles at each age will not form a random sample of the entire family. The same individuals will tend to move into vial B every time, others will tend not to move at all, and the distribution of individuals according to the number of times they are found in vial B will deviate from the expected values given above.

Table 3 gives the observed and expected numbers of dispersing and non- dispersing beetles, for 17 of the families of the wild population (families 10 and 17 were omitted because of errors in scoring). A X test on the totals shows that the deviations between “observed” and “expected” is found in the categories of “0 times” (16 families) and “3 times” (11 families), strongly suggests that all individuals of the same family cannot be considered identical with respect to their tendency to disperse.

3. Differences between sexes. Because the marking of a dispersing beetle  
 obliterated its original marking according to sex, the comparison between the two sexes with respect to their rate of dispersal can be done, at each age, only with the beetles that have dispersed at that age for the first time. At 10 days the 402 dispersing beetles (at this age all dispersants do so for the first time) included 214 males and 188 females. At 20 days 129 males dispersed for the first time, compared to 123 females, and at 30 days the values were 51 males and 43 females. In none of ages was the difference between the sexes   
 statistically significant, but the excess of dispersing males was consistent.

Discussion

The main purpose of the present work was to see whether variability, with respect to the tendency to disperse, exists in natural populations of Tribolium. We decided to measure dispersal under optimal conditions, where it can be described as spontaneous, and not as a reaction to an environmental factor. Spontaneous dispersal is probably   
an adaptation to life in unstable or unpredictable habitats (Southwood, 1962). If the environment is going to become inhospitable, it is better not to wait until conditions actually deteriorate. There will be an advantage to a certain amount of dispersal when conditions are still good, ad the chances and to find a suitable habitat are not reduced by a lowered physical state.

Our results indicate that variability for dispersal behavior indeed exists, at least in the one population studied by us. The fact that the variation between families is much higher than within families (Table 2) strongly suggests that a large part of the variation is genetic. In addition, each family, although characterized by its own average rate of dispersal, seems to segregate for dispersants, that tend to disperse at every opportunity, and non-dispersants, that do not disperse at all (Table 3).

The problem of the forces which are responsible for maintaining this variability is interesting, but it cannot be solved without a better knowledge of the genetic basis of dispersal behavior and the dynamics of Tribolium populations in nature. A recent simulation study (Roff, 1975) has shown that, in a heterogenous environment,   
a variety of genetic models can account for the maintenance of variation within  
a local population, with respect to dispersal behavior.

For the last several thousand years Tribolium has been associated completely, or almost completely, with human facilities, such as flour mills and grain storage sites. Life in these habitats, and in particular as the war against Tribolium intensifies, must include many cases of establishment of new populations on one hand, and extinction of local populations on the other hand. The variability for dispersal ability can well be   
an adaption to this way of life.

The dependence of dispersal rate on age is also consistent with the assumption that spontaneous dispersal is mostly colonizatory. Natural selection is expected to time colonization to a pre-reproductive stage (Lewontin, 1965), and the findings in many migrating insects support this expectation (Dingle, 1972). In Tribolium, efficient reproduction starts in the second week of adult life (e.g. Young, 1970). Our finding that dispersal activity reaches a peak at the age of 2-3 weeks suggests that also inTribolium natural selection favored dispersal at an age in which reproductive potential is at its maximum. It may also be that the slight delay in dispersal activity enables dispersing females to leave some progeny behind, and still maintain the major fraction of their reproductive activity for the new habitat, or at least to become fertilized before entering   
a period in which no mates may be available.

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Table 1 : Number of dispersing beetles, listed according to family, replicate and age. Thirty beetles were tested each time.  
-----------------------------------------------------------------------------------------------------------------  
 10 days 20 days 30 days 48 days  
Family No. rep. 1 rep. 2 rep. 1 rep. 2 rep. 1 rep. 2 rep. 1 rep. 2   
-----------------------------------------------------------------------------------------------------------------  
 1 18 15 15 16 15 22 0 4  
 2 20 12 18 15 16 24 3 4  
 3 12 13 14 19 14 10 4 0  
 4 19 16 10 15 7 8 1 3  
 5 10 9 10 6 12 7 2 -  
 6 10 7 20 5 6 4 2 2  
 7 15 14 17 17 6 4 - 0  
 8 13 14 4 19 10 12 2 0  
 9 1 13 9 11 1 3 1 6  
 10 4 3 17 3 0 4 1 5   
 11 15 12 9 14 15 12 - 5  
 12 3 2 10 5 0 4 0 0  
 13 4 6 10 14 4 8 1 2  
 14 10 9 11 11 12 2 5 1  
 15 3 7 14 4 6 5 0 0  
 16 12 11 13 21 9 14 1 6  
 17 13 12 12 12 11 5 1 2  
 18 13 14 13 14 14 8 2 0  
 19 9 9 11 4 0 7 0 6  
---------------------------------------------------------------------------------------------------------------------  
Total 204 198 237 225 158 163 26 46  
---------------------------------------------------------------------------------------------------------------------

