NO. 1 DECEMBER 1958

TRIBOLIUM INFORMATION BULLETIN

PUBLISHED BY

THE RESEARCH STAFF

THE WILLIAM H. MINER AGRICULTURAL RESEARCH INSTITUTE

CHAZY, NEW YORK

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A group of geneticists interested in Tribolium and closely related forms met on 25 August, during the Xth International Genetics Congress in Montreal, to discuss the activation of an informal mutual assistance group.

It was suggested that the newly organized research unit of The William H. Miner Agricultural Research Institute act as the clearing-house for the group. Mr. A. P. Withall, President of the Institute, has approved this activity including the publication, at irregular intervals, of a Tribolium Information Bulletin (TIB).

This first issue of the TIB is designed to advise interested laboratories of its existence and to solicit items of interest for inclusion in future issues. Please address all communications to Director of Research, The William H. Miner Agricultural Research Institute, Chazy, New York.

It is hoped that each laboratory will submit, from time to time, any technical or allied information on Tribolium and related species that would be of interest to the group. Material presented in the TIB should not be used as references in publications without the consent of the author. If an author is agreeable to having his contribution cited, he should include the statement, "Reference Authorized," along with his news note.

Shortly after the first of the year it is planned to include, in one or more issues, the bibliographic information on Tribolium that Dr. Thomas Park of the Department of Zoology, University of Chicago, has collected. Dr. Park has kindly consented to this activity.

One copy of this initial issue of the TIB will be sent to each laboratory that might be interested. The mailing list used for this purpose is given below. It will be appreciated if the recipients will notify us at once of additional laboratories that should be contacted. There will be no charge for the TIB. ONLY ONE COPY OF FUTURE ISSUES WILL BE SENT TO EACH OF THOSE LABORATORIES SPECIFICALLY ASKING THAT THEIR NAMES BE RETAINED OR PLACED ON THE MAILING LIST.

PERSONS PRESENT AT THE MONTREAL MEETING

25 AUGUST 1958

Agricultural Research Council, Animal Breeding Research Organization, Edinburgh, Scotland: Hugh P. Donald, John W.B. King.

The William H. Miner Agricultural Research Institute, Chazy, New York: James H. Bywaters, Earl L. Lasley, Alexander Sokoloff.

Department of Poultry Science, Purdue University, Lafayette, Indiana: A. Earl Bell, D. F. Bray, Wayne Englert, Grady A. Martin.

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Department of Entomology, University of Kansas, Lawrence, Kansas: G. Schlager, Robert R. Sokal.

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Please help us get this publication to the persons to whom it will be of greatest value.

