TRIBOLIUM INFORMATION BULLETIN

Number 19

March, 1976

Foreword i

Notation on Stock Lists ii

Stock Lists 1

New Mutants 82

Notes – Research 84 – 129

Mortality of mutants reared at different temperatures.

James Albers and A. Sokoloff 84

Lack of parthenogensis in Tribolium audax and T. madens.

Daryl Faustini 86

Absence of a genetic maternal effect on egg surface in

Tribolium audax and T. madens. Daryl Faustini 87

The effect of sex and irradiation on cross-over in

Tribolium castaneum. Daryl Faustini 88

Larval dispersal of three Tribolium species,

D. Jillson, and R.F. Costantino 90

Naturally occurring mutants in stored produce warehouses

In Yugoslavia. Z. Korunic and A. Sokoloff 95

On heterogeneity in cI strain of Tribolium castaneum Herbst.

Tadeusz Prus 97

Dietary effects on population growth rates in Tribolium

W.D.Ryder, F.L. Waterhouse, and R. McHugh 105

Morphological traits and classification of Tribolium.

A.Sokoloff 111

Relative position of the genes aureate, black and light

Ocular diaphragm. A. Sokoloff 113

Artificial selection for food preference in Tribolium castaneum.

M. Hani Soliman 116

Esterase isozyme of some Tribolium strains.

E. Sverdlov, D. Wool, and E. Cohen 120

The response of Tribolium confusum Duv. Between sound

Wheat and wheat flour. T. Yoshida 128

Notes - Technical 130 – 144

A population cage for selection experiments involving

Tribolium. Barbara Howell Keim 130

Method for photographing Tribolium metaphase chromosomes.

Clark D. Taylor 132

Helpful hints for the insectrary. Alberto W. Vazquez 133

The division of microbiology insectary . A.W. Vazquez 137

Bibliography

1. Anatomy, Histology and Morphology 144
2. Behavior Studies 146
3. Insect Tissue Culture and Embryology 148
4. Cytology and Electro Microscopy 150
5. Ecology and Population Ecology 152
6. General 158
7. Genetics and Phenotypic Variation 159
8. Insecticides and Insecticide Resistance 163
9. Irradiation and Use of Isotopes 168
10. Nutrition 171
11. Parasites and Symbionts 172
12. Pests 174
13. Physiology and Biochemistry 190
14. Space and Aerial Ecology

NEW MUTANTS

1. Tribolium castaneum

Reduced juvenile urogomphi #2. ((rju-2, Sokoloff, 1976). Found in decendants of b p + x irradiated . Since recessive, it must have occurred in the b p stock to which the F 1 were backcrossed, especially since it was found in quite a number of vials set up with the same stock.

1. Dachs.

(Dch, Sokoloff, 1976 Dominant). Found in backcrosses of +/+ (irradiated at 4000 r) x au lod p +. F 1 were backcrossed to au lod p. Characterized by a shortening of the legs and the antennae. The legs are not affected at the coxa,

Trochanter or femur, but the tibia is reduced to about half the normal length.

The tarsal segments fuse into a solid mass, which may be definitely separated by a tibio-tarsal joint, or the tibio-tarsal complex may fuse into a solid mass. The antennae segments exhibit a variable degree of fusions: the club segments and the distal funicular segments may be fused into a solid mass but the pedicel and scape are not affected. The first two proximal segments of the funicle may not be affected. Because of their short legs the walking behavior is greatly affected.

1. Confusum-like

(cfl, Sokoloff 1976). In a series of matings in which the F 1 (derived from normal Tribolium castaneum males irradiated with 4000 r x au lod p ) and hence the

F 1 were genotypically ++/au lod; +/p) were crossed back to au lod; p females.

A number of vials yielded this peculiar mutation believed to have arisen in the

au lod p test stock. The mutant head appears somewhat broader and the eye smaller. The interocular space is equivalent to about two eye widths, as in

Tribolium confusum, instead of one eye width. The only other notable modification is a depressed appearance of the gular region. Sometiems the

gular sutures are irregular. No other features of the beetle seem to be affected.

Autosomal recessive.

1. Fused antennal segments – 9

(fas – 9, Sokoloff 1976). Experiments involving au lod p (Exp. 950) found

a “fused antennal segments” mutant designated as fas-9. It is an autosomal

recessive showing the distribution of fusions of the atennal segments shown in

Table I. The fusions involve the funicle and the club. There is some variability in expression, (some beetles having abnormal on one side or the other, and the other antenna showing fusions) but on the whole the mutant can be easily recognized by the fact that segments 6 – 8 of the funicle and 9 – 10 of the club

are involved in fusions. Note that the club may be free of fusions while the funicle is affected, while there are no cases in which only the club shows fusions

while the funicle is not affected. Note also that segments 4-5 and 5 -6 may

occasionally be involved in fusions.

Table I

Distribution of antennal fusions of the mutant “fused antennal segments-9” (fas-9) in T.castaneum.

Right Left Males Females

4-5, 6-8, 9-10 4-5, 6- 8 1

4-5, 6-8, 9-10 4-5, 6-8, 9-10 2

5-6, 7-8, 9-10 5-6, 7-8, 9-10 1

5-6, 7-8, 9-10 5-6, 7-8 1 1

6-8 6-8 7 3

6-8. 9-10 12 25

6-8 6-8, 9-10 9 4

7-displaced/ 6-8, 9-10 1

8 absent

Report of A. Sokoloff

Notes - Research

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Mortality of mutants reared at different temperatures.

A preliminary experiment has been carried out to test te effect of temperature on the survival of mutants reared continuously in each of three temperatures.

The mutants were obtained from the Tribolium Stock Center. About 30 adults from each mutant stock were introduced into a jar containing standard medium, and allowed to lay eggs for 24 hours. The eggs were then subdivided into six vials, each vial containing approximately 50 eggs. Two vials were placed in a walk-in chamber maintained at 29 0C., two were placed in a cabinet at room temperature (24 0 C), and two were placed in an incubator maintained at 36 0C. The eggs were allowed to develop to the adult stage, and the number reaching the adult stage was recorded. The results, shown as average mortality for the two vials, are given in Table 1.

While the experimental results need to be greatly expanded for a more firm conclusion, the data clearly show that some mutants have the same mortality under all three temperatures (e.g., py); other mutants have a greater mortality at the higher temperature (e.g., mah., r, and j); others exhibit a greater mortality at the lower temperature (e.g., p, b,; and finally, some exhibit a greater mortality whenever the temperature is raised or lowered (e.g., b, s, j, sq, ptl). Each of the mutants, therefore, appears to have an optimum for its development.

Table1. Percent mortality of mutants reared at different temperatures from egg to adult.  
 Temperature ( 0 C)

Linkage group Mutant 24 0 29 0 36 0

I mah 26.4 37.0 58.7   
 r 35.3 17.3 54.2  
 py 25.9 30.1 27.0  
 II p 34.1 7.0 24.8  
 III b t 42.2 18.2 49.1  
 b 34.9 24.3 13.3  
 IV Be 67.1 55.0 55.8  
 S 40.8 12.5 65.2  
 V j 37.0 16.0 59.4  
 VII ble 41.5 35.0 66.7  
 c 59.5 34.0 69.4  
 sa 82.4 60.2 83.2  
 VIII sq 72.4 39.7 60.4  
 IX ptl 62.4 30.0 57.2

Supported in part by U.S. Army Research Office grant LP 11790-LS and contract 13545L.

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Lack of parthenogensis in Tribolium audax and T. madens.

Introduction

Halstead (1969) has shown that T. audax and t. madens are closely related species, since crosses between them produce a few sterile, hybrids. Although   
a few ecological investigations have been undertaken these species have not been investigated from the genetic standpoint.

Aside from other insect orders in which this phenomenon is known to be common, parthenogenetic reproduction is quite rare in the Coleoptera (Suomalainen, 1969). Although this characteristic has shown to exist in a number of weevil species (Suomalainen, 1969), so far it has not been demonstrated in Triboloum. The purpose of this study was to determine whether parthenogenesis exists in Tribolium.

