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 Notes - Research, Teaching and Technical

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\*Three Mutants of Tribolium castaneum.

1. tar (tar) – Contents of the prothoracic quinine gland reservoirs are more darkly pigmented than normal, and are not secreted. The gland contents in the mutant are usually red-brown to purple-brown compared to the usual wax-yellow color characteristic of wild type. Posterior glands are seldom affected. We speculate that some faulty mechanism prevents the anterior glands from excreting their contents, which oxidize and become marker with time.

2. broken antennae (ba) – Club segments are often pale and undersclerotized or unevenly sclerotized with a patchy appearance. The funicle has uneven sclerotization, often with patches of excess pigment. Both club and funicle are brittle and subject to breakage.

3. Eyeless (Ey) – Resembles microcephalic (mc), but has a more severe phenotype. The eye-bearing portion of the head capsule is reduced, with an accompanying reduction of the eye itself. In the most strongly expressed individuals, no ommatidia develop. The gena is unaffected.

For illustration of these mutants see the accompanying Fig. 1.

Fig. 1. Upper Row: Left, a normal teneral adult: right, a tar/tar homozygote. Note the dark masses within the prothorax.

Lower Row: Left, an “eyeless” Ey/Es’ translocation heterozygote. Note the complete absence of ommatidia. Right, a “broken antenna” (ba/ba) teneral adult. Note the right antenna with missing terminal club segments and left antenna with reduced club segments.

 

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\*New mutants in Tribolium castaneum.

1.Cleft gula (Cg). Dominant, radiation induced, homozygous lethan. Strongly indented or cleft gular sutures on ventral head, causing a slight widening of the head capsule, giving the eyes a “walleyed” look from the ventrum. Excellent penetrance and viability. It appears to be unlinked to any currently documented linkage group.

2.Unsclerotized elytra (ue). Spontaneous recessive. The mutation is characterized by
a membranous stippling along the midline margin of each elytron. The prothoracic sternellum is often slightly indented at the posterior midline, giving it a lightly cleft appearance. It has complete penetrance, variable expression, and excellent viability as a homozygous stock. Linkage is undetermined at this time.

3.Pretzel (Pz) – EMS-induced, dominant, homozygous semi-lethal. Excellent viability and incomplete penetrance in the heterozvgous condition. The original mutant was
a male with warped prothoracic tibiae. Heterozygotes have normal antennae and gnarled, thickened legs (one or more legs may be affected). The homozygotes have very short antennae and radically reduced legs, consisting of only coxae, tarsal claws, and a hint of some intervening segment. Linkage is undetermined at this time.

4.Displaced sternellum (Ps) – Radiation induced, dominant, fully penetrant, viable, and tightly linked to Reindeer (Rd) on LG2. The prothoracic sternellum has a “pinched” appearance, i.e., laterally narrowed and dorso-ventrally thickened. The mesothoracic sternellum is also enlarged. The pronotum has generalized dorsal dents, usually with
a dorsal anterior midline dip, and abnormally pointed anterio-lateral corners. The ventral anterior of the pronotum also has a midline dip which is often devoid of the anteriorly projecting setae usually found at the anterior margin. Metathoracic antecoxae are disrupted along the common margin with the enlarged posterior metepisternum. All coxal socket, including those of the maxillary palps, are enlarged, with a poorer fit to the coxae. This “looseness” at the maxillary palps gives the ventral head a “walleyed” look, and may also be responsible for an escape of saliva resulting in a crust of flour accumulating around the mouth region. Large setae are commonly found on coxae, with setae and spikes on antennal scape, and maxillary palps occasionally branched.

6.Crab (Cr) – Radiation-induced, dominant, homozygous lethal, fully penetrant. Moderately viable (has difficulty eclosing and moving about due to warped legs): Crab is located on LG7, with 0% crossover with chestnut eye ( c ). It is currently maintained as balanced stock of Crab/PL4. The tibiae of all three pairs of legs are enlarged, and bowed. Males are occasionally seen with a “sex patch” on the prothoracic tibiae, indicating this mutant is a tibia to femur transformation. Random tarsomeres are also often fused.

7.Folded elytra (fe). Spontaneous recessive derived from the dominant eu (euD) stock. It has incomplete penetrance with excellent viability. It is characterized by elytra which are folded under at the tips. The trait is visible in pupae. Linkage is undetermined at this time.

\*Podapolipid Mites (Acari: Podapolipidae) Associated with Tenebrionidae
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Podapolipus tribolii Feldman-Muhsam and Havivi 1972 was described from laboratory specimens of T. confusum maintained in the Medical Entomology Laboratory, Nebrew University, Jerusalem, Israel in 1961. The specimens were removed from under the elytra and from the tergites of the abdomen of the beetle. This was the first and last report of Podapolipus from T. confusum.

Sokoloff (1974) lists several parasitic mites of Tribolium including another mite in Tarsonemoidea, Pyemotes ventricosus. The tenebrionid genera Akis, Pimelia, Blapstinus, Gonocephalum and Alphitobius are hosts for these ectoparasites and it is very likely that additional tenebrionid beetles may serve as hosts. Keys to species of Podapolipus associated with tenebrionid beetles appear in Husband and Baker (1992). A new genus of podapolipid mite was recently discovered by Kurosa on a tenebrionid beetle collected in Japan.

Although many podapolipid mites may parasitize one beetle, the mites are seldom seen because the incidence may be very low in a population. The life cycle of podapolipid mites is abbreviated. Adult male Podapolipus with 3 pairs of legs hatch directly from the egg. Larval female and adult male mites mate under the elytra of beetles. The larval female mite is the stage which migrates to new hosts. This may occur when beetles mate or cluster. Feldman-Mushsam and Havivi (1972) point out that low humidity is detrimental to the survival of the mites. When reaching a new host, the larvae attach with cheliceral stylets and molt to the adult stage. Adult females have one pair of legs and are not capable of much movement. Larval female exoskeletons may be seen attached to adult females. This has resulted in confusion when parts of larval females have been mistaken for structures of the adult females. It is likely that a few larval females (about 0.15 mm) or males (about 0.01 mm) will be overlooked. However, adult females appear as round clear or white spheres about 0.5 mm in diameter and many eggs may remain attached to the posterior of the abdomen. Feldman-Mushsam (personal communication, 1989) noted raised elytra in some parasitized T. confusum. Sokoloff (personal communication, 195) noted raised elytra in Tribolium due to alyffal blisters of genetic origin. Thus, raised elytra in Tribolium may not be due to parasitism.

Feldman-Mushsam, B. and Y. Havivi, 1972. Two new species of the genus
 Podapolipus (Podapolipidae: Acarina), redescription of P. aharonii Hirst 1921 and
 some notes on the genus. Acarologia 14: 657-674.

Husband, R.W. and A. Baker, 1992. A new species of Podapolipus (Acari :
 Podapolipidae) ectoparasitic on Alphitobium laevigatus (Tenebrionidae)
 from Trinidad. Internat. J. Acarol. 18 (2) : 83-87.

Sokoloff, A. 1974. The Biology of Tribolium. Volume 2 : 276-277.

\*A request for help in Collecting Additional Podapolipus tribolii from
Tribolium confusum
by Robert W. Husband, Biology Dept., Adrian College,Adrian mi 49221,

Podapolipus tribolii was collected once, in 1961, and reported by
Feldman-Mushsam and Havivi (1972). It seems unlikely that only a single incidence of ectoparssitism by P. Tribolii under elytra of T. confusum exists. Your assistance in finding additional cases of parasitism by dpodapolipid mites is requested.

I maintain a reference collection of nearly 100 species of mites in this family for my own study and for use by others. Some mites are maintained in alcohol and have been used instudies involving electron microscopy and electrophoresis. At present I have no
P. tribolii in alcohol. If you notice these parasites, please contact me. If abundant, the long caudal setae (setae h ) of larval females will be conspicuous. Females will appear as clear or white spheres about 0.5 mm (+ 0.2 mm) in diameter and will be located on the abdominal tergites under the elytra. Larval females and males have 6 legs while adult females have 2 legs. Two small anterior lobes are characteristic of female Podapolipus from tenebrionid beetles. Your help will be very much appreciated.

I hope the paragraphs above may be appropriate. Please change them as you see fit.

Thank you for your help.
Sincerely


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\*Experimental models available in mice.

Two experimental models, one resulting in cardiomyositis and the other producing experimental primary glaucoma in inbred mice DBA/2 have been developed by the author. A summary of the models is included. The Editor of TIB has been informed by the author that he does not have the required financial support to continue working on these models, hence he is making the models available for sale. If you are interested contact Dr. Listov at the above address.

M.V. Listov, Ph. D. (Biology)
Experimental Model of Cardimyositis in Mice

 Summary

The disease is manifested in mice of two lines (DBA/2 and C57B1) after peroral injection of 2 substances one of which is inhibiotor of the known enzyme and the other one is metabolic precursor of the substrate of this enzyme. The substances are injected with water (0.2 ml/mouse) daily during 3 to 4 weeks.

The pathology has been originally registered by the electrocardiography method. Hisological study has shown diffuse injury of the whole of the myocardium: absence of the cross lines in some fibers, necrosis and destruction of some muscular fibers, a small edema. Lymphohistocystic and inflammatory reactions are moderately expressed. Observations of the experimental mice have demonstrated that some of the mice had their motion functions disturbed and spine crooked. An assumption arises whether the pathology in question is the model of one of the widely spread forms of polimyositis (Wagner – Unferricht form).

The advantage of this method of reproduction of the heart pathology is that it is easy to implement and also the fact that one of the injected substances is an inhibitor of the enzyme which is especially interesting in connection with the opportunity to understand the natural mechanism of the origin of the pathology in question.

M. V. Listov, Ph.D. (Biology)
Experimental Model of Primary Glaucoma in Linear Mice DBA/2

 Summary

The disease is manifested in the form of acute bout in 5 to 20% of mice taken for the experiment (mass of the animals is from 18 to 20 g, animals of both sexes) after intraperitoneal injection of aqueous solutions (0.2 ml/mouse) of two substances. Within 1 – 1.5 hours after injection of the solutions, pathological process (stable increase of the intraocular pressure resulting in the clearly visible increase of the size of the eyeball) occupies one or both eyes and is accompanied by the cornea turbidity. Within 24 hours the increased eyeball assumes the form of a cone and wrinkles, the animal becomes blind.

