TRIB0LIUM INFORMATION BULLETIN

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 Notes - Research, Teaching and Technical

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\*A simple technique for minimizing suicide in Tribolium

All species of Tribolium available in the laboratory secrete quinines which they store in the reservoirs of stink glands located in the prothorax and in the posterior portion of the abdomen. Some species of Tribolium secrete greater quantities of quinines per unit body weight than other species. For example, T. castaneum, weighing about 2.3 mg secretes 39.5 + 3.43 ug quinines (Q) and 2.38 + 0.25 hydroquinones (HQ), while
T. confusum, weighing about 2.5 mg secretes 33.08 + 2.62 ug HQ and 2.72 + 0.2 ug HQ, and T. breviornis, weighing 7.5 mg on the average produces 2.25 + 28.72 ug Q and 15.26 + 1.04 ug HQ. (For further details see Table 15.6 in Sokoloff 1975).

Previous observations on T. castaneum and T. confusum have shown that when these beetles are taken out of the flour and examined under the dissecting microscope without etherization they will release a liquid when they are poked or squeezed with forceps. This liquid has the odor of quinines. On occasion, if the contents of the various stadia of a culture are placed in the etherizer, the ether fumes may cause the beetles to release quinines. The effect of these secretions is evident on the eggs and the immature stadia and the teneral adults; these change in color from white or tan, acquiring a pinkish or purplish color. The eggs usually fail to hatch, and the larvae, pupae and teneral adults may also die depending on the concentration of the quinines. If the exposure is only slight, the parts affected may become black and necrotic, and the imagoes emerging from them may be highly deformed; the antennae may be completely or partially missing, and the distal segments of the legs may be missing.

If the adults and other stages of development are taken out of the flour and placed in
an empty vial for a period of time, the beetles may be found dead or dying. It is assumed that if there are may adults, some may become irritated, relese quinines, and because the quinines are denser than air, the quinines will cause their death. (The beetles will show some crystallization of the quinines on their rear ends, and the vial will definitely hae the odor of quinines). There is some species differences in this suicidal behavior: T. castaneum is least likely while T. confusum is more likely to commit suicide in this manner, judging from the number of vials in which death of beetles has occurred.

With the availability of T. freeman for research, we can add another species which will also commit suicide through the release of quinines. This species has not been investigated in regard to the amounts of quinines per unit body weight, but when the contents of a T. freeman culture are sifted and placed in a soup dish, the adults will release their quinines, and this release becomes evident by the behavior of the larvae, which begin to wiggle violently in response to the presence of these chemicals, and the odor of quinines becomes very evident if the contents of the soup dish are examined under the dissecting microscope.

During the course of an experiment to induce mutations through the use of EMS we have routinely isolated 100 males in an incubator maintained at 30 C. for 24 hours in an empty vial before they are fed a solution of sucrose mixed with a small amount of EMS. This procedure has been on the whole successful. The majority of these 100 male samples have survived for this period without mishap. However, a small number of samples was lost because of the beetles’ release of quinines resulting in the death of all beetles. We attempted to reduce the incidence of this phenomenon by placing 50 males in each of two vials, thus reducing the density. This procedure improved the situation somewhat, but in some cases the beetles in one or both of these vials still committed suicide.

We then tried placing the container upside down in the incubator on the assumption that the slippery surface of glass vials caused some stimulation to the beetles to relese their quinines. By placing the beetles on the coarse surface of a paper towel or a piece of Kimwipe, the beetles apparently feel more secure, and they are not as prone to release their quinines.

One more word on this subject. If you are scoring beetles from single pair matings, it is better to sift, etherize and count the contents of each vial separately. By sifting the contents of manyvials in a single operation before etherizing and counting. one risks the possibility that the adults may become irritated at other adults or larvae and release quinines. And since a mutation may manifest itself in a single beetle and it may not occur for a long time, why risk the loss of this single individual through their exposure to quinines?

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\*Preliminary Characterization of the Male Accesory Reproductive Glands of
Tribolium brevicornis (Coleoptera : Tenebrionidae)

Introduction

The contribution of reproductive glands other than the testes or the ovaries to the process of insect reproduction has often been overlooked (Leopold, 1976; Happ, 1984). This is true for the tenebrionid beetles, where the morphology and biochemistry of accessory reproductive glands have not been extensively studied, except for Tenebrio molitor, the mealworm beetle, Murad and Ahmad (1977) reported on a histological study of the accessory glands of Tribolium castaneum. We became interested in examining the reproductive accessory complex of T. castaneum and to Tenebrio molitor.