Materials and methods

From about 2000 pupae of T. audax and of T. madens allowed to develop into adults, twenty adult beetles were selected and isolated in vials containing fresh wholewheat flour and brewer’s yeast. These females were allowed to lay eggs for three days, then transferred to new vials for four days. The four-day egg-lay flour was discarded. After the beetles were transferred into a new vial containing fresh flour the old three-day vial was examined for larval activity. If no activity was observed after two weeks the flour was discarded. At the end of two months, a new series of virgin female adults was started and the old series sacrificed. The vials were kept in an environmental chamber maintained at 29 0C and 70 percent relative humidiey.

Results and discussion

In the period of six months, over which this experiment was conducted I isolated 1866 T. audax and 2061 T. madens. Not one beetle of either species exhibited parthenogenetic reproduction. Based on these preliminary observations, it appears that in both Tribolium audax and T. madens the phenomenon of parthenogenesis is absent.

Literature cited

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My thanks to Professor A. Sokoloff for his valuable advice and assistance in this experiment. (This project was funded by Army Grant RDRD LP 11790-LS.).

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Absence of a genetic maternal effect of egg surgace in Tribolium audax and T. madens.

Dawson and Riddle (1975) discovered that females of T.castaneum lay sticky or non-sticky eggs. If the eggs are sticky they become flour-covered; if they are non-sticky the surface of the egg appears wet and transparent-like. They have termed this phenotypic trait as “weird egg”. This trait is due to a maternal effect exhibited by females homozygous for a single recesive gene. The purpose of this study was to determine whether this trait exits in either T. audax or T. madens and to what extent.

Materials and methods

The following procedure was followed for both T. audax and T. madens: 40 single pair adult matings were isolated in small vials containing approximately two grams of wholewheat flour and brewer’s yeast. Every three days the eggs were removed through the use of a fine sifter and examined to determine if the weird egg characteristic existed. If none of the eggs was of the weird type the flour and eggs were discarded and the parent returned to the vial. At the end of two weeks a new group of 40 pair-matings was started and the old group discarded. The vials were kept in an environmental chamber maintained at 29 0 C and 70 percent relative humidity.

Results and discussion

This experiment was conducted over a five month period at which time approximately 840 sigle pair matings were crossed for each species. The “weird” egg phenotypic characteristic was not found in any of the beetle eggs examined.

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The effect of sex and irradiation on crossing-over in Tribolium Castaneum

Markers on limkate group III of Tribolium castaneum were used to determine whether (1) sex has any influence on recombination ad (2) whether irradiation has any influence on recombination in this linkage group.

Four wild type male Tribolium castaneum beetles were exposed to gamma rays at a dosage of 4000 rads. These males were mated with non-irradiated females homozygous fo aureate (au) and light ocular diaphragm (lod), located on linkage III and pearl (p), located on linkage group II. All the heterozygoud F 1 males and females obtained were crossed back to au lod/au lod; p/p beetles of the opposite sex. The control (non-irradiated) group was treated the same way but the original males were not irradiated.

As seen in Tables 1 and 2, in non-irradiated beetles there was a significantly greater frequency of recombination in males (the heterogametic sex) than in females (28.17% vs. 17.41%, respectively). Irradiated males showed

a significant increase in the frequency of recombination over non-irradiated males (31.41% vs 28.17%, respectively) but the irradiated females did not differ significantly from non-irradiated females (19.31% vs. 17.41%, respectively).

This study shows that, for linkage group III, the frequency of recombination is greater in the male (the heterogametic sex) than in the female, and that irradiation can influence the recombination frequency in the male, but not in the female.

Supported in part by U.s. Army Research Office grant LP 11790-LS and contract 13545L.

Table 1 . Parental and recombinant phenotypes observed in backcrosses of irradiated and non-irradiated female beetles (i.e. ++ /au lod; +/p X au lod/au lod; p/p).

CROSS TOTAL NUMBER PARENTAL RECOMBINANTS  
 OF PROGENY PHENOTYPES au-lod

Females n %

Irradiated 2729 2202 527 19.31  
Non-irradiated 2142 1769 373 17.41

Table 2. Parental and recombinant phenotypes observed in backcrosses of Irradiated and non-irradiated male beetles. (i.e. ++ /au lod; +/p X Au lod/au lod; p/p).

TOTAL NUMBER PARENTAL RECOMBINNTS

CROSS OF PROGENY PHENOTYPES au-lod

Males n %

Irradiated 2566 1760 806 31.41  
Non-irradiated 2581 1854 727 28.17

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Larval dispersal of three Tribolium species

Introduction

Numerous Tribolium researches have conducted studies on dispersal in the flour beetle. The principal focus has been upon the adult, particularly T.confusum and T. castaneum (e.g. Ghent 1966, Naylor 1959, 1961, Prus 1963, Wool 1969). Adults obviously possess the capacity for covering much greater distances than do larvae; however, the more meager dispersal ability of larvae should not be neglected. The ability of larval stages to travel over moderate distances may be an important component of species survival. Avoidance of cannibalism, access to grain of high nutritional quality, and lack of physical disturbances to tunnels may significantly increase larval survival. The laral distribution of three Tribolium species in homogeneous culture medium was investigated.

Materials and Methods

Tribolium castaneum corn-oil sensitive (Costantino, Bell and Rogler 1967), T.madens and T. brevicornis were selected as experimental animals. These species show a gradient in adult body size: T.castaneum is the smallest, T.brevicornis is the largest. A cactorial design was employed, whereby the larval distribution of each species was inspected at three time periods. Plexiglas boxes measuring 12 x 17 cm were filled to a depth of 1 cm with standard culture medium. The shallow depth of medium encourage horizontal dispersal. The experiment was begun by placing 50 eggs of a species into the center of a container. Six days after the eggs were introduced, three replicates of each species was sampled by dividing the container into 16 equal-sized quadrats, and recording the number of larvae in each quadrat. The procedure was repeated at 10 days and 14 days.

Results

Dispersal upon an homogeneous rectangular plane may be viewed as a radial pattern, with distance from the center as an important parameter. The 16 sampling quadrats were lumped into four “distance” categories of four quadrats each, representing distance from the container midpoint: 2.6 cm, 5.1 cm, 6.7 cm, ad 7.9 cm. Table I lists the percentage of each larval population sampled within each distance category. After 6 days, T.brevicornis was found in moderate numbers throughout the containers: T.madens was more frequently sampled in the central quadrats, and fully 95% of the T.castaneum larvae were found in the center of the experimental containers. After 10 days, each species was located noticeably farther from the midpoint; with T.brevicornis found principally in the most distant quadrats. At 14 days, T.madens and T.castaneum larval distributions remained largely unchanged from the 10-day sample, whereas T.brevicornis larvae attained a uniform distribution throughout the containers.

A cactorial analysis of variance was performed on the number of larvae of each species in each quadrat over time (Table II). The species and distance main effects had significant F.values at p .05, as did the time x distance, species x distance and the time x species x distance interactions.

Discussion

T. brevicornis larvae clearly moved greater distances than dis T.madens or T.castaneum larvae. Within 6 days, nearly one-third of the T.brevicornis larvae had reached the farthest corners of their containers, whereas nearly two-thirds of T.madens and practically all of T.castaneum larvae had not moved from their initial location. That differences in dispersal pattern are not due exclusively to larval size is seen by a comparison of mean larval lengths at each sampling period (Table III). At all three sampling periods, T.madens larvae were largest; T.brevicornis were intermediate in size exfept at day 10. However, the differences in length between species at a sampling period was much less than the difference between sampling days.

TABLE - I

Mean percentage of larvae within each distance category at three time periods.

SPECIES DISTANCE DAYS

cm 6 10 14

T.brevicornis 2.6 .32 .15 .27

5.1 .27 .12 .24

6.7 .10 .27 .25

7.9 .31 .47 .24

T.madens 2.6 .61 .41 .46

5.1 .12 .26 .21

6.7 .12 .14 .20

7.9 .14 .18 .14

T.castaneum 2.6 .95 .43 .41

5.1 .04 .27 .17

6.7 .01 .16 .25

7.9 .00 .14 .17

TABLE II  
 Analysis of variance on number of larvae per quadrat.