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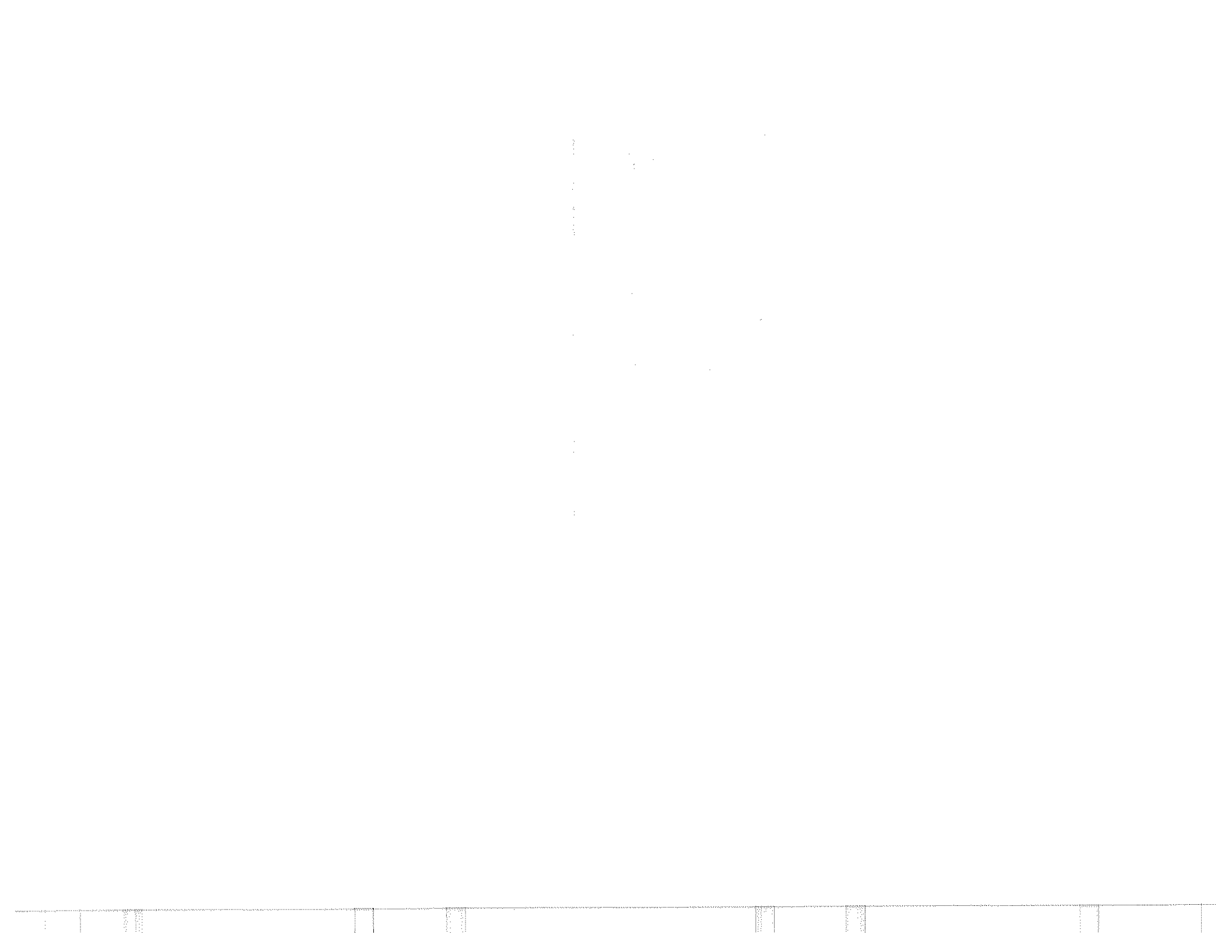

THE TRIBOLIUM INFORMATION BULLETIN

The Tribolium Information Bulletin is prepared and published at irregular intervals by the staff of the Scientific Research Division, The William H. Miner Agricultural Research Institute as a service to agriculture.

Laboratories and individuals using this insect in any type of research are encouraged to submit information for publication in the bulletin.

Information appearing in the bulletin should not be used for publication without the consent of the author unless the note, "Reference Authorized," accompanies the information.

Suggestions concerning the bulletin, including the mailing list, will be welcome and given honest consideration.

* * *

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EDITOR'S NOTES

The Tribolium Information Bulletin is designed and published as a medium for the informal exchange of information of interest to folks using this insect and its close relatives as an experimental animal.

Thus, if you have come up with some gimmick of procedure or equipment that has helped you in your work, the TIB would, we hope, be a good place to tell about it and perhaps help others.

This bulletin is a good place to let others know just who is doing what and where. There are a lot of workers using Tribolium in many areas of research, and we should become better acquainted with each other and our activities. So, write in about yourself and your friends. This invitation includes people working on the control of this insect as well as on its genetics, morphology, physiology, nutrition or ecology.

A brief description of stocks in use at each laboratory would be useful.

Some laboratory may need a publication that is surplus to another. Help may be available through a note in TIB.

Right now, the Miner Institute would like to hear from anyone having the following issues of Genetics for sale. Please indicate the issues and the price desired for each.

Issues of Genetics Needed

Vol.	Nos.	Vol.	Nos.
1	1,2,3,4,5,6	20	2,3
2	1	21	2,3,4,5
3	1,2,3,5	22	1,2,3
4	1,3,4,5,6	24	1
5	1,2,3,4,6	25	1,2
7	2	26	1,2,3,4,6
9	1	31	1,2,3,4,6
10	1	32	1,2,3,4,5
16	2	35	1
17	1	38	2
19	1,2	40	1

SELECTED BIBLIOGRAPHY ON GRAIN INSECTS

Developed by
Thomas Park, Professor of Zoology, University of Chicago

Prepared for this Publication by
Robert R. Shrode, Geneticist, The William H. Miner Agricultural Research Institute

(Editor's Note. Dr. Park and his students have used the flour beetle, Tribolium, and some related forms in studies in animal ecology for over a quarter of a century. They have contributed much to the knowledge of Tribolium and its behavior and have developed an extensive reprint file on grain insects. Dr. Park has generously permitted Dr. Shrode to prepare the titles of these reprints for this publication. They are presented alphabetically by first author.)