Materials and Methods

Colonies of T. brevicornis were raised in petri dishes of white flour at a constant temperature of 32 degree C. Pupae were collected by sifting the media (U.S. Standard 14 sieve) and were sexed by examination of the developing external genitalia. Upon eclosion to the adult stage, the animals were isolated and maintained at 32 degree C. For histological studies, 8-10 day adult glands were dissected in phosphate-buffered saline (PBS), fixed in 3% gluteraldehyde, embedded in Paraplast and sectioned at 7 um. Sections were stained in hematoxylin and eosin or Mallory’s trichrome (Pearse, 1968). Estimates of gland sizes were made using an ocular micrometer. For whole mount age studies, reproductive accessory gland complexes were dissected in PBS at 0, 2, 4, 6 and 8 days after eclosion. Glands were stained in 0.3% Oil Red O (ORO) in 70% ethanol or in 0.5% Sudan Black B in 70% ethanol overnight and destained in 30% ethanol.

RESULTS AND DISCUSSION

The reproductive accessory gland complex of T.brevicornis consists of two sets of paired glands which are located at the junction between the seminal vesicles and the ejaculatory duct (Figure 1). Each gland is roughly circular in cross section, with a single layer of secretory material was observed in the lumen of both glands.

One set of glands, the tubular accessory glands (or TAGs) are long and thin, with uniform thickness throughout their length. The mean cell height for TAG secretory epithelium ws 19.8 + 0.5 um (n = 10), with a lumen of about 40 um, giving the entire gland a width of 80 um. No differences between cells were detected with either hematoxyling or Mallory’s trichrome, indicating a single, uniform cell type is present in the TAG.

The second pair of glands, the pear-shaped accessory glands (PAGs), possess
a thickened wall that bulges outward at the terminus of the gland. A representative PAG had a mean length of 584.3 + 2.7 um (n = 7), with a mean width of 223.3 + 1.3 um (n = 23), and mean depth of 200.8 + 1.3 um (n = 18). The secretory epithelium was composed of long thin cells (mean height 97.0 + 1.6 um, n = 9), surrounding a small lumen (about 20 um). Four distinct cell types were detected through staining of the mature gland with Mallory’s trichrome and hematoxylin.

Intact adult glands stained with Oil Red O (ORO) or Sudan black also showed regional specificity of staining occurred in the PAGs but not the TAGs. Age-related trends in staining pattern were also seen. In the PAGs of newly eclosed adults, a small area of cells along the inner surface took up ORO, but not Sudan black. The staining in this area increased in size and intensity until day 2 of adult life. By day 4, the cap (terminal area ) of each PAG had stained intensely with both ORO and Sudan black. In the mature (8 day) glands, an additional cell type was detected when cells on the shoulder of the gland (near the seminal vesicle attachment site( stained intensely with ORO, but not with Sudan black. The remainder of the gland (body) never accumulated either stain.

CONCLUSIONS

The reproductive accessory gland complex of T. brevicornis is quite similar to that of Tribolium castaneum and Tenebrio molitor. All three species possess two sets of paired glands, with one set generally long, thin and uniform in cell type, and the second gland with a thicker epithelium containing regionally distinct cell types. For both
T. brevicornis and T. molitor, developmental changes occurred in the second gland, although in Tenebrio up to eight cell types have been detected (Dailey, et. al., 1980.

The biochemical changes which result in differential staining of cell types are still unknown and will be the subject of further investigation. The fact that T.brevicornis is one of the larger Tribolium species will be helpful in that regard.

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\*Thermal Requirements for Development of Stored-Product Insects.

INTRODUCTION

Predicting distribution and abundance of stored-product insects is essential for establishing effective control or management strategies of the pests. Climatic factors are often postulated to mainly determine the distribution and abundance of stored-product insects (Freeman, 1962; Sinha, 1974; Banks, 1977), as is the case with insects in general (e.g. Uvarov, 1931; Andrewartha and Birch, 1954). Messenger (1959) suggested that the theory of thermal constant is useful to estimate potential distribution and abundance of insects, The theory presumes a hyperbolic relationship between developmental period and temperature (Peairs, 1914; Bodenheimer, 1926):

 Y (t-a) = K,

where y is the time required for complete development of an organism stage at temperature ‘t’, ‘a’ is threshold temperature above which the organism of the stage can develop, and K is thermal constant in day-degree. The model has some limitations due to its simplicity (e.g. Wagner et al., 1984), but may be also convenient for its simplicity when we compare temperature dependent development of various insects. The model includes only two parameters, each of which has a biological meaning. Howe (1965) listed minimum temperatures for the development of 53 stored-product insects but they do not correspond to the threshold temperature.