Source of Mean  
 variation df Square F

Time (T) 2 1.919 0.634  
 Species (S) 2 33.030 10.909 \*  
 Distance (D) 15 32.138 10.614 \*  
 T x S 4 6.294 2.079  
 T x D 30 9.847 3.252 \*  
 S x D 30 12.981 4.287 \*  
 T x S x D 60 4.900 1.618 \*  
 Residual 288 3.028

\*Significant at the 0.05 level

TABLE III

Mean larval length (mm) of three Tribolium secoes

Species days

6 10 14

T.castaneum 1.42 + 0.04 2.46 + 0.09 4.19 + 0.18

T.madens 1,69 + 0.04 2.54 + 0.08 4.61 + 0.12  
T.brevicornis 1.53 + 0.05 2.40 + 0.05 4.23 + 0.30

If dispersal distance were primarily a function of larval size, one would expect the dispersal distance of T. castaneum at 10 days to be greater thant that of T. brevicornis at 6 days. One would expect T. madens larvae to have covered the greatest distance, for they were consistently the largest. The failure of these expected patterns to appear implies that larval dispersal ability is a complex phenomenon and not a simple function of larval size.

T. brevicornis larvae are notable in that their patterns of dispersal were substantially different from T. madens and T. castaneum. Larvae of the latter two species were distinguishable in dispersal pattern after 6 days, but were similar in the 10 and 14 day samples. Further testing in larger experimental containers might further delicate larval dispersal ability.

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Naturally occurring mutants in stored produce warehouses in yogoslavia.

Examination of samples of flour beetles derived from a number of stored product warehouses near Zagreb, Yogoslavia, have yielded a number of mutation.s

One, previously recorded, is an autosomal semidominant black body color mutant (b z) allelic with the previously described McGill black. A second mutation, referred to as maroon (mar) is a sex-linked recessive mutation modifying the normally black eye into

a reddish one. The mar mutant is a much better marker for the X-chromosome than eyespot (es) or even the es lt allele. The third mutation, christened “fused antennal segments-3 (fas-3)” is an antosomal recessive which produces fusions of the funicular and club segments of the antenna. It differs from fas-1 in that fas-1 does not involve the more proximal segments 3-4 of the funicle. It resembles fas-2 in that segments 3-4 and 5-6 of the funicle and 9-10 of the club are affected, but in additionin fas-3 segments 6-7 or 7-8 are involved in fusions. Often blocks of segments 6-10 or 7-10 or 8-10 are formed, resulting in a fairly solid mass. A further difference is that fas-3 antennae are often elbowed a characteristic seldom seen in fas-2.

Table I shows the distribution of fusions of antennameres in the two sexes.

Linkage studies of fas-3 and mar are in progress.

(This investigation was supported in part by U. S. Army Research Office grant

LP11790-LS and contract 13545L).

TABLE - I

Antennal fustions in fas-3 T. confusum.

Segments fused in antennae Male Female

3-4, 5-6 3

3-4. 5-6, 7-8 6 3

3-4, 5-6, 7-9 3 2

3-4. 5-6, 7-8, 9-10 2 3

3-4, 5-6, 8-9 1 2

3-4, 5-6, 7-10 2

3-4, 5-6, 8-10 1

3-4, 5-7 1

3-4, 5-7, 8-9 3

3-4, 5-8, 9-10 2

3-4, 6-7 3

3-4, 6-7, 8-9 8 6

3-4, 6-7, 8-10 -

3-4, 6-7, 9-10 1

3-4, 6-7, 9-11 1

3-4. 6-7, 8-9, 10-11 1

3-4, 6-8 1

3-4, 6-9 1

3-4, 6-10 2 1

3-4, 7-8 4 1

3-4, 7-8, 9-10 2

3-4, 8-9 6

3-4, 8-9, 10-11 1

3-4, 9-10 2

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On heterogeneity in cI strain of Tribolium castaneum Hbst.

Introduction

Four genetic strains of T. castaneum obtained from Prof. Dr. Thomas Park’s laboratory show differences in may physiological features as fecundity, fertility, cannibalistic predation, longevity, mortality, duration of development and other characteristics (Park, Mertz, Petrusewicz 1961, Park, Martz, Grodzinski, Prus 1965). These strains of

T.castaneum together with 4 genetic strains of T. confusum were brought to Poland in 1963 and have been cultured at 29 degree C and 75% of relative humidity for many years. In 1966 a study of elements of energy budget was initiated (Klekowski, Prus, Zyromska-Rudzka 1967), using strain cI of T. castaneum. During measurements of production a heterogeneity was found within this strain. It is possible that the strain could evolve and change its characteristics or split into two sub-strains.

The aim of this paper was to describe the difference observed with cI strain, the number of larval instars being the criterion for division. Such features as growth curves, maximum weight, and the time of reaching this maximum as well as the duration of development were ascertained.

Methods

Two series of individual cultures, 25 in each, were run for over 100 days. The medium of each larva consisted of 2 g of flour and baker’s powdered yeast (95% and 5%, by weight). The cultures were synchronized, the difference in hatching time of the larvae did not exceed 4 hours. The larvae and other stages were weighted every second day and the number of exuviae was recorded. Each vial content was sifted through fine mesh and the larva with its exuvium were collected. The larva was weighed and placed again into 2 g of fresh medium. The small larvae were weighed with a quartz balance and the large larvae with a sartorius balance.

Results

Out of 50 individuals only 43 survived over 100 days, 22 females and 21 males. Of the 22 females, 8 had 6 larval instars and 14 – 7 larval instars (36.36% and 63.63%, respectively). Of 21 males, 6 had 6 larval instars and 15 had 7 larval instars (28.57% and 71.43%, respectively). In general, there were 14 individuals with 6 larval instars and 29 individuals with 7 larval instars (32.55% and 67.45%, respectively). The ratio is close to 1:2, which may have some genetic implications.

The two groups of animals differed also in their growth curves (Fig.1). The 6-instar larvae grew faster than the 7-instar larvae but they obtained lower and earlier maximum weight. The difference in weight was permanent in prepupa, pupa, and adult stage, the 6-instar larvae were always lighter than 7-instar larvae (Fig. 1).

Statistical comparisons were made between 6 and 7 instar individuals in respect of their maximum weight, the time of reaching this maximum weight, and the weight of adult beetles on the 72nd day of their life using the Student t-test (Table I). All comparisons of these features between 6 and 7 instar individuals showed significant differences at

a probability level of 0.001. This points to differences in the course of growth and development of these two groups. However, the sex differences within these two groups of individuals in respect of the features examined were insignificant with

an exception that the 7-instar females reached the maximum weight one day earlier than did the males (Table II).

The two groups of larvae differed also in duration of their development (Fig. 2). The complete development for a newly hatched larva to the adult stage in 6-instar males lasted 25.6 + 0.82 days and in 6-instar females to 24.88 + 0.97 days, or 25.02 + 1.00 on the average (Table III). The total development of 7-instar males lasted 28.17 + 0.69 days, and that of females – 29.29 + 1.59 days, or 28.73 + 1.13 on the average. The differences between the total duration of development in 6- and 7- instar males and females are highly significant at a probability level of 0.001 (for males – t = 8.41, for females t = 8.86). The difference between average duration of development for both sexes is also highly significant (t = 8.98).

Discussion

The results concerning duration of the development of the cI strain differ somewhat from those reported by Park, Mertz, Petrusewicz (1961). According to these authors the total duration is 28.12 days, whereas in the present paper it was 32.73 days (when the two groups were averaged and when the duration of an egg equaling 4.09 days was added). This discrepancy is obviously due to handling techniques (sifting, weighing, and measuring the larvae). The development occurred not continuously at 29 degree C but for about 8 hours every second day at a room temperature of 22 degree C which prolonged the total development. Beside, it was found by Mertz and Robertson (1970) that the handling of growing larvae has a significant effect on prolongation of the larval stage in T. castaneum (genetic strain cIV-a). Howe (1961) has pointed to heterogenous character of Tribolium cultures in respect to duration of development and individual weight. These results are in agreement with his findings.

Summary

By individual culturing Tribolium castaneum genetic strain cI, differences were found in several features of this strain. Two groups of individuals, both males and females, were distinguished. One group included individuals with 6- larval instars, the other one – 7 instars. The 6-larval instar group had lower maximum weight and shorter development cycle than 7-larval instar group. The differences in the course of growth and in duration of development were statistically significant.

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Dietary effects on population growth rates in Tribolium.

Introduction

Preliminary observation showed that lentil meal, while permitting the survival of Tribolium castaneum adults for many months did not allow population growth. This suggested that, incorporated into favorable foods, lentil would provide a source of heterogeneity whereby the rate of increase in numbers might be restricted. The mixing of lentil with 12:1 combination of wheat flour and yeast caused retardation of the growth of populations from small numbers of virgin adult. Subsequently a 1:1 mixture of yellow maize meal ad fish meal was used as the favorable food.