Table 2 Analysis of variance for rates of dispersal (at 10, 20, 30 ad 48 days)  
------------------------------------------------------------------------------------------------------------------  
Source of variation df SS MS F  
------------------------------------------------------------------------------------------------------------------

Ages (A) 3 2126.553 708.851 83.6\*\*\*  
Replicates ( R ) 1 0.421 0.421 0.06   
Families (F) 18 1594.618 88.590 10.4\*\*\*  
Interaction: A x R 3 5.947 1.982 0.2  
 A x F 54 921.697 17.068 2.0\*\*  
 R x F 18 238.829 13.268 1.6  
Residual 54 457.803 8.478  
  
\*\*\* 0.001 p  
\*\* 0.01 p 0.001

Table 3 : Observed and expected numbers of beetles that moved 0, 1, 2 or 3 times (out of 3 possibilities) to vial B, for 17 of the 19 families. For method of calculation of expected values see text. Expected totals were calculated from average frequencies of dispersing beetles at each age.

---------------------------------------------------------------------------------------------------------------------  
 Number of times  
 0 1 2 3  
Family No. Obs. Exp. Obs. Exp. Obs. Exp. Obs. Exp.  
---------------------------------------------------------------------------------------------------------------------

1 5 5.00 18 19.51 28 24.97 9 10.51  
2. 5 4.20 15 18.33 30 25.73 10 11.73  
3 12 9.45 21 24.60 20 20.45 7 5.50  
4 14 11.18 25 27.04 14 18.37 7 3.40   
5 23 19.04 22 26.77 10 12.33 5 1.86  
6 27 20.90 15 27.37 17 10.54 1 1.18  
7 15 11.19 21 27.35 20 18.72 4 2.74  
8 15 12.89 26 26.02 11 17.30 8 3.79  
9 32 28.62 20 25.07 6 6.00 2 0.31  
11 12 11.19 27 25.27 13 18.88 8 4.66  
12 40 39.19 17 18.69 3 2.06 0 0.06  
13 25 24.00 26 26.80 7 8.40 2 0.80  
14 22 19.91 23 26.81 13 11.66 2 1.63  
15 31 28.58 19 24.38 10 6.48 0 0.55  
16 18 9.89 14 25.22 18 19.89 10 5.00  
18 12 11.49 27 25.46 14 18.58 7 4.45   
19 31 27.82 18 24.87 11 6.77 0 0.52  
---------------------------------------------------------------------------------------------------------------------  
Total 339 281.9 354 457.0 245 240.7 82 40.8

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\*Stage and syndrome of lethality in Tribolium castaneum homozygous for Fta.\*

The Fta allele of Tribolium castaneum (see Sokoloff 1966) exerts its lethal homozygous effect in the egg stage (Sokoloff, pers. Com.). Embryonic lethal potentially represent valuable tools in the study of early steps in development such as specification of the basic body pattern (see Sander 1976). In order to ascertain stages and syndrome of Fta-dependent lethality we studied development in eggs from heterozygous parents (Tab. 1)..

(a)32 deg.C (b)29 deg.C (c) 20 deg.C (d) 20 deg.C (e) total  
number of eggs 1099 921 559 1099 3678   
---------------------------------------------------------------------------------------------------------------------  
% failure before  
 germ band 3.4 5.6 17.0 7.2 7.1  
% legless larvae 25.5 23.9 19.1 24.2 23.7  
% normal larvae 71.2 70.6 63.9 68.6 69.1   
% legless/total larvae 26.4 25.3 23.1 26.1 25.6

Tab. 1 - Developmental results from eggs incubated at different temperatures, parents heterozygous for Fta. A-c offspring of 3 pairs each, d offspring from mass culture tested to see whether deviation noted in c was due to lower temperature.