The injected substances are well soluble, they are injected in relatively low doses, can be natural metabolites of the organisms. The model advances a researcher to understanding of the natural mechanism of the intraocular pressure regulation, outlines ways of development of the early diagnostics of primary glaucoma and search for new drugs to treat this disease.

The model can be used for screening of chemical substances with hypotensive activity as well as substances – inactivators of those natural metabolites analogs of which have been used in our experiment.

The main advantage of the model is that it advances a researcher to understanding of the natural mechanism of the origin of primary glaucoma.

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\*Effects of Annona squamosa Lin. Seed oil on adult emergence and sex-ratio of
Tribolium castaneum (Herbst). (Coleoptera : Tenebrionidae).

 ABSTRACT

The petroleum ether (40-60 C) extracted Annona squamosa Linn. Seed oil was found to be effective for the reduction of adult emergence of Tribolium castaneum (Herbst). But this seed oil did not significantly deviate the sex-ratio from an ideal sex-ratio 1 : 1.

 INTRODUCTION

Annona squamosa Linn., the custard apple, is a small tree or shrub widely distributed mainly in tropical America, but has long been introduced into India and south east Asia. Annona squamosa bears heart-shaped, yellowish-green, juicy, sweet, delicately flavoured and cream, yellow or white fruit. Economically, the family is of appreciable importance as a source of edible fruits. Oils from the seeds of some of these plants may be used for the production of edible oils and soap. Many members of this family are used in folk medicine for various purposes. The seed extracts of this plant have been used as an abortifacient (Shenoy et al., 1968).

The most effective application appears to be against various aphids and human body lice (Reyes and Santos, 1931). Insecticidal materials are precipitated from custard apple seed extract concentrated with ether or petroleum ether at O.C. (Feinstein, 1952).

Tribolium castaneum (Herbst) is a major pest of stored products and is cosmopolitan in distribution (Good, 1933; Sokoloff, 1972, 1974). Both adults and larvae are able to exploit a wide variety of stored commodities (Ziegler, 1977). Infestation by these beetles leads to persistent release of unpleasant odours in the commodity. These are due to the secretion of benzoquinones from two pairs of defence glands, one pair in the thorax, and the other in the abdomen. The species is particularly suitable for many kinds of experiments because both the intra and extra medium conditions can be maintained at a constant level. By using similar flour in all experiments and by ensuring similar weights, surface exposure and external conditions, a total environment can be established which is relatively constant and reproducible (Park, 1934).

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MATERIALS AND METHODS

The insecticidal properties of petroleum ether (40-60 C) extracted Annona squamosa seed oil have been examined. Petroleum ether extracts of the seeds contained.unsaturated fatty acids such as linoleic acid (24.7%) and oleic acid (75.3%) in the seed oil, and it has insecticidal properties (Kumar and Thakur, 1988).

Newly hatched T. castaneum larvae were reared in either fresh or treated flour medium. Larvae were reqularly observed until pupated. The pupae were collected by sieving the medium through a 250 micrometer aperture sieve and sexed by microscopic examination of the exogenital processes of the male and female pupae (Ho, 1969). The genital lobes in the female pupae are large, bifid and flexible, whereas in the male pupae the lobes are minute. The pupae are easily sexed on the basis of these lobes.

After the pupae were sexed, the flour particles were removed from the pupae with a fine soft brush. The sexed pupae were returned to the individual tubes and observed daily for adult emergence. The experiments were conducted with four replicates for each treatment and each replicate consisted of ten newly hatched larvae.

 RESULTS

Adult emergence and sex ratio : The results and statistical analysis of the experiments are shown in Table 1. The effect of different doses of A. squamosa seed oil on adult emergence was tested by analysis of variance. The significant differences between the doses were determined by a Student-Newman-Keul’s multiple comparison test (Zar,1984).

Deviations of the sex-ratio from an ideal 1:1 ratio were determined using the binomial test (Zar, 1984). The percentage emergence of adults was measured from the numbers of adults emerging in relation to the total numbers of larvae used. All pupae produced adults. Arcsine transformed data for the percentage emergence of adults were used here for analysis of variance. There was a significant (P 0.001) reduction in adult emergence from the larvae treated with A. squamosa seed oil compared to the control. The average percentage of adult emergence with A. squamosa seed oil was not dose-dependent. In the controls and in the some chemical treatments the sex-ratio deviated from the typical 1 : 1 sex-ratio but not significantly (P 0.05) (Table 2).

 DISCUSSION

There was a significant reduction in adult emergence from the larvae treated with A.squamosa seed oil compared to the control (Table 1). In the present experiment the sex-ratio deviated from the typical 1 : 1 sex ratio, but the deviation of the sex-ratio was not significant (P 0.05) in either the control or treatments (Table 2).

In nature, natural selection favours a 1 : 1 sex-ratio at conception for most species (Leigh, 1970) but some organisms show a deviation from this typical sex ratio. These deviations may be due to the influence of environmental factors on the physiology of the offspring after conception (Anderson, 1961: Trivers and Willard, 1973: White 1973: Charnov and Bull, 1977.

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Table 1. The effects of A. squamosa seed oil on the percentage of T. castaneum
 adult emergence after the larvae exposed to the seed oil treated medium.
---------------------------------------------------------------------------------------------------------------------
Dose Mean percentage of + S.E.
(ppm) adult emergence
---------------------------------------------------------------------------------------------------------------------
Control 85.39 + 4.61 a
 90 60.64 + 4.47 b
 180 59.19 + 5.61 b
 360 54.22 + 5.32 b
 720 47.89 + 5.09 b
 1440 43.50 + 3.69 b

Student-Newman-Keul (SNK) multiple comparison test values followed by the same letters are not significantly different at 5% level (P 0.05).

Table 2 – The percentage of T. castaneum adult emergence and sex-ratio from larvae reared on fresh medium and medium treated with different concentrations of
 Annona squamoss seed oil. The values of ‘t’, a test for significant departure
 from a 1 : 1 sex-ratio.
---------------------------------------------------------------------------------------------------------------------
Treatments % of total % of emergence Sex-ratio t”
(ppm) emergene M F M F
---------------------------------------------------------------------------------------------------------------------
Control 97.50 43.59 56.41 1: 1.29 0.644 N.S.
90 75.00 50.00 50.00 1: 1 0.187 N.S.
180 72.50 44.83 55.17 1 : 1.23 0.563 N.S.
360 65.00 46.15 53.85 1 : 1.17 0.192 N.S.
720 55.00 45.45 54.55 1 : 1.17 0.223 N.S.
--------------------------------------------------------------------------------------------------------------------
Four replicates for each dose, each replicate consisting of 10 larvae (N = 20 x 4 = 40), M = Male, F = Female, N.S. = Not significant, P 0.05.

“t” is based on the formula

Where ‘n’ is the total number of insects emerged ‘p’ & ‘c’ are the proportions of insects of each sex (p being the greater), ‘c’ is the expected value of ‘p’, ie, 0.5.

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Efficacy of Annona squamosa Linn. Seed oil on the reduction of pupal and adult weight of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae).

 ABSTRACT

The effect of Annona squamosa L. seed extracted oil was found to be highly significant (P 0.001) for the reduction in the weight of pupae and adults of Tribolium castaneum (Herbst). The prolongation of the pupal period with the treatment of Annona squamosa L. seed oil wa highly significant (P 0.001).

 INTRODUCTION

Annona squamosa Linn, the custard apple, widely distributed naturally in tropical America, has long been available in India and southeast Asia. It bears heart-shaped, yellowish green fruit which are juicy sweet, delicately flavoured and cream, yellow or white. The seeds are many, brownish black, smooth and oblong. In many countries locally available plant materials are widely used as protectants of stored products against insect pests. The effectiveness of many derivatives for use against grain pests has been reviewed by Jacobson (1958, 1975, 1990). The seeds, leaves and immature dried fruits are used as an insecticide against bedbugs, head and body lice (Harper et al, 1947). The leaves of A. squamosa have a disagreeable odour, while the seeds contain an acrid principle fatal to insects (Lindley, 1946). The insecticidal principles of Annona spp. have received considerable investigation summarized by Harper et al (1947). The seeds and roots of custard apple A. squamosa contain an insecticidal material which when concentrated with ether appears to be as potent against several insect species as rotenone (Harper et al, 1947). The seed extract of this plant was used as an abortifacient (Shenoy et al. 1968). Annona extracts have been claimed to act as both contact and stomach poisons (Harper et al., 1947).

Tribolium castaneum (Herbst) is one of the most serious pests of stored products and it occurs all over the world wherever stored products are found. The effect of temperature and humidity on the rate of development and mortality of T. castaneum over a series of temperatures was found to be between 15 C and 35 C at 70% relative humidity (Howe, 1956). Pupal development can be completed in 4-5 days. Adults are small, flat elongate, red brown beetles 3 – 4 mm long. They are singed and fly well (Hill, 1990). The life of this beetle is longest at 25 C. and 70-80% relative humidity (Simwat and Chahal, 1970).

The following experiments were undertaken to study the efficacy of locally available plant materials on the pupal and adult growth of T. castaneum which have not been investigated or published elsewhere before.

MATERIALS AND METHODS

The insecticidal properties of petroleum ether (40-60 C) extracted Annona squamosa Linn. Seed oil have been examined in this and the accompanying article (Parveen and Selman, 1995).

PUPAE : Newly hatched T. castaneum larvae were reared in flour medium, either fresh or treated. Larvae were regularly observed until all pupated. The pupae were collected by sifting the medium through a 250 micrometer aperture sieve and sexed by microscopic examination of the exogenital processes of the male and female pupae (Ho,1969).