Experiment 1 considers the effects on population growth of adding different amounts of lentil to a fixed amount of the maize meal/Fish meal mixture, whereas in Experiment 2 the total weight of incredients remains constant ad the proportions of lentil and favorable food vary. Experiments 3 and 4 are concerned with the effects of ingredient particle size variation.

Materials and Methods

Experiments 1 & 2: the ingrediens were ground and sieved to obtain particles of 420-710 microns, and were placed in glass jars whih were 5 cm in diameter and 6.5 cm high. The surface was seeded with five (Experiment 1) or four (Experiment 2) virgin adults of each sex aged up to five days. The jars were closed with organdie and kept in darkness. Randomized blocks designs with six replications were used. Experiment 1 was conducted at 25 degree and 70% RH, Experiment 2 at 30 degree and 54% RH. Live final instar larvae, pupae and adults were counted at intervals. Analyses of variance were performed on the square roots of the counts. Means were compared using Duncan’s (1955) multiple range test. In Experiment 1 all treatments contained 5 g each of maize meal and fish meal; one treatment lacked lentil, the rest included 2.5, 5, 10, 20 and 40 g of lentil meal. In Experiment 2 there were 30 g of total ingredients per treatment; one treatment contained 15 g each of maize meal and fish meal; in the others the weights of each of these materials were 12, 10, 7.5, 5 and 3 g, in addition to which there were 6, 10, 15, 20 and 24 g respectively of lentil.

Experiment 3: the basic medium consisted of 20 g of a 1:1 mixture of maize meal ad fish meal of particle size 420-710 microns. 10 g lentil were incorporated as particles of 0-150, 150-420, 420-710 or 710-1000 microns, or as split lentils. The media were seeded with four virgin adults of each sex.

Experiment 4: particles of 180-250 and 420-710 microns were used. 10 g of lentil of each size range were mixed with 20 g of 1:1 maize meal/fish meal of each size range. Five virgin females and three virgin males were used for seeding. The experiment was conducted at 30 degree and 75% RH.

Other details for Experiments 3 & 4 were as in Experiment 1.

Results

Experiment 1 & 2: numbers of larvae and pupae increased rapidly, then fell to zero. Adult numbers also peaked, but their decline was relatively gradual. In Experiment 1 the population growth rate fell with increase in lentil content. Peak adult numbers in the treatment containing 2.5 g lentil were anomalous in that they were smaller than those in the treatment containing 5 g lentil (Table 1). In Experiment 2 there was little evidence of a graded response to lentil content by the immaturestages. Adult numbers tended to fall with increase in lentil proportion (T^able II).

Experiment 3: 27 days after seeding there was marked retardation of larval development where the finest lentil particles were used. Overall retardation of population growth wa greatest where the size of the lentil particles equaled that of the favourable food (Table III).

Experiment 4: 41 days after seeding there were more pupae in the lentil-free treatments than in those containing lentil. In the lentil-free treatments, adult numbers were higher where the finer particles were used. The largest reduction in adult numbers ws produced by the coarser grade of lentil in maize meal/Fish meal, there was a slight tendency for the finer lentil to depress adult numbers more than the coarser grade

(Table IV).

Discussion

The mixing of lentil with maize meal and fish meal substantially retarded population growth, even where the amount of favorable food remained constant. Bhattacharya and Pant (1969) found that lentil had a low food value for Trogoderma granarium because of a cholesterol deficiency and the presence of a growth-inhibiting factor. The possibility exists that in our experiments lentil ingestion occurred due to an inability to distinguish between or to separate different materials of similar particle size. Population growth retardation could have followed because of the nutritional qualities of the lentil. Reduced intake of favorable food might alo have had an effect. Variation in environmental volume and packing density appeared not to be responsible for the results obtained. Extra consumption of time and energy during searching activities in lentil-containing mixtures conceivably led to diminished populations. Overcrowding seemed not to be a factor in the results, since population density was usually lower in lentil-containing treatments than in those lacking lentil. There was no evidence of increased cannibalism in the presence of lentil.

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Tables

In the above tables, means in the same row lacking a letter in common are significantly different.

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Infestibility of faba bean by Tribolium and other storage insects

Infestation potential of 12 species of storage insects on two cultivars of faba bean (Vicia faba L. var. minor), which has recently been introduced into Canada as a potential potein supplement for animal feeds, was determined at 27 – 30 degree C and 70% RH. When survival and reproduction was used as criteria, whole seeds were resistant to attacks by all insect species except Tribolium castaneum (Herbst). Even T. castaneum died on whole beans of ‘Ackerperle’ and ‘Diana’ cultivars in 10 weeks, other 11 species died in 1-6 weeks – all without reproducing.

Cryptolestes ferrugineus (Steph.), Oryzaephilus surinamensis (L.), T. castaneum and T. confusum (du Val) reproduced on crushed beans; F 2 generation, however, was rarely produced even after 20 weeks. T. castaneum was the most successful pest of faba beans, continued multiplying slowly on crushed beans even after 32 weeks and performed equally well on both bean cultivars.

The following storage insects did not reproduce at all on crushed faba beans, but survived between 2 and 16 weeks: Acanthoscelides obtectus (Say), Cryptolestes

Turcicus (Grouv.), Oryzaephilus Mercator (Fauvel), Sitophilus granaries (L.), S. oryzae (L.), S.zeamais Mots., Tribolium audax Holstead..

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Morphological traits and classification of Tribolium

Taxonomists rely on many morphological traits to place an organism in its proper family, genus and species, and variation in one or more attributes still enables the taxonomist to place a specimen in its proper category.

To cite an extreme case as an example from Tribolium, a specimen of labiopedia, which modifies the labial polps into legs bringing the total legs to four pairs, would still enable even the most inexperienced taxonomist to place such a specimen among the insects instead of the arachnids, since the specimen looks more like an insect than a spider, and the possession of two pairs of wings, the anterior pair modified into elytra, would place it in the Coleoptera.

Mutants affecting the antennae and the tarsi present a special problem because the segment number in the antennae may be reduced (from the normal one of 11 segments to a much smaller number) and the number of tarsal segments may be considerably modified so that the characteristic formula of 5-5-4 tarsal segments in the first, second and third pair of legs no longer holds. Thus, for example, the tarsus may consist of at most at most one segment in Fta (Fused tarsi and antennae) and in many specimens of Dachs Dch) the tibia is reduced in size and the tarsi fuse into a solid mass which may also be intimately fused with the tibia.

One of the reliable traits for separating T. castaneum from T. confusum is the size of and distance between the eyes; T.castaneum can be identified by having an interocular distance equivalent to one eye width (when the ventral aspect of the head of the beetle is examined) while T. confusum is recognized as having an interocular distance equivalent to two eye widths.

The interocular distance attribute is no longer reliable to classify one mutant of T. castaneum from T. confusum; in the “confusum-like” (cfl) mutasion the head is considerably modified in shape so that the beetle resembles T. confusum more than T. castaneum (see Fig. 1). Indeed, when I first discovered it among some normal T. castaneum beetles I thought it was a contamination: Without the antennae as an accessory sid in classification I am certain the reader would have difficulty in determining whether the mutant be ongs to T. castaneum or T. confusum.

I thank Mr. Daryl Faustini for helping to obtain SEM micrographs of this interesting mutant.

This investigation was supported by U.S. Army Research Office grant LP11790-LS and contract 13545L.

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Relative position of the genes aureate, black and light ocular diaphragm.

The relative position of the genes black (b) light ocular diaphragm (lod) and aureate (au) in linkage group III of T. castaneum is b-lod-au. The distances between the genes varies, depending on the cross: b++/+lod au + lod au/+lod au give these recombination values: au-lod = 18.32; b-lod = 21.05; b-au = 37.93. The reciprocal crosses give au-lod =27.67; b-lod = 13.97 and b-au = 39.79.

Clearly, recombination values between b – lod in the female are larger than in the male, while in lod-au they are larger in the female. For the larger distance covered by b-au the sex differences in recombination are not significant.

This investigation was supported by U.S.A.R.O. grant LP11790-LS and contract 13545L.

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Maze-learning in T. castaneum as influenced by selection for food preference.