METHODS

The thermal constant (K) and the threshold temperature (a) for the development from egg deposition to adult emergence of 58 stored-product insect species including a predator and parasitoids were estimated by fitting a linear regression line to the temperature against reciprocal of developmental period (developmental rate, 1/y) data from literatures. When the data points deviated from the regression line at low or high temperature ranges, they were omitted from the analysis. The number of data and temperature range analyzed are shown in Table 1. Standard error of ‘K’ and ‘a’ were estimated by the equations proposed by Campbell et al. (1974).

RESULTS

The results are summarized in Table 1.

H. pseudspretella had the largest thermal constant K and the lowest threshold temperature ‘a’. another oechophorid E. sarcitella had also a relatively large K.E.kuhniella had the largest ‘K’ and lowest ‘a” among the pyralid moths, although the figures of ‘K’ varied with strain. These species occur in temperate regions. While
a Mediterranean or a tropical moth species, E.calidella and C. cephalonica had
a smaller ‘K’ and higher ‘a’.

Among coleoperous species, ptinid species had a large ’K’ and low ‘a’ with the exception of P. pusillus. While, ‘K’s of L. oryzae and O. surinamensis were small and some gigures of ‘K’ were less than 300 day-degree. Several cucujid species had also
a small ‘K’ ‘a’ s of tenebrionid species were higher than 15 degree C except for those of T. destructor and P. laesicollis. Particularly L. oryzae which prefers hot climate (Freeman, 1962), had the highest ‘a’, which was higher than 20 degree C.

Braconid species required much smaller heat to complete development, but ichneumonid V. canescens required heat more than twice as much as the braconids.

Lepidopterous species had the largest mean K (774.9 + 381.0, mean + s.d. based on the data measured at an optimal condition for each species and those of males when the data of both sexes were available), followed by Coleoptera (502.3 + 179.7) and predator and parasitoid (203.1 + 86.2), although the means had large variations. While, mean ‘a’ of Lepidoptera (10.7 + 3.1) was lower than that of Coleoptera (14.3 + 3.1 and ‘a’s of predator and parasitoid ranged 10.5 – 16.3 degree C.

DISCUSSION

‘K’ and ‘a’ differ depending on strains of a species. There are two distinctly different strains in E.kuhniella: a strain with a larger thermal requirement and a strain with
a smaller thermal requirement. Payne (1934) reported that there were at least two strains of E. kuhniella, a fast development strain and a slow one and all moths obtained in Germany belonged to the slow strain and those from the United States contained both strains. The strain of Jacob and Cox (1977) corresponds to the slow strain and that of Imura (1986) to the fast strain. The slow strains have a lower ‘a’ than the fast ones. A similar strain difference in ‘K’ is also observed in L. oryzae, in which, however, the difference in ‘a’ is not significant.

Relative humidity or moisture content of medium alters ‘K’. With the exception of L.oryzae and P.truncatus which are tolerant of dry condition, the drier the rearing condition, the larger the ‘K’ of the insects. Insects possibly require more energy to maintain body fluid at drier condition. This must be critical particularly for stored-product insects which rely on dry foods. However, these condition may not basically affect ‘a’ of stored-product insects, with the exception of C. chinensis in which ‘a’ seems to decrease with humidity reduction.

‘K’ depends on diet. O.surinamensis had much smaller ‘K’ on oats than on walnuts. Insects possibly require more heat on less efficient or nutritious media. The fact that predator and parasitoids have a smaller ‘K’ than other species may also reflect a nutritional difference between sarcophagi and phytophagy. The production efficiency of carnivores is significantly higher than herbivores in insects (Hunphreys, 1979). In addition, Hagstrum and Milliken (1988) stated that moisture and diet seem to have more significant effect on the development of insects around optimal temperature for their development.

Females require more heat to complete development than males. Production of more costly gamete, eggs by females must be responsible for their larger ‘K’ ‘a’ however, dose not fundamentally differ with sexes.