The percentage of eggs which failed to reach the germ band stage (Tab. 1) was somewhat lower in most test samples than in the wild-type controls. Eggs which continued development produced either normal larvae or larvae with extremely reduced appendages. All appendages from antennae to urogomphi were uniformly affected but apart from this the larvae looked quite normal. Closer observation of leg development revealed normal-looking leg buds in the early germ band stage, but these failed to grow

1)  
The authors are indebted to Dr.A.Sokoloff for providing the Fta strain and for valuable advice, ad to the Deutsche Forschungsgemeinschaft for financial support (SFB 46).

thereafter. At the larval level of differentiation the knob-like legs were found to consist of only the more or less well developed coxa; there were some coxal bristles and muscles but none of the more distal leg structures. Some of the malformed larvae hatched but apparently were unable to feed properly; none reached the 2nd larval stage. The percentages presented in Tab. 1 indicate that, independent of rearing temperature, lethality caused by the Fta allele in the homozygous state is entirely due to the very uniform syndrome of arrest of appendage growth at the late germ band stage. Similar malformations were also observed in assumedly homozygous offspring from parents heterozygous for the allele Sa-2 (Kuld 1976); Krause et al. (1963) had already established the egg stage as the period where lethality of this allele is expressed.

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Subpopulations of Tribolium castaneum (Host.) resulting from routine culture maintenance.

The “Idaho” strain of T.castaneum was started in 1959 from a relatively small initial number of individuals and maintained on an irregular but active subculturing basis until mid-1962. Subsequent subculturing was sporadic until 1966 when the strain was nearly lost by neglect. It was recovered from a very few individuals (ca. 10) at which time a more routine culture maintenance was established; reculturing at 3-5 week intervals from 1-3 lines. From mid-1978 to July 1971 new cultures were established, usually at weekly intervals but occasionally at intervals of 2 weeks. No records were kept as to whether parent adults were 1, 2, 3 or 4 weeks old. Since July 1971 cultures have been started on a weekly basis from parents approximately 2 weeks old. At ca. 90 degree F and 50% R.H. This has resulted in a 6 week sequence of cultures.

By mid-1973 it became apparent that this culture system had produced 6 subcultures, as indicated by mean pupal weights (100 male, 100 female individually weighed to +   
1 microgram) statistically distinct t the 1% level. Segregation into sub-strains was probably to be expected, but that this was noted in terms of pupal eight was less to be anticipated. Although there are differences in the extremes of variation from the overall mean, these differences are small. Each sub-strain has shown the “capability” to have approximately the same high or low mean pupal weight. The differences seem to derive from the frequency of high or low mean weights and not the capability to attain maximal or minimal values. Male and female mean pupal weights usually varied in the same direction, but often not to the same degree. Weekly means varied randomly within a + 15% or greater range from the overall mean. Sequential sub-culture mean pupal weights, however, apparently do not very randomly. A possible explanation for this observation is currently being studied.

Other T. castaneum strains (Georgis, Georgia Blind-sooty, Kansas and Kansas State University) have been similarly maintained since mid-1971. Although some pupal weight data have been obtained from these strains they have not been analyzed. Subjective evaluation indicates that each is now represented by 6 subpopulations.

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\*Observations on a natural population of Tribolium brevicornis LeC  
  
In 1859 John Le Conte discovered a beetle in Ft. Tejon, California which he named Aphanotus brevicornis. Hinton (1948) included this species in the genus Tribolium. In adition to Tribolium brevicornis the brevicornis species group of Tribolium includes:

Species Locality

T. brevicornis Le C California  
 T. linsleyi Hinton Mexico  
 T. parallelus (Casey) Western N. Americ  
 T. gebieni Uyttenb ?  
 T. carinatum Hinton Argentina  
 T. carinatum dubium Hinton Argentina  
 T. uezumii Nakane Honshu, Japan

Linsley (1944) pointed out that T. (Aphanotus) brevicornis is a species which is often found in bee nests. His literature survey included the observations of Davidson (1893) who recorded Aphanotus brevicornis from Xylocopa bee nests; Nininger (1916) who extracted these beetles from nests of carpenter bees and Hicks (1929) who reported Aphanotus brevicornis from nests of the bee Anthidium mormonum fragariellum. Linsley himself (1943) had found that cells of Xylocopa orpifex found on an old log of   
an incense cedar, Libocedrus decurrens were infested with Aphanotus brevicornis.