By repeated runs in a T-shaped maze Lerner and Inouye (1968) demonstrated that T. castaneum adults are able to learn and that this ability could be increased by artificial selection. In their experiment no aversive stimulation was used. However, learning is known to play an important role in feeding of insects where feeding preferences could be modified by the previous experience (Dethier, 1966). Thus the present report is concerned with the effect of the presence of garlic powder, which seems to have an aversive effect on the behavior of T. castaneum, on the learning ability of the beetles to discriminate between garlic (40%) and standard medium.

The runs were performed in the same way as in the selection experiment (this issue of TIB). The only difference was that the beetles assigned for the learning experiment were run 3 times, each time using insects that had chosen the specified medium. The progeny of the beetles in each generation was split into two groups, one for the selection experiment (run once) and the other group for the learning experiment (run thrice.) Individuals used in the learning experiment were not used for the artificial selection for food preference. If the number of insects for any given run was extremely small the runs were not continued, as was the case in G4 for the garlic line.

Table 1 indicates that rerunning the beetles tha chose the standard medium had improved their performance. This improvement was more pronounced in the initial generation GO (from 58% to 94%) than for G3 and G4. These results indicate that selection for desirable behavior improves thae ability to learn this behavior.

On the other hand, selection for an undesirable medium, such as garlic, resulted in an aversive response and did not change the learning ability of the beetles concerned. On the contrary, in G4 males learned not to choose garlic (negative learning). This negative response was more pronounced for females than for males. The improvement from GO to G3 in the learning ability of the males in the garlic line may be due to the ability of the males to adapt to garlic in their breeding environment up to G3. Lerner and Inouye (1968) have also found that selection for running speed can also result in selecting for ability to learn in the males but not in the females. The present observations led to the conclusion that food preference, although it has a genetic basis, could be altered by behavioral factors; one of which is learning.

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Artificial selection for food preference in t. castaneum.

In the present experiment, artificial selection was applied to examine the genetic basis of good preference for either standard medium (95%) whole wheat flour and 5% dried yeast) or garlic medium (40% garlic powder).

Material and Methods

Beetles les than one week old were used. They were starved for 24 hours before being introduced into the empty arm (Source) of aY-shaped maze (Soliman, 1975). The beetles selecting the arm of the maze that contained the standard medium (Normal line) in the initial generation, GO, and subsequent generations were reared in that medium. Beetles selecting the garlic medium (Garlic line) were bred on garlic up to G3 after which they were bred on standard medium to produce progeny tested in G4. This was done due to the low viability of the beetles reared on garlic medium. In each generation after maze selection, the selected insects were left on the appropriate medium for

3 days after which eggs were collected for a period of 24 hours. Progeny of G3 and 4 were separated into sexes. No more than approximately 50 individuals were used in

a single run. All beetles were incubated at 30 degree C and 70% R.H.

Results and Discussion

The results for the preference test in each generation of selection are summarized in Table 1 for the complete data. Fi. 1 presents the results of the percentages of the beetles that chose either medium calculated from the total number of beetles that left the source (active). From this graph it is evident that the preference of the Normal line for the standard medium has increased from an initial frequency of 50% at GO to a maximum of 96.7% for females and 94.2% for males at G3. However, there is a slight decrease at G4, where 88.4% of the active males and 86.1% of the active females preferred standard medium. It appears that both sexes behaved in a similar manner (X = 0.2 and 1.6 for G3 and 4).

Chi-squared values for the difference between the two media within each generation and line showed increasing significance from G1 to G4. In GO and G1 there was no difference in the preference of adults for either medium. The decrease in the garlic preference of adults for either medium. The decrease in the garlic preference for the line selected for garlic preference is unexpected. From the selection results and the observed low viability of this line. It seems that the concentration of 40% of garlic has a harmful effect, possibly as that previously observed for phenyl-thi-carbamide (Soliman, 1974). Therefore, it could be postulated that the observed results for the behavioral selection in this line are due to a negative feedback mechanism to counteract this harmful effect. The negative response to garlic was more pronounced for males than females. This sex difference could be due to some secondary sexual characteristic interfering with chemoperception of the two media which is probably localized in the antennae (Soliman, 1975). In the silk work moth (Bombyx) the male shows a strong behavioral reaction to bombykol while the female does not, which indicates that the female is lacking a receptor specific to bombykol and related substances (Schneider, 1963).

The present results indicate that the avility of the beetles to distinguish between the two media is under genetic control. It is worth mentioning that the rapid response obtained in such a short period of time has also been observed by Lerner and Inouye (1968) who selected for speed of maze-running in both T. castaneum and T. confusum. This rapid response may indicate that the number of genes which control food selection may be small in the present case. More detailed and controlled experiments will undoubtedly reveal useful information about the genetics of good and habitat selection by the flour beetles as a behavioral mechanism for adaptation to their variable and available environments under natural and semi-natural conditions. The use of the wide range of antennal mutants in Tribolium will enhance this field of investigation since food seeking and selection is mediated by the antennae, the sensory organ for the olfactory cues in the flour beetles (Soliman, 1975).

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Esterase isozyme of some Tribolium strains

Introduction

Isozyme patterns detected by gel electrophoresis are useful genetic markers in a large number of animal and plant species. Separation of proteins by electrophoresis is now the most sensitive method available for detecting genetic variation, in animal and plant populations.

We know of only two preliminary reports on the use of this method with Tribolium. Since this beetle is one of the most commonly used laboratory insect in population research, we feel that additional information on isozyme patterns in this insect may be useful for many colleagues.

In this report we describe the results of extensive work with one group of enzymes—namely Esterases—in two species of Tribolium.

Materials and Methods

A general description and discussion of the methodology of gel electrophoresis from

a genetic viewpoint is given by Lewontin (1974). We shall assume that the reader is familiar with the principles, and describe only those aspects relevant to our own work.

1. Gel Preparation. We used 6% Acrylamide slab gels and a continuous, 0.1 M Borate buffer system pH 8.2.
2. Homogenates. Single beetles were homogenized in 0.1 ml of 10% sucrose in 0.1 M Tris-Borate-EDTA buffer, pH 9.2 (Shaw and Prasad, 1970). Bromphenol Blue was added to th homogenizing solution to form an anodally fast-migrating front line.
3. Separation. Samples of about 25 microliters of the homogenate were introduced into pockets in he acrylamide gel. Gels were run in constant current of about 4 mA per cm of gel width, for about 2 hours, in the refrigerator.
4. Staining was done in 0.1 M phosphate buffer, pH 6.5, using -Naphthyl Acetate as substrate and Fast Blue RR as dye (Shaw and Prasad, 1970). At room temperature, bands appeared within 15-30 minutes.
5. Fixation and preservation of the gels. After staining for about 2 hours, the gels were fixed overnight in a 5:5:1 mixture of water: Methanol: Acetic Acid. They were then placed on filter paper and held tight by a piece of Nylon bolting cloth at room temperature for about 3 days. The gels dried and remained attached to the filter paper. (Acrylamide gels do not adhere to the nylon). The dry gels may be stored in this way for extended periods.

Results

All esterases we measured migrated to the anode (in other work, using starch gels, we found indications of some cathodally-migrating esterases. but these are not included in the report since they were not analysed in any detail). Four esterase systems could be detected which we numbered from the fastest (Est-1) to the slowest (Est-4).

There were differences in esterase patterns between adults, larvae and pupae

I Adult esterases

(a)Tribolium castaneum, the black strain CS bb (see stock list).

In the course of our work with this strain, several inbred lines were propagated in   
 which different variants of Est-1 pre-dominated. Four of these variants are   
 illustrated in Fig. 1. The variants 1 (S), 2 (F), and 4 (FS) were the most ommon.  
 In some in-bred lines there were two S bands. Very rarely, a fifth variant was   
 found with a weak fast and migrating as fast as the anodal end of the F band.

EST-2 appeared as one or two bands (Figure 1).

EST-3 and EST-4 usually appeared as diffuse bands, and staining was   
 variable.

(b)T.castaneum – the wild type strain, CS ++ (see stock list).

In this strain we found only two of the EST-1 variants; either F or FS variants  
 2 and 4 in Figure 1. These variants were electrophoretically identical to those of CS bb. In more than 200 beetles electrophoresed the slow variant (S) was never  
 found.

(c)T.castaneum – the eu strain (see stock list).