The results reveal that species or strains of warmer regions generally have smaller ‘K’ and higher ‘a’ than those of cooler regions, although there are discrepancies for some species. There are statistically significant negative correlations (p 0.01) between ‘K’ and ‘a’ in Lepidoptera (r = -0.836, a = -0.006K + 15.1) and Coleoptera (r = - 0.621,
 a = 0.011K + 19.6) (Fig. 1), as Utida (1957) suggested. This relationship is anticipated, because on parameter is a function of the other; a - -d-K, where ‘d’ is y-intercept of the regression line fitted to the developmental rate against temperature data used for estimation of ‘K’ and ‘a’. Consequently, y-intercept, ‘d’ represents the slope of ‘a’ against ‘K’ regression line. The smaller ‘r’ for Coleoptera is due to inclusion of species from various families. Fig. 2 shows the regression lines of two families of Coleoptera, Tenebrionidae and Cucujidae. Regression equation for Tenebrionidae is a = -0.013K + 22.9 (r = 0.848\*\*) and that for Cucujidae is a = -0.013K + 19.6 (r = -0.721\*). Tenebriomid beetles which are much larger in size than cucujid ones apparently require more heat for development. Despite such an allometric effect on the thermal requirement, each taxonomic insect group may have its own characteristic slope of regression line (Fig. 2). In fact, the slope of the regression line for Coleoptera was significantly steeper tan that for Lepidoptera (p 0.05).

The data analyzed in this study was based on those measured at constant temperatures. Fluctuating temperature may affect ‘K’ and ‘a’ of an insect but such effect is not so remarkable for a stored-product insect (Siddiqui and Barlow, 1973). Estimates of ‘a’ and ‘K’ from each developmental stage do not always coincide with those estimated from the total developmental period in an insect, but the difference between the sum of stage-specific ‘K’s and ‘a’ ‘K’ estimated from the total developmental period is not significant in Tribolium species (Imura and Nakakita, 1984). Diapause is another important factor which affects distribution and abundance of insects. Diapause development of insects is, however, a much more complicated process than non-diapause development which was studied in this paper (Hodec, 1983). Therefore construction of an unified model which incorporates these two developmental processes has to wait future studies.

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THE EFFECT OF VARIOUS CONCENTRATIONS OF TRICALCIUM PHOSPHATE
ON FECUNDITY AND HATCHABILITY IN TWO SPECIES OF TRIBOLIUM

INTRODUCTION

Food for stored product pests is a very important ecological factor that limits, among other factors, the population numbers. Insects need not only the presence of particular components in the diet for normal growth and development, but also suitable proportions of these components. An unsuitable ratio of the components causes
a disturbance in insect populations such as delay of development, a change in fecundity and hatchability. Pratt et al. (1972) reported that the use of mineral salts offers a new promising method for insect control. These authors suggested to increase the level of different elements (e.g. Fe, Cu, F, and Mg) to required high concentrations controlling pest. It was found that the strongest inhibitory effect for stored product pests was that of ammonium nitrate and tricalcium phosphate (Boczel 1984). For example, the tricalcium phosphate (TCP) has proven to be a good source of calcium and phosphate for man and domestic animals and at the same time a 1 – 3% concentration of this compound is enough to protect the stored flour and grain from the insect infestation.

The paper aimed at learning about response of 6- and 7-instar groups of strain of Tribolium castaneum cI (discerned earlier by Prus 1976) and T. confusum Duval bIV (discerned earlier by Bijok 1984) to different concentrations of TCP in the food. Both fecundity and hatchability were examined.

MATERIAL AND METHODS

Experiments were carried out on cI strain of T. castaneum and bIV strain of
T. confusum. In this strain, 6- and 7-instar groups were discerned and cultured at 29 degree C and relative humidity 75% in dark incubator. Standard mixture of wheat flour and baker’s yeast in weight proportion 20:1 was used as culture medium. To culture medium tricalcium phosphate was added in adequate proportions to obtain three concentrations: 0.5, 1.0 and 1.5% (by weight). The substance originated from USDA Laboratory, Savanah, Georgia. The standard culture medium was used as a control sample.