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RESEARCH NOTES

Sokoloff, A. Effect of ether on fecundity of T. castaneum.

Chicago wild-type beetles two months old were subdivided into lots of 10 males and 10 females and subjected to etherization for 0, 1, 2, 3 and 5 minutes. After observing the process of recovery, the beetles were placed in creamers containing 4 grams of flour, and the cultures introduced into an incubator maintained at 29° C and 70% relative humidity. Beetles were transferred almost daily to fresh medium, except that on the sixth day the adults were left in the same creamer for a period of 18 days. The number of progeny in each creamer was recorded as larvae at the end of 19-24 days. The data are summarized in the following table. It is assumed that ether has no effect on the viability of the eggs and that all eggs survive to hatching.

Number of Larvae Produced by Beetles Exposed to Ether

Days Elapsed After Etherization	Time of Exposure to Ether (Min.)				
	0	1	2	3	5
1	6	4	3	2	1
2	27	23	33	35	7
3	47	41	65	79	42
4	74	64	63	69	89
6-24	127	120	122	116	69

It is evident that no appreciable effect is produced when beetles are exposed to ether for a short time: Beetles exposed for one, two, or three minutes lay about as many eggs (as early as 24 or 48 hours after exposure) as the controls (column two). However, if beetles are exposed to ether for five minutes, the effects are longer lasting: At the end of 48 hours these beetles have laid about as many eggs as females exposed to ether for a shorter period had laid for the first 24 hours after etherization.

Beetles exposed for five minutes apparently recover fully by the end of 48 hours, so that by the end of the third day they have laid as many eggs as the controls.

Other data not included in the table suggest that exposure to ether for a longer time will have longer lasting effects: Beetles etherized for 10 minutes will not lay any eggs the first 48 hours, very few by the end of the third day, and achieve normal egg laying by the sixth day. A

15-minute exposure will postpone egg laying 24 hours over the preceding period: Egg laying begins on the fifth day, and may decrease sharply on the sixth day. A 20-minute exposure will delay egg laying about as much as the preceding period, but females that survive this prolonged exposure may recover full egg production by the sixth day. A 25-minute exposure will prevent females from laying for a full five days, and few eggs are produced on the sixth. A pair surviving etherization for 30 minutes had not produced any progeny by the end of 17 days. Beetles subjected to ether for 45 minutes may recover, but they subsequently die without leaving progeny.

The sudden rise in egg production on the second and third day by beetles exposed to ether for two or three minutes requires further investigation.

Sokoloff, A. A technique for censusing large populations of T. castaneum.

Populations started with 50 pairs of beetles in 100 g of flour yield very large numbers of offspring in one generation. A method for handling large populations has been developed in our laboratory which greatly shortens the time spent in censusing a single population. The following procedure takes advantage of the clinging ability of larvae and adults, and the tendency, on the part of the adults, to seek shelter in a dark corner (negative phototactism).

Place a porcelain pie plate with scalloped edge in a 200 mm nesting dish. Prop one end of the plate. (A creamer lying on its side will give the plate the proper angle). Sift the culture through #2 silk bolting cloth, placing the sifted material (adults, larvae, pupae and frass) on the plate. Resift the flour through #5 silk bolting cloth to separate eggs and small larvae from the flour. Spread this material thinly over black construction paper and spill the frass onto another piece of black construction paper. Larvae will cling to the first piece of paper, and they can be dropped into the dish by tapping it vigorously. Repeat the process five times. Over 99% of the small larvae will thus be recovered from the frass. Since eggs and fecal pellets are of about the same size, the eggs are sacrificed and discarded with the frass.

The nesting dish is coded to identify the population and oriented so that the uptilted edge of the plate faces the strongest source of light. The adults climb the edge of the plate and fall into the dish. In this manner about 95% of the adults separate themselves out of the frass. The rest of the beetles have to be separated with a brush: Aliquots from the plate are transferred to a clean dish. The live adults are grouped together. The dead adults (including pieces of beetles) are placed in a separate dish. The frass is blown off gently, and the larvae and pupae counted and returned to a bottle containing fresh medium.