The genital lobes in the female pupae are large, bifid and flexible whereas in the male pupae the lobes are minute. The pupae were easily sexed on the basis of these lobes. After the pupae were sexed, the flour particles were removed from the pupae with
a fine soft brush. Then the pupae were individually weighed.

The sexed pupae were returned to individual tubes (50 x 25 mm) with 0.3 g of either fresh or treated media into each tube, and the tubes were capped with cotton wool. Adult emergence was observed regularly and the pupal period was recorded. The pupal period was recorded from the time of pupal formation to the time of adult emergence.

ADULTS : When the adults emerged, they were separated from the medium by sieving through a 500 micrometer sieve and they were then cleaned and weighed individually

 RESULTS

PUPAL WEIGHT AND DEVELOPMENT: The results and statistical analysis for the pupal weight and pupal period of T. castaneum are shown in Table 1. The effects of the treatment with A. squamosa on the decrease in the weight (in micrograms) and the pupal period were analyzed by analysis of variance. The significant difference between the means of the pupal weight and period was tested using the Student-Newman-Keul (SNK) MULTIPLE COMPARISON METHOD. All the treated media significantly reduced the pupal weight (P 0.001) and this reduction in weight was found to be dose-dependent. The SNK multiple comparison showed that the pupal weight loss at the different doses was statistically significant.

ADULT WEIGHT AND DEVELOPMENT: The effect of the treatment with A. squamosa seed oil on the reduction in the weight (in micrograms) of the adults of T. castaneum, compared to the control, was significant (P 0.001) (Table 2). The reduction in the adult weight after treatment with A. squamosa seed oil was dose dependent.

The results were analyzed by analysis of variance. All the treated media significantly reduced the adult weight in comparison with the control. The significant difference between the means of the adult weight was tested by using the Student-Newman-Keul multiple comparison test. The adult weight reduction at the higher dose, for example at 1440 ppm concentration of A. squamosa seed oil, was very high compared to the weight at the other doses.

 DISCUSSION

PUPAL, AND ADULT WEIGHT AND DEVELOPMENT: The ether and petroleum ether insoluble resins have previously been extracted from A. squamosa seed oil and fed to the beetles. It was found that the oil has a profound effect, reducing the weight of all stages of T. castaneum. It also lengthened significantly the developmental period compared to the control.

Turmeric oil, sweetflag oil, neem oil and margosan “O” have repellent and growth inhibiting effects on the larvae, pupae and adults of T. castaneum (Jilani et al., 1988). These investigators found that the body weight of T. castaneum larvae, pupae and adults reared in treated wheat flour was significantly lower than that of the control, and that the reduction in body weight was dose dependent. The results of the present experiments using A. squamosa seed oil agree with the results obtained by Jilani et al (1988).

Other investigations have shown that nicotine incorporated into an artificial diet significantly reduced the 7 day old larval and pupal weights and prolonged the pupation time of the tobacco budworm Heliothis virescens F. (Gunnasena et al., 1990). Larval development to the adult stage was greatly delayed by Azadirachtin, and the reduction was dose dependent in Epilachna varivestis M., Ephestia kuehniella Zell., and Apis mellifera L. (Rembold et al. 1982). Nine different oils (almond, shark liver, khaskhas, groundnut, sesamum, castor, mustard, coconut and cucurbit) have been found to retard the growth and development of the larvae, pupae and adults of Trogoderma granarium and T. castaneum (Punji et al., 1970). The present studies, therefore, adds one more oil, that extracted from A. squamosa seeds, is an agent which significantly delayed larval development and reduced body weight of the adults, and the effect was dose dependent.

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Table 1 – The efficacy of Annona squamosa Linn. Seed oil on the pupal weight (ug)
 and developmental period (days) of Tribolium castaneum Herbst.

---------------------------------------------------------------------------------------------------------------------
Dose Mean weight (ug) + S.E. Mean pupal + S.E.
(ppm) of pupae period (days)
---------------------------------------------------------------------------------------------------------------------

Control 2486.25 a + 85.20 3.75 a + 0.48
90 2031.75 b + 98.48 6.08 b + 0.71
180 1855.75 be + 68.63 7.31 b + 0.63
360 1539.50 cd + 24.70 8.17 b + 0.56
20 1213.50 de +182.42 10.17 c + 0.22
1440 934.75 e +151.21 10.59 c + 1.03
--------------------------------------------------------------------------------------------------------------------
Student – Newman – Keul (SNK) multiple comparison test values followed by the same letters are not significantly different at 5% level (P 0.05).

Table 2 – The effect of Annona squamosa Linn. Seed oil on the adult weight (ug) of
 Tribolium castaneum Herbst.

---------------------------------------------------------------------------------------------------------------------
 Dose (ppm) Mean weight (ug) of adult + S. E.
---------------------------------------------------------------------------------------------------------------------
Control 1992.20 a + 64.48
90 1864.73 ab + 43.00
180 1786.53 abc + 86.96
360 1703.50 bc + 85.73
720 1552.25 c + 46.38
1440 1352.25 c + 60.41
--------------------------------------------------------------------------------------------------------------------
Student – Newman – Keul (SNK) multiple comparison test values followed by the same
letters are not significantly different at 5% level (P 0.05). S.E. = Standard Error.

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\*Criteria for identifying the intensity of intra-species competition in Tribolium.

Weight and survival are attributes usually employed in studies of the effect of density on interspecies or intraspecies competition. Biomass usually describes the amount of living material directly related to the amount of energy fixed by the producers of an ecosystem. However, there is no reason why the use of this term cannot be extended to experimental studies of competition if used to inquire how much living material is produced by an organism from a given amount of food. In the present investigation we have used a known amount of flour to rear T. castaneum under different conditions of crowding and determined the individual and the gross weight of the adult survivors. It is in this context that we use the term biomass.

This note is part of a more extensive investigation on intra-species competition in Tribolium castaneum which will be published elsewhere. But the use of biomass (rather than individual weights of survivors as a criterion for determining the intensity of competition, and a method of identifying cohorts in intraspecies competition studies may be of interest to population biologists.

 MATERIALS AND METHODS

1. Strains and substrains. - The material used in this investigation was derived from two basic strains differing in body color and substrains were selected from them as follows:

 1.Strains.

 a. Berkeley synthetic strain. This is a highly heterozygous strain derived from seven laboratory strains (for method of synthesis see Lerner and Ho 1961
 or Sokoloff 1974). Its phenotype is referred to as chestnut or red rust, the
 normal body color of the majority of species of the genus Tribolium. In this
 study, these beetles are referred to simply as strain E.

 b. Black synthetic strain. This strain was obtained by crossing the semidominant black mutant with beetles from the Berkeley strain. The bronze F1 beetles
 were intercrossed, and the F2 black beetles resulting from these crosses were
 selected to obtain strain G, a black strain with the highly heterozygous Berkeley synthetic background.

 2.Substrains.

 Ian Franklin, using the Berkeley synthetic strain, selected four strains for high
 body weight (HEW) and four strains for low body weight (LBW). These
 strains had been selected for 7 generations of brother-sister mating. For our
 study, Franklin made available one strain selected for HBW which weighed about twice as much as the strain from which it was originally derived, and another
 strain selected for LBW which weighed about half as much as the normal strain.
 At the time when these two strains became available for this study selection had
 been relaxed because of loss in viability (for details see Franklin, 1967). To
 eliminate the inbreeding effects and to detect possible maternal effects in our
 study, HBW females were crossed with the Berkeley synthetic wild type males
 and HBW females giving A strain larvae heterozygous for the blak gene. The
 reciprocal cross gave larvae which were likewise heterozygous for black and
 referred to as strain B larvae. Berkeley synthetic wild type males were crossed with LBW females to obtain C strain larvae, and the reciprocal cross gave
 D strain larvae. F strain larvae were obtained by crossing Berkeley synthetic
 males with black females; the reciprocal cross gave F strain larvae. Finally,
 black beetles with Berkeley synthetic background were obtained by crossing
 Berkeley synthetic beetles with black, crossing the F1 to each other. In the
 F2 black beetles were crossed with each other to obtain the G strain. Although
 A-G are substrains derived from strain E, they will be referred to as strains in the rest of the paper. For a graphic representation of the relationship between the original and the derived strains see Fig. 1.

1. Procedure for controls and experimental.

1. CONTROLS.

 Larvae of each strain were allowed to pupate in corn plusyeast medium and allowed to hatch as imagoes. When they were 10 days old, 100 pairs of beetles of each needed strain were distributed over five oviposition jars containing
 corn flour enriched with brewers yeast in a proportion of 19:1 respectively.
 The adults were transferred to fresh food daily to minimize cannibalism of the
 eggs. The eggs were allowed to hatch, and 0-4 hours-old larvae were aspirated into an empty vial and transferred to another vial containing 1 gram of medium.
 For each strain or combination of strains there were three densities (10, 40 and
 100 larvae/gram). Ten replicates were set up for each a strain or combination of strains. The larvae were reared in an incubator maintained at 32 degree C. and
 70% relative humidity. When the beetles hatched, they were sexed, identified
 to body color, and placed in separate empty vials according to sex and phenol-
 type, and killed by placing the vials in a high temperature oven. The adults
 were then stored until time became available to weigh them. Prior to weighing
 them, the beetles in their vials were left overnight in an oven to dehydrate them.
 The contents were then placed en masse in an analytical balance. And the
 number of beetles producing the dry weight recorded.

 2.Experimentals.

 The sources of larvae for the experimental were the same as for the eight strains
 just described for the controls. They were introduced into vials containing 1 gram
 of medium enriched with brewers yeast in the following combinations: AF, AF’ AG, BF, BF’, BG, CF, CF’ CG, DF, DF’ AND DG in equal proportions, i.e.,
 5A and 5F to total a density of 10, 20A and 20F to equal a density of 40, and 50A
 and 50F for a density of 100, and the same for the other combinations of strains.
 The E strain was used only as a “control”. It was not used in the experimental
 because its phenotype is chestnut, the same as that of the A-D strains.