Other than Le Conte’s paper, the only citations which give a geographic location for Tribolium brevicornis are those of Okumura and Strong(1965) (who found this species in Alpine and Mono Counties in California, infesting chicken feed, honeycomb and oat seeds) and Strong (1970) who trapped this flour beetle in nine unspecified localities in California.

T. brevicornis is maintained in stock at the University of Idaho, Moscow, Idaho, and at   
a few other laboratories including the Tribolium Stock Center, California State College, San Bernardino, California. The stock at the University of Idaho was found in Parma, Idaho. The one at the Tribolium Stock Center was kindly supplied by Dr. R.G.Strong, University of California, Riverside. This strain was derived from a sample collected at Bishop (Strong, personal communication 1977).

We report now the discovery of a natural population of Tribolium brevicornis. This population was originally discovered by one of us (A.S.) in April, 1977 under the bark of an alder tree which lay on the bank of the Waterman Canyon Creek just outside of the northern limits of the City of San Bernardino, about a mile north from te junction of California State Highway 18 and the old Waterman Canyon Road.

The log in question had been cosmetically removed from the top of a dead alder tree by the County Highway Department. The bark of the log was cracked in numerous places, but much of it was still firmly attached, so that, by hand, pieces of bark could be removed only with difficulty. Under a fairly loose piece of bark were found two beetles in close contact. The third beetle was found under another piece of bark 30 centimeters away. Aside from these beetles there was a branch and twig boring beetle (Bostrichidae), a rather flat cucujid (?), a snail and an unidentified bee.

The three beetles were brought back to the laboratory, examined under the microscope, and placed in standard flour beetle medium since they appeared Tribolium-like. Every few days the flour was sifted and checked for the presence of eggs. Occasionally   
a piece of apple was placed on the surface in case the beetles required moisture. Except for their small size, these beetles largely resembled Tribolium brevicornis (see Table 1). These beetles began to lay eggs, and from the eggs emerged larvae with distinct dark bands on the tergites. Subsequently one of the beetles died. The other two were weighed individually and their weight determined at about 4.8 mg. At a later date (June 27) the original vials containing eggs were examined. By then there were large larvae, pupae, and a few adults which were about three times heavier than their parents.

On the same date we returned to Waterman Canyon, and under the bark of the stump of the same tree and in another area of the original downed trunk we found five additional beetles. These, however, were much larger than the original ones found. (See Table 1).

A sample of T. brevicornis was obtained from the U. of California at Riverside culture, and 10 males and 10 females weighed individually. The mean value for the 10 males was 11.57 + .63 mg. and a coefficient of variation of 17.30%, and for females 11.82 + .63 mg. with a coefficient of variation of 16.73%.

Thus, judging by the female sample, the laboratory population is considerably more uniform in size (than the population collected in nature), even though the beetles were derived from a culture in which the flour was already badly used up.

The interesting thing about this sample, of course, lies in the fact that the weight of females varies considerably, the largest females being about three times heavier   
(14 mg) than the smallest females (4.5 mg).

A further report on this natural population of Tribolium brevicornis will be made at   
a later date.

Table 1 : Individual weights (in mg) of beetles derived from a natural population at Waterman Canyon (left) and from a laboratory population.

Sex Waterman population Laboratory population

\*1 4.5 13.0  
 \*2 4.5 10.0  
 3 9.4 8.1  
 4 14.0 10.3  
 5 10.7 10.5  
 6 11.0 14.0  
 7 13.5  
 8 12.2  
 9 12.8  
 10 13.8

1 8.4 14.9  
 2 -- 12.7  
 3 -- 10.7  
 4 -- 11.6  
 5 -- 9.3  
 6 -- 13.7  
 7 -- 12.7  
 8 -- 10.6  
 9 -- 8.8  
 10 -- 10.8

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\*The effect of culturing together on adult longevity of Tribolium audax.

Adults 2-6 days old were reared on one gram of flour/beetle in various numbers: 1, 2, 4,10, 40 with corresponding number of replications of 40, 20, 10, 4, 2, 1. (Actual number in each experimental unit = 40 beetles). 1, 2 or 4 beetles were raised in vials   
(1 cm in diameter and 3 cm high); 10 and 20 beetles were raised in vials (2 cm in diameter and 3 cm high); and 40 beetles in ¼ pint milk bottle (5 cm in diameter and   
10 cm high).Males and females were reared separately. Constant conditions of   
35 degree C and 60% R.H. were maintained throughout the experiment. The medium consisted of 95% whole wheat flour and 5% dried yeast. Table 1 shows the effect of rearing together on the mean longevity of adults. It is evident that individually reared beetles have higher longevity than beetles reared together, even though the amount of bood/beetle is the same. Males seem to live longer than females when they are raised individually or in pairs and shorter when 4, 10, 20 or 40 beetles are raised together. This indicates that males are more sensitive to the presence of other males than females. There was no evidence of cannibalism. It is possible that the size of the container may be a contributing factor.