This strain was obtained from F 2 of a cross between a mutant CS bb female  
 and a normal, CS ++ male (Wool and Mendlinger, 1972, 1973). We have tested  
 two substrains derived from it – eu++ and eu bb (phenotypically showing wild type or black body color) for esterases.

The esterase pattern of eu -+ was similar to CS ++, and that of eu bb was similar   
 to CS bb.

(d)Tribolium confusum, the black strain CF bb (see stock list).

The esterase pattern of CF bb adults was clearly different from all CS strains.  
 (Fig. 2, compare cells 4-5 with 6-7). The fast-migrating esterases migrated faster than the F band of EST-1.

II Esterases of Immatures

The esterase patterns of immature were different from the adult pattern. In all   
 T.castaneum strains the changes in esterase patterns during ontogeny were  
 similar. (Figure 3). EST-1 stained more strongly in larvae than in adults. In the   
 pupal stage EST-1 stained more strongly in larvae than in adults. In the pupal   
 stage EST-1 disappeared (in fact, this happened already in the quiescent period before pupation) and reappearedin the older pupae, before adult emergence.  
 The first band to appear in the pupa was the one described as variant 3. Another clear difference between larvae and adults was apparent in EST-4 which stained  
 much more strongly in larvae.

In T. confusum (Fig. 2) the two fast migrating bands stained clearly in the larva,  
 disappeared in the pupa, and reappeared as strongly-stained bands in the adult. However, in addition, a strong band appeared in the pupal stage, which was not  
 visible in either larvae or adult. (Cells 1-3 in Fig.2).

Those esterases which changed activity during ontogeny were located, in both  
 species, in the alimentary canal. When the intestine was removed from the larvae and electrophoresed separately, all activity was found in the intestinal  
 homogenate and not in the rest of the body.

III Enzyme-substrate specificity.

The esterases were routinely stained using - Naphthyl Acetate as substrate,  
 but they stained just as well using - Naphthyl Acetate and - Naphthyl propionate. When - Naphthyl Laurate were substituted, the EST-1 F bands  
 stained well, but the S bands were weak or invisible.

Discussion

Esterases were chosen for this study because they are easily separated and stained. Further studies on other enzymes are under way.

Our experience with esterases shows that they can be useful genetic markers for Tribolium strains and species. One advantage of these markers is that they can be identified in the immature stages (most available morphological markers are detectable only in the adult stage). The esterase patterns of T. castaneum and T. confusum are clearly different from each other, while different T.castaneum strains have the same bands, although not in the same frequencies.

The choice of esterases as markers must be done carefully, to avoid confounding genetic with ontogenetic differences. EST-1 bands cannot be used as markers in T.castaneum pupa because they disappear just before pupation. A similar behavior of esterase patterns during ontogenesis was described in Drosophila (e.g. Berger and Canter, 1973, Korochkin, 1974).

Genetically, we analyzed only EST-1 bands in detail. The F and S bands were first thought to represent two alleles at the same locus. The frequencies of S, FS, and F in the CS bb stock population were approximately 1 : 2 : 1; (about 150 beetles were electrophoresed). From the stock we easily derived strains with only F and only S phenotypes. However, later work forced us to reject this hypothesis, because of the following evidence.

1)We have 7 lines (derived from single pairs of a cross of CS++ x CS bb, both with FS phenotype) which consistently showed the FS phenotype in all their offspring for several generations (as though they are fixed for the “Heterozygous” condition).

2)When these “mixed” lines were crossed back to the S parent, there still was no segregation ad all the offspring were FS.

3)In the collection of approximately 40 CS bb inbred lines derived from the stock, there are some which segregate only S and FS, or alternatively on F and FS, but not all three genotypes, as should be the case in a two allwle, one locus model. These phenomena could be explained if homozygous FF or SS were severely selected against (lethal), but this is not the case since we have flourishing “homozygous” F and S strains.

We now suggest the following genetic model to explain our results. The EST-1 bands represent two loci, F and S, and each has n alternative “null” allele. The F site is composed of two closely-linked genes which we label F1 and Fs. The S site is located away from F so that some recombination is possible. The F and S genotypes could be

If the model is correct, the “fixed” FS lines could arise from a recombination event in  
a hybrid between F and S:

The “double null” (no EST-1) case may be almost lethal because such cases were not detected. Recombinants having only one F band (such as variant 3 in fig. 1) are rare because linkage between F and F I very tight. We have some evidence to the effect that both F and S sites may be linked to the black locus (the similarity of eu bb and of eu++ to CS++ also hints in this direction). More data are being gathered to verify the genetic model.

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Acknowledgements : This work was supported by the Israel Commission for Basic Science.

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The response of Tribolium confusum to Penicillium isolates in the medium.

As part of a study of the relationship of storage fungi to stored product insects, we screened Penicillium isolates for nutritional and/or toxic effects on Tribolium confusum. Penicillium-wheat cultures were ground into a g our and fed to neonatal larvae.   
Table 1 lists some examples of the varying effects of the 18 species (26 is lates) tested. Enhancement of larval growth from an isolate like P. chrysogenum meant rapid larval growth, early pupation and early adult emergence. The adult was not larger than the control adult beetle. Some isolates had little or no effect on the beetles (P. citrinum). Others were definitely inhibitory with adults averaging somewhat smaller and larval periods longer. Mortality in this group was high. One isolate of P. expansum caused 52% mortality and one of P. purpurogenum 98%.

Many of the isolates were known mycotoxin producers. Several isolates of one species with known differences in production of toxic metabolites (example; P.viridicatum) gave variable results. These results were not consistent with toxic metabolite production. Consequently, we found no co-relation between the ability of a Penicillium isolate to produce a mycotoxin and its effect on T. confusum.

Table 1 - Some examples of the effects of Penicillium isolates on the growth of Tribolium confusum

Penicillium Average weight (mg)

Isolate Larva (20 da.) Pupa Adult  
viridicatum 2.3 3.5 2.7  
viridicatum 2.2 3.4 2.7  
chrysogenum 2.3 3.4 2.6  
cyclopium 2.1 3.4 2.7  
patulum 1.9 3.5 2.8  
CONTROL 1.2 3.5 2.7  
citrinum 1.3 3.4 2.7  
cyclopium 1.1 3.4 2.6  
viridicatum 0.9 3.0 2.4  
rubrum 0.4 2.9 2.5  
expansum 0.2 2.8 2.2  
purpurogenum 0.2 2.7 2.1  
expansum 0.1 2.7 2.0

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Food preference of Tribolium confusum Duv. Between sound wheat and wheat flour

Tribolium confusum Duv. Is considered to be a secondary pest which is associated with crushed or ground cereals and cereal products. In the present paper the food preference of T. confusum on two kinds of food, sound wheat and wheat flour, was studied by means of technique whereby the insects were free to chose their food.

An apparatus similar to that described by Loschiavo (1952) was used. It consisted of 2, 4 or 8 tin small sections 2 cm in height that were put together in a cylindrical chamber   
14 cm in diameter. In the center of the chamber was a hole 2 cm in diameter. The chamber was put in a plastic vessel with cover (Yoshida, 1975). Each section was filled with equal weight of foods: 68, 34 or 17 g in 2, 4 or 8 sections respectively; soun d wheat alternated with wheat flour. Ten unsexed adults of the beetle were introduced into the center hole and allowed to choose their food freely. The test insects were confined to the chamber 7 days at about 30 degree C and 75 per cent R.H. The chambers were then opened and the number of beetles in each section was counted. Some beetles were found outside of the chamber in the vessel. The experiments were replicated 5 times.

The result of the experiment was shown in Table 1.

Table 1 - Distribution of 10 adults of Tribolium confusum in food preference between sound wheat and wheat flour

Chamber divided Number of Beetles   
 into Sound Wheat wheat Flour Outside  
 Mean + S. E. Mean + S.E. Mean + S. E.

2 Sections 0.2 + 0.447 9.0 + 1.225 0.8 + 0.837  
4 Sections 0.6 + 0.548 9.2 + 0.447 0.2 + 0.447  
8 Sections 0.6 + 0.894 8.2 + 1.643 1.2 + 1.095

Almost all beetles preferred wheat flour to sound wheat. In addition to this it is worthy of note that the beetle showed a marked tendency to aggregation. The values of Morisita’s I index, a measure of dispersion of individuals in a population, were calculated (Morisita, 1959). The values are shown in Table 2.

Table 2 – The values of 1 for distribution of Tribolium confusum among selected sections.