For experiments on fecundity the animals that were cultured previously for developmental time and survival studies (Prus 1989) were mated and kept in vials as
a single pairs in 8 g of either pure culture medium or with different concentration of TCP. For each concentration 10 replicates were set up. The experiments were carried out for 30 days and in every 3-day period the number of eggs laid by female was counted. The eggs collected from vials were transferred to small vials and left over in incubator for 6 days to develop. After this period the hatchability of eggs was estimated.

An additional series of experiments were performed where animals cultured initially in TCP concentrations of 0.5 and 1.0% in the medium were transferred to pure medium in which their fecundity and hatchability were tested. This allowed to evaluate the effect of temporary lack of harmful agent in the medium.

The data obtained for fecundity were statistically elaborated using three factor design of analysis of variance (Simpson et al. 1960). The computer programme specially outlined for these data by Dr. P. Bijok of the Institute of Ecology was used.

RESULTS

The fecundity of T. castaneum is restricted under the effect of TCP. The concentration of 0.5% limits fecundity by about 15% as compared with control series in 6-instar group and by about 20% in 7-instar group (Fig. 1). The decrease by more than 50% in 6-instar and by 30% in 7-instar group at concentration 1.0% was observed. At concentration 1.5% of TCP in 6-instar individuals mortality was the highest. It resulted in reduction of replications from 10 to 2. In 7-instar group the latter concentration caused a decrease of fecundity by about 60%.

In series where animals were transferred to control medium from 0.5 and 1.0% concentration of TCP, the fecundity was similar to control one irrespective to the concentration at which the development of animals previously took place.

The data obtained for fecundity were statistically elaborated using analysis of variance. In T. castaneum (Table 1) all effects and their interaction are highly significant at probability level of 0.005. This means that fecundity of T. castaneum is a very susceptible trait affected significantly by all factors.

The hatchability of eggs in T. castaneum in all series of experiment is practically the same as in control (Fig. 2). The hatchability sometimes is higher in various series of TCP concentration than in control one.

In T. confusum this salt also decreases the rate of fecundity but to a lesser degree. In 6-instar group the concentration of 1.0% of TCP effects fecundity stronger than concentration 1.5% (Fig. 3). Similarly as in T. castaneum, the fecundity in this species after transferring the beetles from TCP to control medium is practically the same as in the control series.

The results for fecundity in T. confusum were also elaborated using analysis of variance (Table 2). On the contrary to results obtained for T. castaneum, in T. confusum the effect of time is weak one (F = 10.07 as compared with F = 55.55 for T. castaneum) which has some bearing on results, bringing insignificant interaction: time x instar group and time x instar group x concentration.

The hatchability of eggs in T. confusum in all series of experiments similarly as in T.castaneum is practically the same as in the control series (Fig. 4). Hatchability being a very variable parameter, does not show any consistent differences within instar groups in both species. It is also independent of TCP concentration. Due to this fact no statistical analysis was performed with material on hatchability.

The time effect on fecundity and hatchability is different. Fecundity decreases with time gradually whereas hatchability maintains the same level from the beginning of experiment to its end.

DISCUSSION

It is known from previous paper (Prus et al. 1988) that in T. castaneum cI females of
 6-instar group lay more eggs than females of 7-instar group. This phenomenon is also observed in the present paper, where in spite of various concentration of TCP in each series females from 6-instar group lay more eggs than those from 7-instar group. From the above mentioned paper it is also evident that maximum fecundity occurs during the first month of adult life, so in this experiment only this period was examined. The results, as presented in Fig. 1 show that TCP decreases fecundity and that this decrease is highest in concentration 1.5% in both groups of this species. It is very difficult to explain why concentration of 1.0% affects 6- and 7-instar groups of
T. confusum (Fig. 3) stronger than that of concentration of 1.5%. A similar phenomenon was observed in Oryzaephilus surinnamensis (L) under the effect of potassium nitrate in different concentrations (Hassan 1981). In this case concentration of 1.0% of this salt was decreasing strongly the number of total progeny, while the concentration of 3% show the opposite effect.

In both species and both discerned instar groups the transfer of animals from medium with TCP to pure medium caused recovery of fecundity, practically to the same level as in control (Fig. 1 and 3). The same results were obtained by Ignatowicz (1980) for Sitophilus granaries (L) fed with wheat grains impregnated by various concentrations of potassium iodine. The animals which were transferred to the pure wheat grains recovered their reproductive abilities rather quickly during their further life.