Table 1 : Longevity of T. audax as influenced by number of beetles cultured together.

--------------------------------------------------------------------------------------------------------------------- Number of beetles Sex Mean longevity Coefficient of  
cultured together (days) variation (%)  
---------------------------------------------------------------------------------------------------------------------

1 Male 96.6 37.1  
 Female 93.8 35.0

2 Male 91.32 30.1  
 Female 89.30 28.7

4 Male 84.18 33.8  
 Female 87.68 27.9

10 Male 81.55 35.8  
 Female 93.98 29.0

20 Male 67.73 40.1  
 Female 68.63 41.5

40 Male 73.68 34.3  
 Female 84.88 35.1

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\*Rate of egg production in fecundated and virgin females of Tribolium castaneum

The foundation population (NDRI-1) of Tribolium castneum was used to study the rate of egg laying in fecundated and virgin females. This population was collected from areas around Karnal (Haryana) and had been maintained in the laboratory over  
a period of 9 months in mass culture at 32 degree C + 1.0 degree C. Culture media used consisted of 95% wheat flour ad 5% yeast.

100 newly emerged virgin females and 50 males were collected. Each such female was assigned randomly to the two groups and were kept in a 5g glass vial with 2 g media. Males were assigned at random to each female in group one, while the females in the group 2 were not provided with any males. The egg production in the two groups was recorded from 8th day onwards over a period of 21 days. 24 hrs. egg collection was recorded every day at 10.00 A.M. The average daily egg production in the two groups have been shown graphically in Fig. 1. An increase in egg production was noted till 12th to 13th day in both the groups with a decline, thereafter. Significant difference in the rate of egg production between the two groups were noted for 1st, 2nd and 3rd week production. The total weekly egg production averages in fecundated and virgin females were 82.47 + 3.02, 19.80 + 1.76; 69.07 + 2.73, 18.15 + 2.36 and 64.33 + 2.86,   
22.71 + 4.34 respectively for 1st, 2nd and 3rd week. The rate of egg production was highest in first week followed by a decline thereafter, which was not significant.

The average daily egg production in the fecundated and virgin females in 1st, 2nd and 3rd week was 11.78, 2.83; 9.87, 2.59 and 9.19, 3.24 respectively. Females with males (pair mated group) showed two to five fold increase in egg production compared to virgin group. It can, therefore, be concluded that males are necessary for complete egg laying. This is in conformity with the findings of Ruano and Orozco (1966), Orozco et al (1970 and Bhat and Bhat (1974).

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Clustering of nuclear pores to “nuclear sieves” in some Oedionychina fleabeetles.  
  
The phylogenetically young subtribe Oedionychina of the fleabeetles posses as unusual cytological features like unequal mitosis in spermatogonia, the lowest known number of spermatocytes I per cyst, and spermatozoa per bundle (4 and 16, respectively), size of spermatocytes and spermatozoa unusual for Coleoptera (about 50 um across, and over 4 mm, respectively) and large, presumably heterochromatic sex chromosomes, that in the male segregate from a distance-bivalent or multivalent condition (1). The Oedionychina genera fall in two categories in relation to the structure of the nuclear envelope of spermatocyte I, and of the epinuclear material synthetized during diplotene (2, 3). Omophoita shows a more conventional structure with an even and probably random distribution of nuclear pores, and a layer of polysomes separated from the outer nuclear membrane by an intermediate layer about 400 A thick. The nucleus is surrounded by a continuous layer of epinuclear material. In Oedionychina and related genera, all nuclear pores are clustered to nuclear sieves: shallow, cup-like invaginations of nuclear envelope. The sieves are filled with polysomal aggregates kept at a distance of about 400 A from the outer nuclear membrane. The sieves with their contents are called nuclear sieve complexes (NSC). Epinuclear material is formed only at the NSCs, being thus discontinuous.