 In all the 12 types of mixed-strain vials listed above, we could distinguish wild
 type adults from bronze adults by their color and not by their size, which may be
 unreliable as a criterion for identifying beetles to their respective strain when
 competition conditions are imposed at the higher densities. For example, A or
 B HBW beetles may be reduced in size to such an extent as to be confused
 with F strain beetles, and strain C or D LBW beetles may likewise be confused for F beetles at the high densities. However, since the F beetles are bronze
 (heterozygous for black) mistaken identification of the beetles to a wrong
 strain is not likely to occur. Except for a few inadvertent technical errors,
 there were 10 replicates for each strain or strain combination, and density.

 C.Analysis of data.

 The data were analyzed by using Student’s t-test, followed by Analysis of
 Variance and Multiple Regression Analyses to reinforce the conclusions,
 taking advantage of the SPSS (Statistical Package for the Social Sciences)
 developed for the IBM personal computer. Because of certain limitations of the
 program and because of the very nature of inter-density comparisons of
 biomass which will be discussed later, biomass data had to be converted into
 biomass per individual to be able to obtain the significance between any
 differences in the means. This conversion leads to values similar to those
 obtained when mean weight of individuals is considered and can be used to compare data across densities for each of the strains. The main difference lies
 in that, where weights are available the effect of sex on weight can be determined. With these introductory remarks out of the way, we can proceed
 to examine the actual results.

 RESULTS

 A.Effect of density on Survival and Biomass.

 1.Single strains.

 a)Survival.

 (i) Density 10.

 Table 1 shows the symbols for the single strains used (column 1); the number of
 replicates (column 2) and the mean number of survivors to the adult stage. Due
 to technical errors the number of survivors in column 3 exceeds 100% for strains
 A and D, Strains B, C had an excess of 95% survival; strains E, F, F’, and G had
 a survival rate of over 83%.

 (2) Density 40.

 Table 1 shows that all of the strains had a modicum of mortality. The largest
 mortality was observed in the G strain. Nevertheless, 78.75% of the beetles
 survived to the adult stage. The remaining strains had a survival rate of
 89-98%.

 (3) Density 100.

 Table 1 shows that C, D, F, F’ had the greatest survival rates (over 90%). The
 E strain had about 89% survival and the G strain had 72% survival. The lowest
 survival was observed in strains A and B, the HBW strains, which showed
 45 and 65% survival, respectively.

 (4) Other observations.

 (a) Variance.

 Columns 4, 5, and 6 in Table 1 show the standard deviation, the variance and
 the standard error, respectively, for all densities. Most notable are the variance
 values. At density 10, the variance is 2.1 units for strain D and much less for
 strains A, B, C, F, and F’. The highest variance (6.5) is seen for the G strain.
 In density 40, the variance of all strains remains less than 11 units. At density
 100, the losest variances are observed in the LBW strains (less than 20 units).
 Larger than 20 but less than 80 units are the variances obtained for strains F, and F’. A, B and E have a variance larger than 50, but less than 80 units.
 But strain G has a variance astonishingly valued at 457 units.

 (b) Correction factor for survival.

 Table 1a shows the strains shown in Table 1 (except for E) in column 1; the
 mean number of survivors (column (2) and their biomass (column 3). Because
 the mean number of survivors is not equal to density 10, we have corrected the
 biomass (given in column 3) to show, in column 4, the value the biomass should
 be had all the beetles survived to the adult stage. Thus, for example, we have
 a mean of 10.1 survivors of strain A which produced 12.67 mg of biomass.
 Multiplying 12.67 by a factor of 10/10, 1 = 12.52 mg for the biomass of A at density 10. On the other hand, a mean of 9.7 B strain beetles produced
 13.82 mg. Therefore, 10/9.7 = 14.25 mg is the corrected value for the B strain if we assume 100% survival. And so on for the other strains. For Density 40
 (D-40), strain A, we divide the biomass by the number of survivors and multiply
 by 40 (49.56 x 40/37.7 = 52.58. The same procedure for data under density 100.
 The results will be like those shown in Table 1a.

 We now ask the question; to what extent are the values in Table 1a consistent
 with expectation? In the experimental protocol we have increased the density
 from 10 to 40, a four- fold increase and from 10 to 100, a 10-fold increase. All
 things being equal, the biomass for D-40 is expected to be four times greater
 than the biomass at D-40. (We chose D-10 as our standard on the assumption
 that at this density there is no competition between the beetles because there is
 excess of food). The first four columns of Table 1b are identical to Table 1a.
 Column 5 in Table 1b shows the expected values of biomass corrected to 100%
 survival using the D-10 values as a standard. Actually we have two standards
 to compare the observed values: one is an “internal” correction: the biomass of a particular strain is multiplied by a ratio of potential survivors to actual survivors.
 The other is an “external” correaction, and it utilizes the corrected results
 obtained at D-10 as a standard. An example will show the difference between the two types of correction:

 1)Internal Correction.

 Strain Col. 3 Correction factor = Column 4

 A 49.56 40/37.7 = 52.58
 B 52.87 40/37.3 = 56.70 (Under Col. 4 are the
 Expected values for A and B at D-40).

 For D-100 A = 48.49; B + 77.86. Correcting for mortality:

 A = 48.9 x 100/44.9 = 108.9
 B = 77.8 x 100/95.4 = 119.4

 2) External correction:

 D-40 : A = 49.56; B = 52.87

 Basing calculations on values in D-10, column 4:

 A = 12.52 x 4 = 50.08
 B = 14.25 x 4 = 57.00

 D-100: Standard from values in D-10:

 A = 12.52 x 10 = 125.2
 B = 14.25 x 10 = 142.5

 For the comparisons at D-40, when mortality is low, sometimes the internal
 and other times the external correction results will give a closer value to the
 observed value.

 For D-100, if the survival is high, there will be little difference between the
 observed and either the internal or external correction. If the survival is low,
 the observed biomass will be so different from the corrected values that it
 makes little difference whether the observed values are compared to the
 internally or externally corrected values.

 2.Mixed-strains.

 a) Survival.

 Table 2 shows the basic statistics for survivors for each of the strains involved.
 For each of the three densities, the strains involved are shown in column 1, the number of successful replicates is shown in column 2. Columns 3 to 7 show
 the symbol of strain 1 (also referred to as genotype 1) in column 3, the mean
 number of survivors in column 4 and in columns 5, 6, and 7 are shown the
 corresponding standard deviation, the variance, and the standard error.
 Columns 8-12 show the same statistics for genotype 2. The survival of the various strains at the three densities can be summarized as follows:

 1) Density 10. The mean survival values for strains A and B (the HBW strains)
 are comparable: For the HBW strains the average survival value is 96.3% for
 the LBW strains it is 96.7%. For the F strains 95.5, for F’ 91% and for the
 G strain only 77%.

 2) Density 40. The mean survival value for the HBW strains is 97.7%, for the
 LBW 98.1%, and for the F, F’ and G strains the survival values are 79.4, 89.4
 and 69.1% respectively.

 3) Density 100.

 The mean survival value for the HBW strains drops
 markedly to 66%. The LBW strains maintain a high survival value of over
 93.8%. The F and F’ strains have a mean survival value of 73.4 and 72.2%, respectively, and G drops in mean survival to 58.7%.

 A glance at Table 2 shows that there is a decrease in survival as density
 increases. The HBW strains are most affected by the increase in density, and
 of these, strain A is more affected than strain B. The C and D LBW strains are
 minimally affected. Of the intermediate weight strains, the F and F’ 95.5 and
 91%, respectively at D-10, drop to 79.4 and 89.4 at D-40, and to 73.4 and
 72.2% respectively at D-100. The G strain shows a gradual drop from 77%
 average survival at D-10, to 69.1% at D-40, and 58.7% at D-100.

 4) Other observations.

 (a) Variance.

 Analysis of the data in the single strains showed that the variance was a useful
 statistic to determine the effect of density on survival. Examination of the
 variance in Table 2 shows that generally, at D-10, the variance is very low (less
 than one unit) for most strains, and less than 2 units for strains F’ and G. As
 density increases, the variance at D-40 increases to 2.5 – 5.1 units for most strains. The only exception is the G strain whose variance becomes 7.5 units.

 For D-100, the variance of the A and B strains is about 58 and 27 units,
 respectively. For the C and D strains is about 12 and 16 units, respectively.
 For the F and F’ strains the variance is about 24 units.

 Although the variances in Table 2 are high, those in Table 1 (for the single strains) are much higher. We can conclude from these values that both strains
 benefit by being reared in association with another strain in mixed-strain vials.

 (b) Biomass.

 Table 2a summarizes the basic statistics of biomass for each strain (genotype).
 The mean and its standard deviation are given for 10 replicates of each strain except as noted. As we have done for survival, column 1 gives the symbols for the strains reared together; column 2 gives the symbol of one of the strains;
 column 3 gives the number of replicates; column 4 and 5, the biomass and its
 standard deviation; column 6 gives the symbol of the other strain (genotype 2);
 column 7 the number of replicates; columns 8 and 9 the biomass and standard
 deviation of the other strain (genotype 2). Columns 10, 11 and 12 give the
 t-values, degrees of freedom and p values respectively, obtained when
 genotype 1 and genotype 2 are compared within a density. Column 13 gives
 the significance of the p value obtained. Column 14 gives the total biomass
 produced by genotypes 1 and 2, and columns 15 and 16 the percentage of the
 biomass produced by each of the strains of genotype 1 and 2, respectively.
 (The figures in parenthesis are sums of the six percentages obtained at
 that block of numbers).

 Table 2a shows the overall results of the biomass calculations:

 1)Density 10

 Taking the data at face value, at D-10 the greatest average biomass is that
 produced by HBW strains A and B (7.2 and 6.9 mg, respectively), and the lowest is produced by the LBW strains C and D. (4.0 and 4.4 mg, respectively). The
 biomasses of the F, F’, and G strains are intermediate between the HBW and
 LBW strains (5.8, 5.5 and 4.2 mg. respectively).

 2)Density 40.