Since in this particular "infection" experiment the eye phenotype is essential, the whole adult population is etherized for three minutes, placed on a white glass plate between guide lines imprinted with a wax pencil, and counted under the dissecting microscope.

Once the flour is sifted and the adults sorted out, it takes half an hour to census as many as 2500 adults in a culture.

Sokoloff, A. Safe periods of etherization of T. castaneum.

In contrast to the characteristic posture exhibited by overetherized Drosophila, it is difficult to tell from the appearance of Tribolium whether it has been overetherized. Because etherization is necessary in many phases of work with flour beetles (e.g., sexing of adults, identification to phenotype, etc.) it is desirable to know the tolerance of Tribolium to this narcotic. Two month old Chicago wild-type beetles were subdivided into lots of 10 males and 10 females and exposed to ether fumes for different periods. The behavior of beetles following narcosis was observed (to obtain a reliable criterion on when beetles should be re-etherized), and the number of beetles surviving the various periods of exposure was recorded. The room temperature during the beginning of the experiment was 78°F. An etherizer of the type described by Muller (DIS 6:55, 1936) was used throughout the experiment. The data are summarized in the following table.

Time exposed to ether (min.)	Survival %	Approximate time elapsed before beetles begin to walk (hours)
1	100	1/3
2	100	2/3
3	100	1-1/2
5	100	2
10	100	2-3/4
15	45	6
20	25	Probably 72
25	10	Probably 72 or more
30	55	Undetermined
45	20	Undetermined
60	0	--
90	0	--

It is evident that if beetles are etherized for as long as 10 minutes all will recover. Following longer exposures to ether beetles may walk, but they may die a few days later without leaving progeny. Those that survive may have long-lasting effects (see accompanying article on the effect of ether on fecundity).

A few remarks on the behavior of beetles following short periods of etherization may help other investigators to plan their work.

1. Ether for 1 minute

When observed immediately after removal from the etherizer, the beetles are not completely narcotized: legs and antennae are moving, and those on their feet attempt feeble walking movements.

5 min. later - Those on their legs attempt to walk, but some may fall on their backs.

20 min. later - Beetles will walk and stay on their feet if righted.

32 min. later - Beetles can cling to bristles showing coordinated movement. If poked, will take a few steps back.

40 min. later - Righted beetles walk, back, forward or in circles.

60 min. later - About half of the beetles walk.

70 min. later - About 90% of the beetles walk.

2. Ether for 3 minutes

No movement at all when observed immediately after removal from etherizing bottle.

6 min. later - Tarsi and antennae twitch.

13 min. later - Movement of the head ventrally.

15 min. later - Ovipositors may be extruded. Eggs may be laid by gravid females.

25 min. later - If beetle is poked, the legs move faster.

60 min. later - If beetle is righted, it will fall on its back.

80 min. later - If beetle is righted, it will stay on its feet.

95 min. later - Beetles begin to walk.

120 min. later - Feeble walking, with no fixed direction.

3. Longer exposures to ether

The "waking up" pattern is the same, but movement of beetles is greatly delayed: If etherized for five minutes, most beetles will be motionless at the end of 25 minutes and will fall on their backs after attempting to crawl even at the end of 100 minutes. If etherized for 10 minutes, some may crawl if righted at the end of 150 minutes. Half of the beetles will walk normally 5-1/2 hours after treatment, and 90% will be fully recovered at the end of 23 hours.