In this experiment hatchability in both species and in both discerned groups has shown no consistent differences caused by the effect of TCP (Fig. 2 and 4), whereas the significant decrease of this parameter was observed in 0. surinamensis at
a concentration 1.0 of TCP and in T. granarium at a concentration 2.0% of this salt was observed by Hassan (1981).

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 EXPLANATIONS TO FIGURES

Fig. 1. The effect of various concentration of tricalcium phosphate on fecundity of
 Tribolium castaneum Hbst cI strain 1 – concentration 0.5%, 2 – 1.0%,
 3-1.5%, 4 – control, 5 – 0.5% ---- C. 6 – 1.0% ---- C.
Fig. 2. The effect of various concentration of tricalcium phosphate on hatchability of
 Tribolium castaneum Hbst cI strain.
 Explanations – as in Fig. 1.

Fig. 3. The effect of various concentration of tricalcium phosphate on fecundity of
 Tribolium confusum Duval bIV strain.
 Explanations – as in Fig.1.

Fig. 4. The effect of various concentration of tricalcium phosphate on hatchability of
 Tribolium confusum Duval bIV strain.
 Explanations – as in Fig. 1.

Table 1. Analysis of variance of fecundity in Tribolium castaneum Hbst.

Source of Sum of d.f. Mean F P
variation squares square

time 4219.3 10 421.9 55.55 0.00
concentration 7715.2 4 1928.8 263.95 0.00
instar group 189.3 1 189.3 24.93 0.00
interaction:
time x concentration 1233.3 40 30.8 4.06 0.00
concentration x )
instar group ) 337.3 4 84.3 11.10 0.00
time x instar )
group ) 1374.2 10 137.4 18.09 0.00
time x instar group x)
concentration ) 400.2 40 10.0 1.32 0.00

deviations 7519.2 990 7.6
total 22988.0 1099
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Table 2 – Analysis of variance of fecundity in Tribolium confusum Duval.
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Source of Sum of d.f Mean F P
variation squares square
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Time 479.1 10 47.9 10.07 0.00
concentration 2170.0 5 434.0 91.24 0.00
instar group 156.7 1 156.7 32.58 0.00

Interaction :
Time x
concentration 213.7 50 4.3 0.90 NS
concentration x )
instar group ) 1195.4 5 239.1 50.27 0.0
time x )
instar group ) 32.3 10 3.2 0.68 NS
time x instar group)
x concentration ) 202.1 50 4.0 0.85 NS

deviations 5650.6 1188 4.7
total 10099.9 1319
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INTRAPOPULATION DIFFERENTIATION OF TRIBOLIUM CASTANEUM HBST.
cIV and T. CONFUSUM DUVAL bI

INTRODUCTION

Searching for intrapopulation differentiation within populations of flour beetles as a possible mean of response to environmental factors leading to better survival under certain conditions the other genetic strains: T. castaneum cIV and T. confusum bI were tested. In our previous papers we have tested in this respect T.castaneum cI (Prus 1976. Prus and Prus 1987). T. confusum bIV (Bijok 1984, Prus et al. 1988) and two black mutants: T. castaneum “sooty” and T. confusum “ebony-2” (Prus – in print).

The strain cIV of T. castaneum is characterized by the lowest productivity whereas the strain bI of T. confusum - by the highest one (Park et al. 1961, 1965).

The aim of the present paper was to check two strains of flour beetles whether they also show differentiation similar to that observed in previously tested strains.

The whole experimental work for this paper has been done in the Whitman Laboratory, Department of Biology, University of Chicago during our one-month visit in 1989.

MATERIALS AND METHODS

The material used for this work were strains of Tribolium from Whitman Laboratory, University of Chicago. The method used to test the strains for heterogeneity was similar to that applied earlier by Prus (1976) and Bijok (1984). It depended on individual culturing of single specimens from egg to the adult stage, each at 1 g of culture medium consisting of wheat flour and dry powdered yeast at proportion 20:1 by weight. The cultures were run in a dark incubator at a temperature of 29 C and relative humidity of 75%.

The census was done every day consisting of looking for the skin cast in the culture vials. Every second day the animals were weighed starting from the 11th (T.confusum) or 12th (T.castaneum) day of development of larvae. A total of 120 replicates was set up, 60 for each of the two strains. The results obtained were later drawn as individual growth curves of wet weight grouped according to sex (determined at the pupal stage) within each strain. Depending on the number of exuviae, the individuals were classified
either as 6- or 7-instar group.