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\*The Effect of Propagule Sex Ratio Upon the Number and Sex Ratio of the Adults Produced in a 37 day interval by Tribolium castaneum

In some experiments it is necessary to begin each generation with a sample of adults chosen at random from a much larger adult population. Deviations in the sex ratios of these propagules can result in large fluctuations in the population density from generation to generation. For this reason, it became necessary in our work to determine the effect of propagule sex ratio upon the number and sex ratio of the adults produced by that propagule during a 37 day interval.

A series of populations was established with groups of 16 adults whose sex ratio had been determined at the pupal stage (Park, 1934). The sex ratio of these propagules of 16 adults was varied from 4 and 12 to 12 and 4 (see Table). Five replicates were established for each sex ratio treatment. After 37 days the populations were censused for adults and the adults were preserved in 95% ethanol. Within three days of preservation, the adults were sexed with the aid of a dissecting microscope by the technique of everting the genitalia (Stanley and Grundmann, 1965).

The results of this sex ratio-productivity assay are presented in Figure 1. The net result of increasing the number of females in a propagule by oen is to increase the number of adults at day 37 by approximately 14. This result was arrived at by determining the slope of the line drawn in Figure 1. (The X’s represent the treatment means and the brackets are one standard deviation about the mean.)

The sex ratio of the founding propagule was found to have no effect upon the sex ratio of the adults produced by that propagule. In no case did the sex ratio of the populations at 37 days differ significantly from a one-to-one ratio of males to females.

TABLE  
 SEX RATIO TREATMENTS

4 females + 12 males 5 replicates

6 females + 10 males 5 replicates

8 females + 8 males 5 replicates

10 females + 6 males 5 replicates

12 females + 4 males 5 replicates

25 vials total  
 -------------------------------------------------------------

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\* Tribolium confusum brei in vitro.

While developing a tissue culture technique to determine quantities of mycotoxin carried within stored product insect, it was observed that adult T. confusum prepared as a sterile brei were highly toxic to mammalian cell lines while a brei of larvae had to toxicity (Figure 1). The brei were prepared by grinding 1 g (about 400) insects with mortar and pestle. The sample was extracted with a 10% solution of dimethyl-sulfoxide in phosphate buffered saline (PBS). After centrifugation the supernatant was passed through a series of Millipore filters (1.2-0.3 u). Several dilutions of the sterile filtrate in PBS were added to cultures of African green monkey kidney cells (VERO) at the time of passage. The number of cells/culture flask was estimated with an ocular reticule as the mean of 10 random cell counts/flask 24 hours after passage.

Two major differences between adults and larvae of T. confusum are the presence of sclerotized cuticle and the odoriferous, quinine-producing glands in the adult. Both are absent in the larva. Dissected cuticle consisting of elytra, head and appendages from 100 adults was prepared as a sterile brei. The paired, abdominal odoriferous gland preparation caused 100% destruction of the cells as an undiluted brei (10 degree). One tenfold dilution prevented this toxicity. Two tenfold dilutions were necessary to prevent toxicity from the 1 g brei. The effect of the adult odoriferous glands masks any other toxic material that might be present within the adult in small quantity. Therefore the larva is a better test organism for this purpose.

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Automated cultivation of a chain of polymiktic generations of flour beetles.

To achieve a chain of separate, polymiktic generations of Tribolium in an automated manner the following construction is used. Cylindrical chambers occluded with sieves, each for one generation, are stored together. The mesh-width of the sieves is sufficient to allow freshly hatched larvae of the beetles to pass through. The generation succession is achieved by special arrangement of the food and/or substrate within the chambers. Main components of this arrangement are:

1 Difficult decomposable wheat-groats suspended in Perlon-wool for   
 maintaining the adult beetles.  
2 A layer of a nourishment powder the beetles prefer for depositing eggs, behind the sieve of the next chamber.

The set of cylinders is aerated with 40% oxygen, 60% nitrogen in the direction F to p.

With n chambers we hope to achieve a pure F. The other generations may be in part mixed together, yet it is hoped to prevent this by a proper reduction of feeding. The system works already for three generations and is projected for use in a free-flyer experiment of the NASA.

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Some Modifications to Cultureing Techniques for Tribolium

Over the past couple of years, we have developed several modifications to standard culturing techniques (Sokoloff, TIB; Vol. 3, 1960) which have significantly increased the ease and efficiency of collecting certain types of data in quantitative genetics studies with Tribolium castaneum. Workers in Quantitative genetics or in other fields may find these modifications useful also.