 The relative standings of the biomasses at this density are not altered: the HBW
 strains produce the greatest biomasses, both producing about 28 mg.; the C and D strains produce the lowest biomasses (16 and 18 mg, respectively) and the
 G strain produced 15 mg of biomass. All things being equal and basing the
 theoretical values on those obtained in D-10, the expected values for A = 28.8;
 B = 27.6; C = 16; D = 18; F = 23; F’ =22; and G = 16.5 mg., so the observed and the theoretical (uncorrected) values are very close.

 3)Density 100.

 The observed biomasses at D-100 do not change their relative standing from that observed at D-10 and D-40, but the expected and the observed values have become highly disparate. In the following we list the strains alphabetically, and
 the observed and expected values in that order; A 34 and 72; B 53 and 69;
 C 40 and 40; D 41 and 44; F 41 and 58; F’ 39 and 55; and G 31 and 42. The
 fact that C and D agree between their observed and calculated values suggests
 that the LBW strains are the only ones obtaining sufficient food at this density.
 The remaining strains are actively competing for the available food supply, but
 A and B are the strains showing the most obvious effects of starvation.

 (c) Ratio of biomasses of coexisting strains.

 Before undertaking long series of calculations using statistical methods, a few simple calculations were carried out to understand the data better : Ratios were
 calculated to obtain a trend, if any, other than a visual observation that as density
 increases the variance of the biomass increases. It was expected that ratios of
 Hi over intermediate strains would give a ratio greater than 1, and ratios of
 Lo overintermediate would give a ratio less than 1 under normal conditions, but,
 it might be modified under abnormal conditions such as high density, and it might be altered at the higher densities in an unknown way, since all of the strains were
 highly heterozygous but differed in their body-weight determining genes.

 The results were interesting: On face value, the HBW strains appeared to be more efficient in the production of biomass than the F, and F’ strains by 10-28%
 (the difference in their body weights) and more so than the G strain by a factor
 of 54-65%. The C and D strains were less efficient in producing biomass than the F and F’ strains by a factor of .01 to + .20, and by a factor of - .01 to .12%
 compared with the G strain. As crowding increased to D-40, the A and B strains increased in biomass 32-53% over the F and F’ strains and 71-100% over the
 G strain. The C strain was less efficient than the F or F’ in producing biomass by a factor of 16%, while the D strain was less efficient than the F’ strain by 16%, but more efficient than F by 11%. Both C and D were more successful than the
 G strain in producing a biomass by a factor of 10 and 26%, respectively.
 At D-100 the level of efficiency in biomass production of the A and B strains over
 the F and F’ strains was between 23 and 37% for A and between 36 and 64%
 for B. A was inferior in biomass production to G by 12%, while B was superior to G by 84%. C was inferior to F and F’ in biomass production by 5 and 6%
 respectively, while D was inferior by 18 and 25%, respectively. Both C and D
 were superior to G in biomass production by a factor of 28 and 31%,
 respectively.

 (e) Ratios of biomass using D-10 as standard.

 Assuming the biomass values given in D-10, columns 4 and 9 are correct, we have calculated the expected values of biomass for genotype 1 and genotype 2
 combinations in columns 17, 18, and 19 and the corresponding percentages for
 each strain in columns 20 and 21 in Table 2a, part 2, and from columns 17 and 18 we have re-estimated the ratios with the following results:

 1)Density 40.

 For D-40. The ratios of A and B with F and F’ show that the biomass of A is
 greater than F and F’ by 16 and 20%. Respectively, while that of B is greater
 by 10 and 28%. The ratios of A with G and B with G show that biomass of the
 HBW strains is 70 and 54%, respectively, than the G strain. The C and D strains
 biomass is less than the biomass of F and F’ by 7 and 20% for C, while that for D is less than F and F’ by 6 and 19% respectively. The biomass of G, when reared with , is equal. When G is reared with D the biomass of G is less by 12.5%.

 (2) Density 100.

 For D-100, the ratio of biomass is about 16 and 21% greater for A and 10 and
 27% greater for B over F and F’. The ratio of A and B is 63 and 56% greater, respectively over G. The C and D ratios of biomass are below by 17 and 20%
 with F, respectively, and 6 or 19% lower, respectively when compared with
 F’. Finally, the biomasses of C and G are equal, while that of strain D is
 greater than the biomass of G by 12 per cent.

 The above estimates for biomass ratios have been determined without any
 correction. Table 2b shows the corrected values of genotype 1, genotype 2 and the sum of genotypes 1 and 2. The corrected values are based on observed numbers of survivors of each genotype. For example, for D-10 in the AF vials, there was a mean of 4.5 beetles out of 5 A larvae originally introduced, a 90% survival which weighed 6.95 mg. Therefore to correct this for 100% survival we multiply 5/4.5 x 6.95 = 7.72 for genotype 1; 5/4.9 x 5.99 = 6.11 for genotype 2 and 10/9.4 x 12.94 = 13.83 for the the corrected values of genotypes 1 + 2.
 Density 10 provides the standard values for the determination of the theoretical
 values for D-40 and D-100 just as we did for single strains.

 (3) Correction factor for biomass.

 Table 2c is an abbreviated version of Table 2b. Column 1 shows the combination of mixed strains; column 2 the strain referred to as genotype 1;
 column 3 the mean number of survivors for genotype 1. Column 4 the mean
 weight of survivors for genotype 1. Column 5 gives the corrected weight assuming 100% survival, Column 6 gives the symbol for genotype 2;
 Column 7 the number of survivors; Column 8 the mean weight of biomass of
 genotype 2; Column 9 the corrected value based on 100% survival; and
 Column 10 the total biomass (adding columns 4 and 8). We can now see how
 close the observed and the calculated values of biomass are to each other.

 Density 10.

 All the comparisons between biomasses 1 and 2 are very close to each other.
 The greatest difference between the genotypes grown together in mixed strain
 vials is observed for strain G when grown with C (0.88 mg). The remaining
 biomasses differ much less than that.

 Density 40.

 At this density there is a greater gap between the observed and the calculated
 biomasses, especially if there is a large drop in the number of survivors.
 A, reared with F has the greater difference in biomass. But the difference in
 biomass between A and F’ and A and G is less than 0.2 mg. B with F, F’ and G
 have a difference between observed and calculated biomass of the order of
 0.4 – 2.2 mg. C and D differ from F, F’ and G by 0-1.2 mg. On the other hand,
 F, F’ and G range in difference from 0 to almost 8 at this density.

 Density 100.

 At this density the difference between the biomass of co-exising strains becomes manifest : The differences in biomass between F and F’ observed and calculated
 values when reared with A is 11.7 – 25.5 mg; 10-19 when reared with B; 9-14 mg
 when reared with C and 5.5 – 6.3 when raised with D. The difference between
 G and A is 17.5 mg; with B it is 24.7 mg; and with C and D the difference is about
 22 units. Occasionally we may observe that the weight of biomass of the

 C and D strains may significantly exceed the weight of the G strain, which under
 less dense conditions behaves as an intermediate weight strain.

 Table 3a summarizes the number of replicates, the mean weight of the biomasses produced at three densities with their accompanying statistics
 (mean, standard deviation, variance and standard error) by the single strains,
 and Table 3b does the same for the two strains in mixed strain vials. Tables
 4A and 4b do the same as Tables 3a and 3b, except that instead of number of replicates the number of individuals (survivors) contributing to the biomass is given. In Table 4a the biomass estimated from values in D-10 are used as
 standard to contain the calculated values in biomass for D-40 and D-100.

 d) Paired comparisons.

 The statistics given in Tables 4a and 4b have been used in paired comparisons to determine whether the difference between the means is significant or not by
 applying Student’s t-test.

 The results of the t-tests have been summarized in graphic form in Fig. 4 for the single strains: in Fig. 5 for the biomasses of mixed-strains of genotype 1 and
 genotype 2 within a vial, and in Fig. 6 for comparisons between a given genotype between single and mixed-strains.

 1)Single strain comparisons.

 a..Density 10

 Fig.4, D-10 shows that the A and B strains are significantly different from each other and from every other strain used. None of the other paired comparisons of the other strain used. None of the other paired comparisons of the other strains

 (i.e. LBW and Intermediate weight strains) is significant.

 b.Density 40.

 The A and B strains are significantly different from each other and from every other strain. For the other paired comparisons, C is not significantly different from D, E, or G, but it is significantly different from F and F’, Strain D is not
 significantly different from E, nor from G, but it is significantly different from F’ and G. F is significantly different from strains F’ and G, and F’ is significantly

 different from G.

 c..Density 100.

 A is significantly different from every other strain; B is significantly different from

 F and F’; C is not significantly different from D, but both C and D are significantly

 Different from E, F, F’ and G. E is significantly different from F and F’, but not from G. F is not significantly different from F’, but both F and F’ are significantly different from G.

 2. Mixed strain comparisons of genotypes 1 and 2.

 a)Density 10.

 Fig. 5 shows that the HBW strains A and B and the LBW strains differ significantly from the intermediate weight strains F, F’ and G.

 b)Density 40.

 The HBW and LBW strains differ significantly from each other and from the Intermediate strains F, F’ and G.

 c)Density 100.

 The D strain is not significantly different from the F’ strain, nor from the G strain. Every other paired comparison is significantly different.

 3.Comparisons between single and mixed-strains.

 The only useful comparisons are those between single and mixed-strains of the same genotype, and these are summarized in Fig. 6.

 a)Density 10.

The single strain A in group 1 differs significantly from A in groups 9, 10, 11. The B strain in group 2 differs significantly from B in group 13 but not from Groups 12 or 14. C from group 3 differs from C in groups 15, 16, 17. D from group 4 differs significantly from D in group 20, but not in groups 18 or 19. F in group 6 differs from F in groups 9, 12, 15 and 18. F’ in group 7 differs significantly from F’ in groups 10, 13, 16 and 19. G in group 8 differs from G in groups 11, 14, and 20, but not from G in 17.

 b)Density 40.

 A in group 1 differs significantly from A in groups 9, 10, 11.

 B in group 2 differs from B in group 14, but not from B in groups 12 and 13.

 C in group 3 differs from C in group 15 but does not differ from C in groups

 16 and 17.

 D in group 4 differs from D in groups 18 and 20 but not from D in group 19.