No. of experiments Values of I  
 Chamber Divided Into  
 4 Sections 8 Sections  
1 1.600 2.857  
2 1.422 2.667  
3 3.111 1.556  
4 3.200 3.022  
5 3.101 2.932

All of the values were larger than unity. The departure from randomness of the distribution was significant at the 5 per cent levels in all of the cases. This means that the distribution of beetles was contagious; 4 to 9 beetles were found frequently in one flour section.

References

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A population cage for selection experiments involving Tribolium

The population cages to be described were designed for use in selection experiments. The design fulfills several important criteria needed in our experiments; 1) allows clear separation between populations, 2) permits natural movement of beetles within and between chambers, 3) facilitates removal and replacement of vials of medium for census of progeny.

Covered, four-chambered plastic boxe, 8 . 3/8” x 2.3/4”, (obtainable from Tri-State Plastics) are used (Figure 1). The extreme chambers house the two separate populations. Each chamber is large enough to contain five 40 x 25 mm shell vials which can be filled with the appropriate medium. The vials are easily removed for censusing progeny and for replacement with fresh medium. To allow for movement from one vial to another and from one chamber to another, simple additions to this basic cage are made. A small piece of paper, cut to size is put on the floor of each chamber to facilitate movement of the beetles on the smooth plastic. Movement of the beetles between chambers is facilitated by the use of adhesive tape “ladders” which can be attached to the partition walls and to the sides of the vials. The beetles are able tot easily move from one vial to the next and from one chamber to te next.

Though these population cages were specifically designed for selection experiment, they can be adapted for use in many experiments which require some separation of populations while at the same time allow for beetle movement between populations.

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Method for photographing Tribolium metaphase chromosomes.

Living male pupae of the species under investigation were isolated andheld for dissection. The best results were obtained from pupae which appeared to be in the middle of the pupal stage. Pupae which are too young for this procedure appear white and glisten, those which are tool old have attained dark melanization of their mandibles and compound eyes, and are generally of a darker color than most mid-stage pupae.

The best metaphase chromosomes were found in the pupal testes. The testes were dissected out of the pupae in a solution of 1% sodium citrate and placed immediately into a drop of acetocarmine dye solution on a glass slide. After five to ten minutes, the drop of dye containing the pupal testes was covered with a glass cover slip and then observed under the low power of a compound microscope. If any of the testicular lobes were present and undamaged, then the cover slip was covered with a piece of paper toweling and pressed upon gently with a finger or an eraser for approximately five seconds. After cleaning up any excess dye squeezed out from beneath the cover slip, the slide was once again observed under the compound microscope. If any areas were found showing metaphase activity under high-dry magnification (4000X), the cover slip was ringed with paraffin in preparation for oil immersion observation.

Oil immersion observation was done using a phase-contrast microscope at 1250X magnification. Cells exhibiting the best metaphase plates were photographed, using   
4” by 5” cut film.

In order to increase the number of metaphase cells available, a method of administering colchicines to the pupae might prove useful. As of yet, this has not been tried.

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Helpful hints for the insectary.

A number of useful techniques have been developed at the Division of Microbiology insectary which may be helpful to other individuals working with stored product pests. Some are believed to have originated there, others have been gleaned from the entomological literature. It is hoped that the following items will facilitate culture propagation and handling by the reader.

A superior aspirator. Possibly the most useful tool for handling fast moving, or delicate insects, the aspirator poses at least a potential hazard. Even with a fine gauze or wire screen filter around the suction opening, the possibility of inhalation or ingestion of insect eggs occurs (1). Consequently the following fritted glass construction was developed. It also has the advantage of permitting high air flow rates when handling cultures by mechanical suction.

**NOTES -TECHNICAL**

Separation techniques for insect cultures. In addition to their obvious use in separating materials by size, sieves can be otherwise utilized in the insectary. After appropriate size-range separation a sieve fraction can still consist of insect larvae plus cast skins and culture medium residues. The first operation should be to blow most of the ery light cast skins out of the sieve. If appreciable undesirable material remains, living insects can be separated by placing the retained material on a $60 sieve and allowing them to cling to the sieve fabric. The sieve is then tipped over to allow non-clinging items to fall into a pan. The live material is then jarred into a separate receiver. Repetitive handling in this manner can nearly quantitatively isolate live insects from culture debris. Pupae may similarly be isolated “by default”.

Other unusual techniques deserving mention include placing a mixture of adults, larvae and debris in a watch glass placed on a small beaker nested inside a considerably larger one. The most active fraction, usually adult, will be concentrated in the outer beaker. Similarly the mixture may be placed on a slanted plan and active material segregated at the lowere end. Negative phototropism of certain species such as Alphitobius diaperinus may be utilized by placing the culture in a flat pan one-third of which is covered by a sheet of cardboard. Soon almost all adults will migrate to the dark portion.

Adult Oryzaephilus in crowded culture tend to walk upwards, so one convenient way to harvest them is to place the culture jar in a pail of CO 2, supplied by a regulator at ca 1 lb PSIG. The rim of the jar should be 1 – 1.1/2 inches above the rim of the outer pail. Insects will walk or fall into the pail where they will be rapidly narcotized. Excessive exposure to CO 2 will kill, however.

Maintaining security. Many times the investigator will want to handle fast moving live insect. If they fly, sleeve type cages are probably the only answer to absolute security, and even they have faults. For crawling forms an alternative to the expensive and cumbersome oil-moated handling table is to simply work with the culture jars standing in a tray of 70% alcohol or some reasonable equivalent. A polyethylene was bottle filled with 70-90% isopropanol (more toxic) is handy for “shooting” escapees or generally killing off unnecessary cultures, etc.

Volumetric equipment for insect handling. When stating cultures, one of the most convenient ways of assuring that sufficient seeding adults are added is to use small dippers to measure them out. These may be made by fusing a 3 mm diameter Pyrex rod ca 3” long to the edge of a 1 ml beaker. Brass cartridge cases, thoroughly cleaned and suitably calibrated with soldered-on wire handles are also useful. Cal. .22 cartridges cut very short are useful scoops for measuring out a few mg. of moth eggs. The eggs should be weighed into a paper, transferred to a case, their level marked, and the case filed to approximately the correct height. Test dips of eggs may then be weighed to zero in on correct length.

Lids for culture jars. For quart and pint jars little problem of suitable lids occurs since two piece self –sealing caps are universally available. The outer band can be used to retain a piece of 40-mesh bronze screen and/or a filter paper disc. For larger or smaller jars, however, some easy method of cutting clean holes in metal lids must be sought. Radio chassis punches are the ideal solution. They are relatively inexpensive, and are available in 1/16 inch increments from 3/8 inch to 2 inch diameter. Consequently even small vials may e provided with wire screen closures. For 1 gallon wide mouthed jars the big meter-size punch 2- /32 inches in diameter is excellent. (Greenlee No.730 M or equivalent). This provides the investigator with excellent rearing containers at minimal expense.

Storage of culture media. The large two, three, and five gallon wide-mouthed jars (“glass buckets”) available from various laboratory supply houses, or the Atlantic Glass Co. are excellent storage containers. Their 132 mm cap size permits access by a large ladle or scoop for removal of contents. The screw cap construction excludes insects. They are also adequate substitutes for the more costly desiccators. Normally used for constant humidity chambers. When used for this purpose the investigator;s ingenuity must be taxed to design a suitable receptacle for the satured salt solutions required.

Introduction of egg laying. Certain insects such as the cadelle, Siamese grain beetle and the cigarette beetle prefer to oviposit in crevices. Artificial crevices (3) may be provided by placing a small piece of filter paper between two microscope slides, or 1 x 1 inch acrylic plastic squares held together with rubber bands. The paper should be ca ¼ inch smaller in dimension than the outer member. Stacks of filter paper or blotting paper squares also are useful, but eggs cannot be seen when they are used.

Footnotes

(1)Hurd, P.D. 1954. “Myiasis” resulting from the use of the aspirator for the collection of insects. Science 119: 814-815.

(2)Bond, E.J. and Monro, H.A., 1954. Rearing the cadelle (Tenebroides mauritanicus) as a test insect for insecticidal research. Can. Ent. 86: 402-408.