We now use 30 ml.polyproplyene beakers for rearing individual families or small groups of individuals. These beakers are lightweight, inexpensive, autoclavable and can be stacked for storage when not in use. We use plastic medicine cups for covers to these beakers which permits stacking of the beakers containing Tribolium. We have found this to be very convenient when storing sexed individuals for subsequent mating.

A raised circular center portion in the base of the medicine cup is easily removed by placing a few drops of wylene in the center of an inverted cup. This makes it possible to construct a large number of inexpensive screens to fit these beakers. When we wish to collect eggs from the same females in a number of consecutive time periods, we place the female with flour in the screen (30 mesh) which is then inserted in a beaker. At the end of the egg laying period the flour can be shaken through the screen and the screen with the female can be placed in a fresh beaker for a second egg laying period.

In some recent experiments, we have been weighing individual larvae on day 14, checking daily for pupation and then weighing pupae on day of pupation. If this daily check of pupation is to be meaningful, all larvae must be checked in a relatively short period of time. We have been able to do this by placing the larvae, after weighing, into one of the wells of a falcon tissue culture plate. The type of plate we have been using for larvae have 96 wells and are made of transparent polysterene. Each well measures 6 mm in diameter and has a curved bottom. Because of this curved bottom, pupae generally rest dorsal side down. The sex of the pupae is then easily determined, without handling, by placing the tray on the stage of a dissecting microscope. For the purpose of adding flour to the wells, the tray is covered with a piece of 40 mesh wire cloth and flour is deposited on the screen. Flour can then be transferred fairly uniformly to each well with small paint brush. Exact measurement is not necessary since the flour is provided in excess and replaced daily. The flour is removed in a similar manner, by vacuuming through the screen. We have used a piece of flexible tubing and a one-holed-rubber stopper fitted with glass tubing to reduce the opening of the vacuum cleaner for this purpose. It takes a little practice to become proficient at removing the flour without transferring larvae from one well to the next. However, it takes one experienced worker, depending upon how many larvae have pupated, between 15-90 minutes to check 20 trays (1920 larva) and replace the flour. We have fashioned lids for these trays from flexible tissue culture plates and a piece of plate glass so each well is individually covered. For storage of large populations until individual selection can be performed, we have been placing pupae, after weighing, in similar plates with flat bottoms (wells are slightly larger). This reduces the effort needed to locate the selected individuals as well as the storage space required.

The items mentioned above are probably available from a number of suppliers. We have purchased them through University Medical Stores. However, listed below is one source for each of the items:

Wire cloth Small Parts, Inc.  
 6901 N.E. Third Ave.  
 Miami, Florida 33138

Medicine cups Solo Cup Company  
 Urbana, Illinois 61801

Tissue culture plates Cooke Laboratory Products  
 900 Slaters Lane  
 Alexandria, Virginia 22314

Polyproplyene beakers Cole-Parmer  
 7425 North Oak Park Ave.  
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A SIMPLE TECHNIQUE TO IMMOBILISE TRIBOLIUM ADULTS

Tribolium great activity and mobility can cause considerable technical difficulties in laboratory operations, e.g. adult sexing, antennae clipping, etcl, mostly if the number of individuals subjected to these operations is large.

Adults can be efficiently immobilized by employing adhesive labels commonly used in offices, thus making laboratory manipulations easy enough. One of these labels can be fixed on a slide or on any other surface easy to handle under a binocular microscope. The adhesive surface of the label should be maintained in an upwards position. The insect will then be placed on the adhesive side where it will be trapped (Fig. 1-4). Preferably, the imago will be adhered to the label by its dorsal side in order to avoid damaging its extremities. Adults should be clean of flour and dust in order to make   
a better and longer use of the adhesive material. It is also convenient that this adhesive material be constituted by a thin film, because if the insect was fixed using its own weight, no excess of gun, which could hurt it, will be sticked on its body when it be taken off the label.

Insects can be taken off the label by using a soft forceps made from steel hoop (thickness 0.1 mm) (Fig. 1-1) or with an appropriate needle. Brushes are not recommended as they will not lift the insect but only displacing it on the adhesive side, with the risk of it becoming damaged.

For antennae clipping, steel forceps with very thin tips can be used (Fig. 1-2). As they will clip by pressure rather than tearing off they will give a better result than using   
a small scalpel. In this way it is not necessary a very great adhesive strength nor as excess of pressure of the insect on the label. These forceps are of the type used by watchmakers (5 PEER rust less steel or 4 royal electronic, Switzerland). Adults can be sexed by using a thin brush to touch the profemus.