 F in group 5 differs from F in group 12 but not from d in groups 9, 15 and 18.

 G in group 8 differs from G in groups 11, 14, 17, and 20.

 c)Density 100.

 A in group 1 differs from A in groups 9 and 11, but not from 10.

 B in group 2 differs from B in groups 12, 13 and 14.

 C in group 3 does not differ from C in groups 15, 16, 17.

 D in group 4 differs from D in group 20 but not from groups 18 or 19.

 F’ in group 7 does not differ rrom F’ in groups 10 or 13, but it does differ from

 G in groups 17 and 20.

 E A further model for testing survival and biomass.

 Based on the results obtained for the single strains summarized in Table 1b, column 4, we have created some data to serve as a model against which we could compare the results of the mixed-strains of equivalent density. Since mixed-strain vials consist of larvae of strain 1 and strain 2 in equal numbers, we have added the corrected values of strain A (12.52) and strain F (10.5) in density 10 and divided their sum by 2 (i.e., 12.52 + 10.5/2 = 11.51. This procedure was repeated for all the remaining combinations of strains for all the three densities. Next we have compared each of these values with the values obtained at equivalent densities and combinations of strains with the results summarized in Table 5c, left, under the heading of single strains. Then we have transferred the corrected values of Table 2b, column 12 and transferred them to Table 5c, right column, under the heading mixed-strains for immediate visual comparison. The results are obvious if it is remembered that at D-40 the number of initial larvae is four times the number of larvae introduced into vials at D-10, and the number at D100 is ten times larger than at D-10; Looking at Table 5c it is evident that there is very little difference between the calculated and the observed values as expected. At D-40, the values for combinations Including the C or D strains are closer than the values that include the A or B strains. At D-40 the differences between the observed and the theoretical values, especially for the combinations that include the A and B strains become more obvious. At D-100 the closest data between the calculated and observed values, as expected are those for mixed-strains involving C and D strains. The biomasses of mixed strains involving A and B are generally higher than the calculated values. This, again, leads to the conclusion that at high densities in vials involving C and D strains, both LBW and the intermediate weight strains, by being reared together, are able to produce a greater biomass than single strains of equivalent density. Mathematically, it makes sense that if food is abundant, a large strain and a middle sized strain will produce as much biomass as they are physiologically capable. If food is as much biomass as they are physiologically capable. If food is in short supply, larger body sized strains will show a greater mortality and reduction in body size than smaller sized strains. And 50 A (HBW) larvae, grown with 50 F (intermediate) larvae in one gram of medium will fare much better than 100 A larvae grown in the same amount of food. However, the genotype of the strain is also important as we have seen; Judging from the behavior of the black body color strain G the genetic makeup is Important: Even though this strain had the same heterzozygous background as the other strains apparently G is more sensitive to crowding, resulting in beetles with lower survival even at the lowest – density, and at higher densities it showed a greater reduction in body weight that other strains of comparable intermediate size.

 F)Variance of biomass.

By squaring the standard deviations shown in Tables 6a and 6b we can obtain the variance of each strain and some idea of the effect of density on the biomass of each strain. The results of our analysis are as follows.

 Single strains.

It will be recalled that first instar larvae were introduced into vials containing 1g of flour at densities of 10, 40 and 100 larvae per vial. There were 10 replicates for each strain and density.

 Density 10.

The variance for strains A-E exceeds one unit of variance, but it is less than 3 units. The variance for the F and F’ strains, F1 heterozygotes of crosses between E and G are less than 1 unit. The variance of the G strain exceeds 6 units, and it is the most variable of all strains even in the absence of crowding. We will use these values as a standard.

 Density 40.

There is no increase in the variance of strain D. For the remaining strains, there Is roughly a four-fold increase in variance for strain B and a 10-fold increase for strains A, C and E, an increase of 9-15 times in the variance of F and F’, and a 12-fold increase for the variance of strain G.

 Density 100.

The least increase in variance is exhibited by strains C and D. C increases only 8-fold over the variance observed in density 10, while D shows a 38-fold increase in variance. Strain variance increases about 60X the value observed in density 10. F’ increases by 100X and F’ by more than 200x the values observed in density 10. Strain G increases by 80-fold the variance observed in Density 10.

In the single strain biomass data in Table A we can see that at density 10 the variance of A and B (the Hi body weight strains) and C and D (the Lo body weight strains is comparable. The F and F’ (heterozygous for black) show the lowest variance and G, the black strain, shows the highest variance.

 Density 40.

All the strains show an increase in their respective biomasses, which have become about 4 times larger than those observed in density 10, as expected. The variances of comparable strains have not increased equally: The variance of A has increased five-fold, while the variance of B has increased about four-fold over that observed at D-10. The variance of the C strain has increased about five-fold, while that of the D strain is about the same as that observed in D-10. The variance of the E strain has increased about seven-fold over the variance in D-10. The variance of the heterozygous strain F has increased about 9-fold and that of the strain F’ has increased almost 20-fold. At density 100, the variance of A and B has increased 100-200-fold, compared with that of density 10. The variance of strains C and D, although the lowest for all strains, has increased about 8-fold for strain C and 30-fold for strain D. The E strain has increased 50-fold over that seen in D-10. F and F’ have increased in variance about 200-fold and 300-fold, respectively over that observed at D-10, and the variance of G, the black mutant strain, has increased 80-fold.

 Mixed strains.

We refer now to Table 6b to obtain a rough picture of what is happening to the biomasses and variances of co-existing strains.

 Density 10.

Strain A, co-inhabiting with strains F, F’ and G, has a variance of about 1.5-1.6 units when reared with F and F.. respectively, and about 0.8 units when reared with strain G. Variance of B, when reared with F, gives a variance of 1.000, about 0.7 when reared with F’ and about 0.3 when reared with G. The Lo body weight strains C has a variance which ranges from 1 to about 0.6 when reared with F, F’, ad G, and D, when reared with the same middle weight strains has a variance of 0.1 to 0.6. The F, F’ and G strains have very low variances, ranging from .02 to 0.5.

 Density 40.

The variances for the Hi body weight strains A and B range from 1.5 to 10.6 for the A strain, and from 3 to 6 for the B strain. The Lo body weight strains range in variance from 0.6 to 6.1 for the C strain and from 0.4 to 7.6 for the D strain. None of the strains co-inhabiting with A, B, C, or D has a variance exceeding 1.2 units.

 Density 100.

The variance of the Hi body weight strain A ranges from 80 to 184, while the variance for the Hi body weight strain B ranges from 41 to about 79 units. For the Lo body strains the variance has increased from 4.3 – 11.6 units for the C strain and from 10 to 22 units for the strain D. For the intermediate weight strains, the variances of the F strain range from 2.1 – 16.9; for the F’ from 3.4 to 25.6 and for the G strain from 1.7 to about 35 units.

To simplify the presentation all the variances of each strain have been pooled and averaged for the number of entries (three entries each for the A, B, C, D strains, and four for the F, F’, and G strains with the following results:

 Density 10

 A: 3.93/3 = 1.31 F : 0.686/4 = 0.172

 B: 2.01/3 = 0.67 F’: 0.395/4 = 0.10

 C: 1.04/3 = 0.34 G : 0.576/4 = 0.144

 D: 1.08/3 = 0.36

 Density 40

 A: 22.1/3 = 7.36 F: 1.51/4 = 0.377

 B: 13.7/3 = 4.89 F’: 2.23/4 = 0.558

 C: 9.6/3 = 3.20 G: 3.33/4 = 0.832

 D: 11.8/3 = 3.92

 Density 100

 A: 381.8/3 = 127.3 F: 27.9/4 = 6.98

 B: 193.6/3 = 64.5 F’: 67.45/4 = 16.86

 C: 25.2/3 = 8.4 G: 89.77/4 = 22.44

 D: 42.2/3 = 14.1

The simplified table above shows that at Density 10 only one of the strains (A) exceeds one unit of variance. Strains B, C and D range between 0.36 and 8.67 units of variance, while the F, F’ and G strains have a variance between 0.1 and 0.17 units. At D-40, A and B, the Hi body weight strains have a variance of about 7 and 5 units, respectively, and the Lo weight strains C and D have less than 4 units of variance. The intermediate weight strains F, F’ and G still have a variance lower than one unit.

At D-100, the greatest increase in variance is observed for strains A and B, the Hi body weight strains with 127 and 64 units, respectively. Overall, the A strain produces a lower biomass and a higher variance, and the B strain produces a higher biomass and a lower variance. The C and D Lo body weight strains have a much lower variance than A and B, but comparable to the variance of F and F’. The G strain has a higher variance than F and F’ and C and D, but much lower than the variance of A and B.

When we compare the data of the single strains in Table 6a with the data in 6b it is evident that the highest effect of density becomes manifest in the Hi body weight strains which show a greater mortality, greater loss in biomass, and greatest variability. A similar effect is observed in the performance of G, the black mutant strain, but this effect has a different cause: the black strain does well in low population density conditions, but it has a lowered viability under crowded population conditions as Sokoloff (1977) has shown.

 DISCUSSION AND CONCLUSIONS

The present paper deals only with survival and biomass as criteria for determining the effect of density in intra species competition. These two criteria and two others (weight by gender and disregarding sex, and development) will be the subject of a more comprehensive paper to be published elsewhere.

We have chosen biomass rather than weight of individuals as one of the criteria because biomass data are somewhat easier to obtain than individual weights, and yet, to our knowledge, this criterion has seldom (if ever) been used by population biologists. The section on Results is rather long, but this is due to our desire to see whether statistical models other than variance could be used more readily to detect completion.

The main findings of this investigation are as follows:

1.Survival.

For convenience and to visualize what is happening, we offer a histogram of the single strains (Fig. 2) and one for each density to show survival of the individual strains

(Fig.3, a, b and c).

a)Single strains (See Fig. 2).

(1)A and B, the HBW strains) show about the same percentage of survival at D-10 and D-40, but a significant drop in survival at D-100.

(2)C and D (the LBW strains) show no significant changes in their near 100% survival in all of densities used.