Haydak’s #1 medium

Corn meal thru #30 sieve 4 parts by weight

Powdered skim milk #30 sieve 2 parts by weight

Wheat flour (white) \* 2 parts by weight

Bran thru #30 sieve 2 parts by weight

Brewers’ yeast 1 part by weight

Wheat germ \* thru #30 sieve 1 part by weight

\*or 2 parts whole wheat flour instead of white flour + germ.

Haydak’s #2 medium.

Add enough 1 + 1 honey and U.S.P. glycerin mixture to the above mixture to produce

a very dry, but slightly cohesive paste. (About 30 ml liquid to every 100 g Haydak’s #1).

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The division of microbiology insectary.

The object of this insectary is to provide constant supplies of storage insects, both living and preserved, for research. We ordinarily discourage colonization of stored food pests by District laboratories in order not to compromise their forensic posture by the presence of excessive numbers of the very insects whose fragments they are isolating from samples. It therefore behooves the Division of Microbiology to provide a centralized source of such insects. This is becoming more important recently, as economic considerations have forced many other sources which FDS could have utilized in the past, to curtail rearing activities. As far as can be determined, we now possess a broader spectrum of stored food cultures than any other institution in the United States: Uses of these stocks include reference material for identification of whole, and fragmentary insects, production of authentic insect fragments for recovery experiment, and living insects for life-history, package invasion, and pheromone attraction studies.

The more important beetle and month pests of stored food are listed below, together with comments on the status of those currently maintained in culture.) As of April 1, 1976)

Coleoptera

Anobiidae

Lasioderma serricorne – Cigarette beetle

Cultures are currently in good condition, although they have nearly been destroyed in the past by Pyemotes mites. Considerable preserved material available.

Stegobium paniceum – Drugstore beetle

Cultures are not adequately vigorous. In spite of culturing several isolates of this species on several substrates, the species has never built up to satisfactory levels. Possibly we are dealing with enzootic disease. Experimental rearing on Purina Trout Chow may be successful. This species is susceptible to Pyemotes mites. Considerable preserved material available.

Anthribidae

Araecerus fasciculatus - Coffee bean weevil

Cultures have nearly died twice due to desiccation. This species requires more care than many storage pests. Colony may not survive. Some preserved material available.

Bostrichidae

Rhyzopertha dominica – Less grain borer

Good vigorous cultures. Susceptible to Pyemotes mites. Considerable preserved material available.

Bruchidae

Acanthoscelides obtectus – Bean weevil

Good vigorous culture. Susceptible to Pyemotes mites. Considerable preserved material available.

Callosobruchus analis – never cultured at DM

C.maculatus – Southern cowpea weevil

Formerly in culture, but killed by Pyemotes mites. Considerable preserved material on hand.

C.chinensis – never cultured at DM

Cleridae

Necrobia rufipes – Red legged ham beetle

Good vigorous cultures. Cultures can survive long periods of neglect. Moderate quantity of preserved specimens available. This uncommon species may not be cultured in any other US.S. Laboratory.

Cucujidae

Cryptolestes ferrugineus – Rusty grain beetle

Cultures nearly dead.

C.pusillus - Flat grain beetle

Good vigorous cultures. Some preserved specimens on hand.

C.turcicus - “Turkish grain beetle”

Good vigorous cultures. Some preserved specimens on hand.

Curculionidae

Sitophilus granaries – Granary weevil

Cultures a bit “slow” – may be diseased or parasitized. Susceptible to Pyemotes mites. Considerable preserved material.

S. oryzae – Rice weevil

Cultures and Pyemotes susceptibility as above. Considerable preserved material.

S.zeamais – Maize weevil

Cultures killed by Pyemotes mites. No preserved material available except for ca. 100 adults.

Dermestidae

Anthrenus flavipes – Furniture carpet beetle

Cultures very weak, will probably survive. Cultures can, and usually do, survive long periods of neglect. Some preserved specimens available.

A.verbasci – never cultured at DM

A.scrophulariae – never cultured at DM

Attagenus megatoma – Black carpet beetle

Good vigorous cultures. Some preserved material available.

A.pellio – never cultured at DM

Dermestes frischii – never cultured at DM

D.lardarius – Larder beetle – never cultured at DM

D.maculatus - Cultures died of desiccation. Some preserved material on hand.

Trogoderma glabrum –

Cultures dead, reason not determined. No preserved material available.

T.inclusum – Larger cabinet beetle

Good vigorous cultures. This species survives long periods of neglect. Some preserved material available.

T. variable -

Cultures growing acceptably. No preserved material available yet.

Languriidae

Pharaxonotha kirschii – Mexican grain beetle

Never cultured at DM

Mycetophagidae

Typhaea sterocorea – Hairy fungus beetle

Culture dead, reason not determined. No preserved specimens available.

Nitidulidae

Carpophilus dimidiatus – corn sap beetle

Never cultured at DM

C.hemipterus – Dried fruit beetle – never cultured at DM

C.freemani – never cultured at DM

C.lugubris – Dusky sap beetle

Cultures dead. Carpophilus require considerable care on a day to day basis. Larvae pupate in damp sand. This causes serious mould problems; however, if sand dries out, pupae die. No preserved material available.

Ostomidae

Lophocateres pusillus – Siamese grain beetle

Good vigorous cultures. Cultures survive long periods of neglect. No preserved material available yet.

Tenebroides mauritanicus – Cadelle

Good vigorous cultures. Susceptible to Pyemotes mites.

Some preserved material available.

Ptinidae

Gibbium psylloides – Humped spider beetle

Good vigorous cultures recovered from a total of 311 adults left as a result of Pyemotes infestation. Considerable preserved material on hand.

Mezium americanum – never cultured at DM

Ptinus fur – White marked spider beetle

Never cultured at DM

P.tectus – Australian spider beetle

Never cultured t DM

Trigonogenius globules – never cultured at DM

Silvanidae

Ahasverus advena – Foreign grain beetle

Good vigorous cultures. Some preserved specimens available

Cathartus quadricollis – Square necked grain beetle

Cultures killed by Pyemotes mites. Considerable preserved material on hand.

Oryzaephilus Mercator – Merchant grain beetle

Cultures killed by Pyemotes mites. Considerable preserved material available.

O.surinamensis – Sawtoothed grain beetle

Good vigorous cultures. Susceptible to Pyemotes mites.

Considerable preserved material available.

Tenebrionidae

Alphitobius diaperinus – Lesser meal worm

Good vigorous cultures. Considerable preserved material available.

A.piceus – Black fungus beetle – never cultured at DM

Cynaeus angustus – Larger black flour beetle

Cultures moderately vigorous. We should attempt to maintain large numbers of this uncommon species. A few preserved specimens on hand.

Gnathocerus cornutus – Broad horned flour beetle

Never cultured at DM

G. maxillosus – Slender horned flour beetle

Cultures moderately vigorous. Some preserved material available.

Latheticus oryzae – Long headed flour beetle – never cultured at DM

Palorus ratzeburgi – Small eyed flour beetle

Good vigorous cultures. Some preserved specimens on hand.

P.subdepressus – Depressed flour beetle

Never cultured at DM

Sitophagus hololeptoides

Diseased cultures obtained from ARS labs in Savannah. We were unable to establish

A colony of this species.

Tenebrio molitor – Yellow mealworm

Cultures surviving. This species could be culled from the collection since it is available in most pet shops. Some preserved specimens on hand.

T.obscurus – Dark mealworm

Culture may be too small to permit reproduction, as males and females probably will not mature at the same time. Some preserved material on hand.

Tribolium audax – American black flour beetle

Good vigorous cultures. Considerable preserved material available.

T.castaneum – Red flour beetle

T.confusum – Confused flour beetle

Both of the above available as vigorous cultures and large amounts of preserved material.

T. destructor – False black flour beetle

Cultures a little weak, but appear to be established. Very few preserved specimens on hand.

Lepidoptera

Gelechiidae

Sitotroga cerealella – Angoumois grain moth

Cultures killed by Pyemotes mites. Some preserved larvae available.

Phycitidae

Ephestia kuehniella – Mediterranean flour moth

Never cultured by DM

E.cautella – Almond moth

Cultures killed by Pyemotes mites.

Considerable preserved larvae available.

E.elutella – Tobacco moth

Cultures in acceptable condition.

Considerable preserved larvae available.

E.figulilella – Raisin moth – never cultured at DM

Plodia interpunctella – Indian meal moth

Cultures in acceptable condition. Considerable preserved larvae available.