Although immobilizing pupae is seldom necessary, the same adhesive labels can also be used for this purpose. Special care must be taken in order to avoid damaging the elytra.

Fig. 1 – Antenna clipping technique

The insect remains fixed and motionless on the adhesive side of the label (4) which is mounted on a slide. The insect can be placed on and removed off the label by using   
a flexible soft forceps (1) which can be handled with the left hand. The antennae can be clipped at the third segment level (3) with a watchmaker’s forceps (2) handled with the right hand. To prevent the right hand from shaking it can rest on the left hand free fingers. A binocular with magnifying power from 6 to 16 is enough to carry out this operation.

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A NEW DYE FOR USE IN COLORING TRIBOLIUM EGGS

Introduction

Some studies involving Tribolium eggs require that the eggs be marked by a dye of some sort to differentiate them from newly laid eggs or eggs of other species. The dye customarily used for this purpose is Neutral Red, a substance first used by Rich (1956). It has become necessary, however, to explore the use of additional dyes in order to differentiate the eggs of more than 2 species or strains. While conducting preliminary experiments in a cannibalism study, we have found a second suitable dye as well as one with deleterious effects on the adult beetles.

Materials and Methods

Three dyes, Neutral Red (certified), Brilliant Cresyl Blue, and Janus Green B, were selected for use in an egg viability study of 3 species of Tribolium. The species shosen were T. castaneum (Chicago black), T. confusum (Chicago), and T.madens (wild type). All 3 dyes are certified by the Biological Stain Commission for use as supravital stains for blood.

The medium on which the flour beetles were raised consisted of stone-ground wheat flour, yeast (5% by weight), and dye (1% by weight). All were sifted through a 4XX sieve, and the wheat and yeast were sterilized at 60 degree C for 24 hours. Eight grams of mixed medium were then parceled out in 8 dram vials and placed in an acclimatizing incubator for one week.

Thirty-six vials were set up for each species: 9 replicates for each of 3 dye mixtures and an undyed control. To each vial were added 20 randomly chosen beetles from stock populations. The vials were then incubated at 30 deg. C and 70% R.H.for 3 days.

The original intention was to collect 50 eggs from each vial on the 4th day and tally the number of hatchings. The fecundity of T. madens was over-estimated, however, as fully ½ of the vials yielded less than 50 eggs. All of the Janus Green B vials were included in the under 50 egg group. A similar problem was encountered when collecting eggs from the Janus Green B vials of the other 2 species.

Results

Janus Green B had a marked effect on the fecundity of all 3 species. Only 3 of 9 green T. confusum vials yielded 50 eggs, and not one of the green T.castaneum vials yielded that number. Of the latter, 3 vials yielded 1 egg, 1 vial yielded 2 eggs, and 2 vials yielded no eggs. The probability that this is the result of the sex ratio of the randomly chosen adults is extremely small (P less than 0.025).

Those green eggs which did successfully hatch did so earlier than eggs laid in the other treatments. While no statistical test was conducted to compare mean development time of the eggs, the difference was marked. We can conclude that beetles in the Janus Green B flour ceased ovipositing soon after being introduced into that medium.

Behavioral abnormalities were also noted among the beetles in the green flour. There was a tendency for the beetles to cluster at the top of the flour column rather than tunnel through it. Movement was lethargic. Some of the beetles were dead. These traits were not noticed in the other vials, and for this reason the Janus Green B dyed flour was excluded from any statistical comparison with the control.

Both eh Neutral Red and Brilliant Cresyl Blue compared well with the undyed control.   
A two way anova was performed on the percentage of eggs hatched (using the arcsin square root transformation). No significant dye effect was evident, and there was no significant species-dye interaction. There was a highly significant (P less tan 0.001) difference noted in the egg viability between species: T. castaneum was the most viable and T.confusum the least viable. This last fact is of interest in its own right but is not of any importance insofar as the experimental test of the dyes is concerned.

We can conclude that Brilliant Cresyl Blue provides an alternative to Neutral Red for dyeing Tribolium eggs, while Janus Green B is not suitable. In cases where it is necessary to distinguish 2 kinds of eggs, these dyes permit naked-eye separation.

Rich, Earl R. (1956). Egg cannibalism and fecundity in Tribolium. Ecology 37: 109-120.