(3)The IBW) (intermediate weight strains (=IWS) E, F, F’ and G, show about the same mortality at D-100: there is a significant difference in survival for E and G, but not for

F and F’.

b)Mixed-strains (see Figs. 3a, 3b, and 3c).

(1)D-10. The A, B, C, D, F and F’ strains show about the same percent survival, F has a higher survival than F’ : G has a lower survival than A, B, C or D.

(2)D-40. The survival for strains A, B, C, and D is maintained over 90%. F shows

a significant drop in survival over that shown by A, B, C, and D; F’ has lower survival than B and D; G has a lower survival than A, B, C, and D.

(3)D-100. A and B have a severe drop in survival; C and D maintain their high level of survival. F has a lower survival (about 60%) than at D-10 and D-40. F drops in survival to 50% with A 60% with B, but it stays at 80% or 90% survival with C or D,

G has 60-65% survival with A or B, but higher (about 75% survival) with C or D.

2.Biomass.

a.Single strains.

The data in Fig. 4 show that, at D-10 and D-40, A and B are significantly different from each other and from every other strain used. C and F are not significantly different from each other at D-10, but at D-40, F is significantly different from C, D and E; F’ is significantly different from C, D, and F, and G is significantly different from F and F’. At D-100, A remains significantly different from the remaining strains except G; B is significantly different from F and F’; C and D are significantly different from E-G. E differs from F and F’; F is not significantly different from F’ but is different from G. F’ is also significantly different from G.

b.Mixed strains.

As can be seen in Fig. 5, the two genotypes reared together in combinations AF to DG are significantly different at D-10 and D-40. At D-100 strain combinations AF to DF are still significantly different, but combinations DF’ and DG are not.

c. Comparisons between single strains with same genotype in mixed strain.

We omit the E strain because it was not involved in inter-strain competition. Out of 24 comparisons possible for each density, 5/24 comparisons at D-10 were not significant while 19/24 were significant; at D-40 8/24 were not significantly different while 16/24 were not. At D-100, 13/24 were significantly different, while 11/24 were not. Note that as density increases, the number of significant paired comparisons decreases, meaning that the biomass of single strains becomes of the same order of magnitude at the highest densities.

D. Models.

The discovery that a few technical errors had been made while the experiment was being set up (resulting in survivals of larvae to 100%) led us to attempt to apply some corrections to the data. We also noted, comparing densities, that biomasses of D-40 increased very close to four-fold, over the biomasses obtained at D-10, and the biomasses of D-100 did not increase ten-fold over those at D-10 (all things being equal). Since the survival values of the single strains is not equal, survival was equated by multiplying the biomass by a ratio (see Section on Results for examples), which became a corrected biomass. It was observed that the observed biomass at D-40 was very close to the expected value using the corrected value of the biomasses of each strain at D-10 and multiplied by 4. But the observed and expected biomasses for D-100 and not at D-40 is attributed to the onset of competition at a density somewhere between 40 and 100 larvae per gram. In other words, when competition is taking place at these higher densities, it will become evident because there will be fewer survivors and the biomass values between observed and calculated values will be obviously different. Similar conclusions can be made for the models developed for the mixed-strain vials.

d.Variance.

One of the advantages of the personal computer is that statistics such as mean, standard deviation, variance and standard error, among others, are very easy to obtain. This is very useful because the variance which is the standard deviation squared, greatly magnifies any differences in the standard deviation. In the present paper we have seen that each of the strains, not unexpectedly, had its own variance. The variance may be very low at low densities but increase tremendously at densities where competition is taking place, and it may increase even further in strains which are sensitive to crowding such as the G strain.

Thus, variances can be used as an index of the degree of competition taking place or sensitivity of the strain(s) to crowding. In mixed-strains, the variances shown in Table 2 are high, but they are not as high as those for single strains in Table 1. The highest variances are reached at D-100 for strains A and B (the HBW strains) and the lowest for strains C ad D (the LBW strains). Those for F, F’ and G are intermediate, but G’s variance is higher than the variance of F or F’. Because the variances in Table 2 are lower than the variances in Table 1, we conclude that in mixed strain vials both strains benefit from being reared in association with another than being reared singly at the same density, especially if there is a big difference in the size of the two strains. We believe this is the first time that such a phenomenon has been observed experimentally in the Coleoptera.

Finally a few words about the interaction observed in these experiments.

As the title of the paper indicates, we regard the interactions observed both within and between the highly heterozygous strains of T.castaneum as being examples of competition. Competition and not predator-prey interaction because the interactions occurred for less than one generation in duration (first instar larvae 0-4 hours old were used to initiate the experiments in all single strain and mixed-strain vials) and the experiment was brought to an end when the adults were obviously sexually mature (there were no imagoes that were white or pale in color). Thus, during the experiments, larvae are growing synchronously within vials of the same density. Differences in density 40 and density 100 (as manifested by the total biomass and a decrease in the number of survivors, especially in the HBW strains at D-100, which resulted, in our opinion, from a shortage of food and not from predator-prey interactions. Although predator-prey interactions have been demonstrated in Tribolium (see Sokoloff 1975 and Sokoloff 1977), they are observed in long lasting experiments of several generations or several years duration, when the components of a population (eggs, larval instars, pre-pupae, pupae, teneral adults and mature adults) cannot be controlled. In our view, by starting the experiments with larvae 0-4 hours old we have minimized age differences in both single strain and mixed-strain vials and cannibalistic propensities which may occur; large larvae or adults preying on egg, small larvae, pupae or teneral adults. Since all the individuals were of about the same age in any given vial, the decrease in survivors at the higher densities is ascribed more to the shortage of food or to natural mortality than to predator-prey interactions.

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 LEGENDS FOR TABLES

Table 1 - Survival statistics, single strains.

Table 1a - Abbreviated statistics of survivors and biomasses of strains.

 Column 1 Gives the symbol of the strains;

 Column 2 the mean number of survivors; strains.

 Column 3 gives biomass of survivors as observed.

 Column 4 the corrected biomass assuming 100 per cent survival.

Table 1b - For legends for columns 1 to 4 see Table 1a.

 For Column 5 biomass of the single strains has been calculated

 Using Density values as standard.

Table 2 - Statistics for survival of genotypes 1 and 2 in mixed-strain vials.

Table 2a - Mean (mg) of biomass of genotypes 1, genotype 2, and the total

 Biomass of genotype 1 + 2.

Table 2a - Part 2. Biomass of genotype 1 and genotype 2 and percent of genotype 1

 and percent of genotype 2.

Table 2b - N survivors, biomass and biomass corrected to 100% survival for

 Genotype 1, genotype 2 and genotypes 1 + 2.

Table 2c - Same as 2b, simplified.

Table 3a - Statistics for N replicates and total biomass of genotype 1 and 2.

Table 3b - Statistics for N replicates and biomasses of genotypes 1 + 2.

Table 4a - Statistics for N survivors and biomass of genotypes 1 and 2.

Table 4b - Statistics for strains of genotypes 1 and 2.

Table 4c - Biomass of genotype 1 corrected to 100% survivors and genotype 2 corrected to 100% survivors. Total biomass. Percent of genotype 1 and 2 in the biomass. Ratio of larger biomass/smaller biomass. Column 8,

 Expected biomass using that of D-40 in column 7 as standard and

 assuming 100% survivors.

Table 5a - Simplified survivors and their biomasses (Mixed-strains).

Table 5b - Survivors and biomass expected taking from Density 10 values as

 Standard.

Table 5c - “Corrected” values assuming 100% survivors (left column) vs

 Values derived from Table 1b.

 TABLE 1

 DENSITY 10

 (1)\* (2)\* (3)\* (4)\* (5)\* (6)\*

 A 10 10.1 1.101 1.211 0.348

 B 10 9.7 0.675 0.456 0.213

 C 10 9.6 1.265 1.600 0.400

 D 10 10.1 1.449 2.100 0.458

 E 9 8.444 1.333 1.778 0.444

 F 10 8.600 0.843 0.711 0.267

 F’ 10 8.300 0.675 0.456 0.213

 G 9 7.556 2.555 6.528 0.852

 DENSITY 40

 A 10 37.7 1.494 1.233 0.473

 B 10 37.3 1.418 2.011 0.448

 C 10 39.3 2.111 4.456 0.667

 D 10 39.1 1.912 3.656 0.605

 E 10 35.7 2.669 7.122 0.844

 F 10 36.6 2.271 5.156 0.718

 F’ 10 36.8 3.225 10.400 1.020

 G 10 31.5 8.860 78.500 2.802

 DENSIY 100

 A 10 44.9 8.865 77.878 2.791

 B 10 65.2 7.843 61.511 2.480

 C 10 95.4 3.748 14.044 1.185

 D 10 96.2 4.442 19.733 1.405

 E 8 70.88 8.741 76.411 3.091

 F 9 85.78 6.978 48.694 2.326

 F’ 9 85.78 6.978 48.694 2.326

 G 10 72.00 21.377 456.989 6.760

 \*1. Strains

 2. Replicated number

 3. m;

 4. S.D.

 5. Variance;

 6. S.E.

 TABLE 1a

 DENSITY 10

 (1)\* (2)\* (3)\* (4)\*

 A 10.1 12.67 12.52

 B 9.7 13.82 14.25

 C 9.6 8.04 8.38

 D 10.1 8.35 8.27

 F 7.9 8.30 10.50

 F’ 9.3 8.26 8.88

 G 6.8 6.47 9.51

 DENSITY 40

 A 37.7 49.56 52.58

 B 37.3 52.87 56.70

 C 39.3 33.66 34.26

 D 39.1 32.77 33.52

 F 36.6 42.34 46.27

 F’ 36.8 36.64 39.82

 G 31.5 30.14 38.27

 DENSITY 100

 A 44.9 48.49 108.00

 B 65.2 77.86 119.42

 C 95.4 82.23 86.19

 D 96.2 80.96 84.16

 F 77.2 98.38 127.44

 F’ 83.5 90.94 108.91

 G 65.1 64.33 98.80

 \*The headings for this table are:

1. Strains;
2. Mean number of survivors;
3. Biomass observed;
4. Biomass corrected to 100% survival

 TABLE 1b

 DENSITY 10

 (1)\* (2)\* (3)\* (4)\* (5)\*

 A 10.1 12.67 12.52
 B 9.7 13.82 14.25
 C 9.6 8.04 8.38
 D 10.1 8,35 8.27
 F 7.9 8.30 10.50
 F’ 9.3 8.26 8.88
 G 6.8 6.47 9.51

 DENSITY 40

 A 37.7 49.56 52.58 50.08
 B 37.3 52.87 56.70 57.00
 C 39.3 33.66 34.26 33.52
 D 39.1 32.77 33.52 33.08
 F 36.6 42.34 46.27 42.00
 F’ 36.8 36.64 39.82 35.52
 G 31.5 30.14 38.27 38.04

 DENSITY 100

 A 44.9 48.49 108.00 125.20
 B 65.2 77.86 119.42 142.50
 C 95.4 82.23 86.19 83.80
 D 96.2 80.96 84.16 82.70
 F 77.2 98.38 127.44 105.00
 F’ 83.5 90.94 108.91 88.80
 G 65.1 64.33 98.80 95.10

 \*The headings for this table are :

 1. Strains
 2. Mean number of survivors;
 3. Biomass observed;

 4. Biomass corrected to 100% survival;
 5. Expected biomass using “Density 10” values as standard.

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\*Further experiments to test the possibility of fertility in hybrids of T. castaneum (Herbst) and T. freeman Hinton.