Later on the growth curves were averaged for each instar groups separately, resulting in mean growth curves for 6- and 7-instar group. The incidence of each group within ca 60 individuals of each species was also determined.

Another experiment performed simultaneously aimed at determining the frequency of appearance of pupae in group cultures of these strains. To achieve this, synchronized cultures were set up with eggs 0 to 24 hours old. From the moment of appearance of pupae they were removed, counted, weighed (in order to assess individual pupal weight) and their sex determined. Then, frequencies of both sexes in the two strains were drawn against time which allowed to judge whether there was one or two peaks of pupal appearance in a given strain. This would imply the presence or not of different phenotypic groups within each strain, corroborating thus the results based on individual cultures. Differences in mean weight of pupae with time of their appearance were analyzed as to the existence of the two groups in populations.

Attention should be drawn to the fact that time in days in individual cultures (Fig. 1 and Table 1) was counted from the moment of larvae hatching from eggs, whereas in the group experiments (Figs. 2 and 3) – from the moment when eggs have been laid by females. Thus developmental time in the first case does not include the duration of egg stage (4 or 5 days depending on the species) and in the second one it does include it.

RESULTS AND DISCUSSION

The two strains tested differ in respect of their intrapopulation differentiation, cIV of
T. castaneum being heterogenic and bI of T. confusum – a homogenic strain. Within former two groups of 6- and 7-instar groups were distinguished whereas the latter consisted only of 7-instar individuals (Fig. 1 A). The proportion of 6- to 7-instar group in
T. castaneum was around 1 to 6 similar for both sexes (Table 1). The 6-instar individuals were much lighter than the remaining group and they pupated one and a half day earlier than the 7-instar individuals. More or less similar trends in changes of
coefficient of variation within distinguished groups of T. castaneum vIV strain and that for uniform strain T. confusum bI. Concerning the body weight during development corroborate the rightness of distinguishing these groups in cIV strain and no groups in
bI strain.

Group experiments with T. castaneum cIV strain revealed that the earlier appearing pupae (on 18 to 20th day of development following the hatching from eggs) showed much lower individual dry weight than that of pupae appearing later both in females and males. These two groups of pupae correspond closely to the two peaks of appearance of pupae in time – synchronized cultures of this strain (Fig. 2). By and large, different courses of growth curves, differences in pupal weight and two peak curve of pupal of pupal appearance: all this proves the heterogeneity of cIV strain.

In bI strain of T. confusum only one group of 7-instar individuals was observed. Its growth curves for females and males are presented in Fig. 1B. The mean time of appearance of pupae in individual cultures was 21.2 day of development after the moment of egg hatching for females and 21.5 day for males. It is interesting to note that coefficient of variation is diminishing as the larvae grow reaching the lowest value at the prepupal and pupal stage (Table 1).

The group experiments with bI strain of T. confusum showed rather one peak of appearance of pupae in synchronized cultures with females showing a slight tendency to split into two peaks of appearance (Fig. 3). The individual weight of these pupae against time of their appearance, however, prove that the strain is phenotypically homogenous as evidenced by rather straight lines on the upper graph of Fig. 3. Lighter pupae found on the first day of appearance did not contradict the general conclusion of uniform pupal weight in this strain, but can result from different reasons.

Having investigated the two other strains of Chicago genetic strains in respect of phenotypic heterogeneity the following can be concluded.

T. castaneum reveals higher tendency to phenotypic heterogeneity within a group of four strains than does T. confusum. Both investigated strains cI (Prus 1976, Prus and Prus 1987, Prus et al. 1988) and cIV consist of 6- and 7-instar group, however, their incidences differ. In cI this proportion seems to be 1 : 1 and in cIV 7-instar group is clearly prepondering over the 6-instar group.

In T. confusum such heterogeneity was also observed but only in bIV strain (Bijok 1984, Prus et al. 1988) whereas bI strain is homogenous in this respect. Proportion between the discerned groups in bIV is variable, perhaps on account of methodological error (starting an experiment with newly hatched larvae instead of randomly chosen eggs).

Similar trends were observed in black mutants of these species, semi-dominant “sooty” strain of T. castaneum was much more differentiated than “ebony-2” of T. confusum, which was almost homogenous (Prus in print).

This paper has been support by the National Academy of Sciences USA in the form of scholarship granted to us. We wish to thank Professor Michael Wade for supplying us with laboratory facilities during the tenure of this scholarship.