The genus Tribolium at present consists of over 30 described species distributed unequally over five species-groups (review in Sokoloff, 1972). Within the castaneum species-group two pairs of species resemble each other : T.audax (AU) and T.madens (MD) are blackish in body color. AU and MD were once mistaken for a single species until Halstead (1969) took a closer look, observing differences in various characters in the larvae, pupae and adults. Attempts to hybridize these two species were successful : they can produce a few fertile hybrids. The second pair of species includes T.castaneum (CS) and T.freemani (FR). They resemble each other in their antennal morphology and the inter-ocular distance and their chestnut body color. (For other characteristics see Sokoloff 1966).

FR was found in the Kashmir as a single specimen and it had been described by Hinton. (1948) as a new species included in the castaneum group, but it was not until the Japanese intercepted a contaminated shipment of corn imported from Brazil (Nakakita et al., 1983) that living material became available for research. Nakakita et al. 1981 showed that even though FR weighs about three times more than CS, the two species can mate with each other, producing abundant but strile progeny.

Through the courtesy of Dr. Nakakita, T. freeman became available for genetic studies. One of us (A.S.) and a number of students have undertaken a series of studies to establish to what extent the genomes of CS and FR are similar. Brownlee and Sokoloff (1988) showed by hybridizing CS dominant mutants with recessive lethal effects: semidominants and sex-linked recessive females with FR males and the same kinds of mutants with T. castaneum wild type males for controls that the mutant traits are transmitted in the proper proportion to the F1 hybrids. Furthermore, the expressivity and the degree of penetrance of the genes in the hybrids varied to the same extent as those observed within CS control matings, attesting to the similar genetic library in the two species.

Carrillo and Sokoloff (1991) reported 10 mutations and about 3 dozen teratologies which appeared spontaneously in FR. Spray and Sokoloff (1991) found that the black mutation found in T. freeman was a semi-dominant mutation and the scar (sc) mutation was a semi-dominant gene influenced by temperature in its expression: when scar beetles are reared at 32 C. the F1 beetles are non-scarred, while those reared at 24 are scarred. The same results are obtained when sc FR beetles are mated with sc CS and their hybrid progeny are reared at two temperatures: the hybrid sc CS/sc FR beetles reared at 32 appear normal, while those reared at 24 show a certain proportion of sc beetles.

Henry-Ford and Sokoloff (1992) carried out reciprocal crosses between FR + and CS homozvgous for black and hemi- or homozygous for r and py, and another reciprocal cross involving FR + and CS pd pte. The results were interesting : In the first cross
(CS b : r py male X FR +/+ female) the hybrid F1 were phenotypically bronze body color but normal in respect to the other traits. The reciprocal cross failed to produce progeny even though the b r py females and their FR +/+ mates were moved to fresh medium several times. The second cross involving FR +/+ mates were moved to fresh medium several times. The second cross involving FR +/+ females and CS pd pte males also gave normal F1 progeny, but the reciprocal cross failed to produce any F1 progeny.

Because in these two experiments we expected to obtain viable F1 from both reciprocal crosses, and because we had a great abundance of FR beetles from another experiment, we decided to carry out an extensive experiment to try to explain the results obtained by Henry-Ford and Sokoloff.

 Methods

Crosses were carried out between CS virgin females collected, sexed and allowed to hatch and mature from the stocks shown in Table 2 and FR males of the opposite sex. For the reciprocal crosses we obtained FR virgin females from creamers where large larvae had been introduced in small numbers to avoid crowding (crowding greatly extends the larval period). The creamers containing larvae were periodically sifted to separate the pupae. The pupae were sexed and placed in vials to allow them to hatch to adults before mating. When the F1 from these experiments emerged, the males and females were paired and introduced into vials.

The number of F1 X F1 crosses varied from 1-33 pairs depending on the number of beetles produced by the P1 pairs. The vials were placed in a walk-in incubator for
a period of 6-9 months before checking to see whether the beetles were fertile or sterile.

 Results

Table 1 summarizes the results of crosses between hybrids of reciprocal crosses between the wild type FR and wild type or mutant CS beetles. Table 2 identifies the types of mutants present in T. castaneum in the P1 cross which in column 2 of Table 1 were identified by number.

The results in F2 are given in Table 1 in two columns. The first, headed by STERILE shows whether the mating was successful the letters N/A are given. The fraction shows the number of sterile crosses over the number of crosses attempted. This is followed by a column headed by FERTILE. This column was created should any of the crosses be fertile. (As it turned out, this column was unnecessary because all the F1 X F1 crosses were sterile). In some of the crosses we were unsuccessful in setting up reciprocal matings because a limited number of FR females virgins was available. In other cases abundant F1 progeny were available so larger numbers of vials could be set up.

With these introductory remarks the data in Table 1 speak for themselves. In both series A and B F1 X F1 crosses (about 600 crosses for series A and over 750 crosses for series B) all the crosses gave 100% sterile results. Furthermore, where large numbers of replicates of reciprocal crosses are available, if all the replicates in one cross show sterility, the replicates from the reciprocal cross will also show sterility.

A few of the vials, when sifted, showed larvae of all sizes, sometimes pupae and sometimes living adults in a greater number than hose introduced at the beginning of the experiment. However, these on examination proved to be contaminants belonging to the species T. confusum (CF), escapees which had managed to crawl up on the outer surface of the vial taking advantage of the data gummed label which helped in identifying the type of cross contained therein, and which were large enough to reach the lower surface of the perforated plastic caps we use to close the experimental vials. Once they had reached the top of the vial, they had found access to the inside through the ventilation holes we had made in the cap to allow exchange of gases and thus allow normal respiration and survival of the introduced beetles and their progeny. (Without the holes, the caps are so tight on the vial that asphyxiation of the beetles may take place). Some of the contaminants were males, but they posed no problem in this experiment because, even if they mated with the experimental females, no hybrid progeny can be produced in matings between CF males and CS or FR females. A few vials contained female CF contaminants which must have been virgins when they entered the vials. Had they been inseminated females, they would have been able to produce recognizable CF beetles. There were, in fact, some vials which, in addition to the pair of experimental F1 hybrid beetles contained as many as 40 to 60 CF beetles which had been produced in the vial in the course of the experiment, and the numbers of larvae, pupae and adults attested to the fact that they were contaminants of relative recent invasion. These vials contaminated with CF beetles fortunately were few in number and did not interfere with the initial purpose of the experiment and its conclusion. They served as a warning that such an invasion of the vials is possible, and they permit taking appropriate measures to prevent their recurrence.

As to the results reported by Henry-Ford and Sokoloff (1992), and described above, these experiments do not explain them. Plans are to carry further experiments to determine whether it was a true phenomenon or just an accidental occurrence.

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Table 2 - Stock identification

Wild type strains
11. CSUSB +
14. TEXAS +
15. YUCAIPA +
20. SYNTHETIC +
23. NEW YORK +

Mutant strains

31. p
33. sp
38. r
 Ho
40.
 ph
42. r
44. ag (argentums)
51. dve pd
56. msg, pvr
59. r sp p
68. Malta p
70. pg

74. mas p
83. b McGill
91. lod p
93. Gi
94. Gi ptl
96. mt
 t
98. b
 t
99. b (tawny)
103. apt. mxp
109. ctp-1, ju
122. ptl Rd
123. Be
124. Be s
159. Sa-1
161. Sa c mxp
166. Sa-2
189. apt
196. mas
197. ppas p
200. Davis line 2.
206. ptl Rd
212. Chr Rd
221. bj
236. Dch mxp p
238. mxp
239. mxp
241. nude eggs
254. ty
257. weird eggs
264. sh
272. supergiant
276. Davis low body weight
278. la
284. s umb-1. W
286. p-like
296. b p pd
298. p pv
306. b p pe
310. p s
313. apt Mo p
314. pd p knp (msg)
315. mas p
317. aa p
324. b p
357. pd py sp
376. b ppas
377. b mc
381. b ptl
386. sg
391. j ppas
393. j mc
402. ims s
406. ap s
408. cas s
414. h, s mxp
415. mxp sp
417. h, s
421. Rd ptl p
422. Dch knp p
424. Rd. mas p
428. c Npp
431. Rd ptl knp p
442. Df. Mo s
444. i lod Mo
448. ap Chr
449. ap Chr bt-1
452. fas p
453. Chr cfl
 pk
454. ell p
 6
473. fas
478. Spa p
494. Ag/Es
 3 6
495. Dch / Ev
496. stbd/Es
498. stm – Ey
499. mxp NG/Es

Exp. 1065
Exp. 2063
500. fas-like modif. Dch/mas p

501. (au) Rd (?)