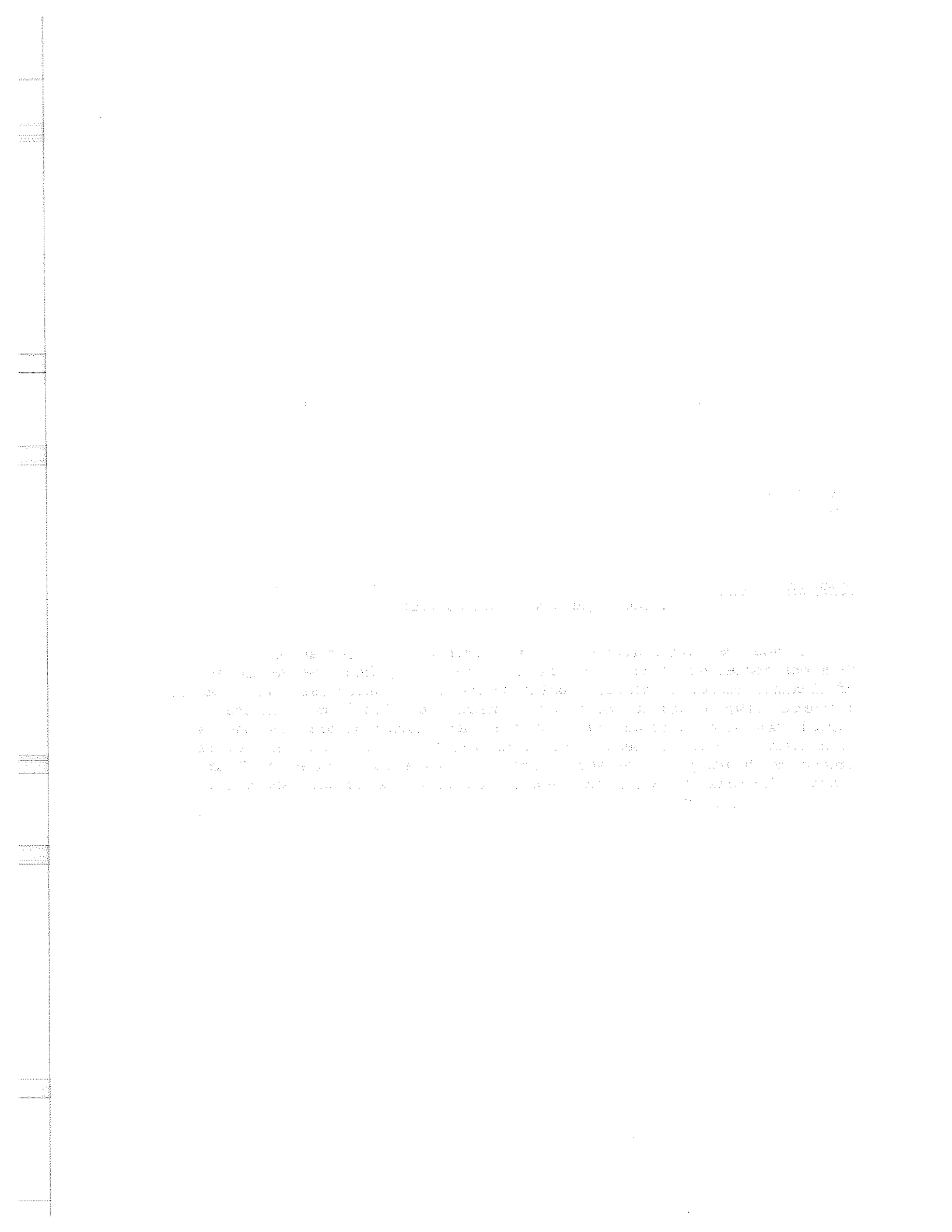
With 15 minute exposure to ether, beetles begin to die. Those that recover may take 90 minutes before tarsi and antennae begin to move and nearly four hours before they begin to crawl if righted. Full recovery may not occur until 50 hours have elapsed.

It is of interest that if beetles receive too much etherization, the color of the soft membranes between sclerites or tergites may change, acquiring a maroon color, which thus differs from the characteristic chestnut color of the normal beetle. This is particularly noticeable in the extruded ovipositor or in the soft parts between head and thorax. This change in color has also been observed in eggs and larvae, some becoming a very deep purple. If beetles are starved too long before etherization, some maroon or purplish material is observed in the rectum of the beetle when it is squeezed with forceps to extrude the ovipositor or the penis. Whether this material is produced following etherization or produced in the course of the beetle's normal metabolism and the nature of the material itself has not been determined.

Since mature females straining to turn themselves upright often lay one or two ripe eggs, etherization may be the solution to obtain flour-free eggs.

Bywaters, James H. Equipment for transferring individual beetles and pupae. (Reference authorized)

If you are having difficulty transferring adult beetles or pupae from one container to another, you might try using about twelve inches of flexible rubber or plastic tubing to one end of which has been attached a common blowpipe and to the other a common pipe stem (some find the curved stem more satisfactory). With a small amount of practice one can soon learn to pick up these forms without difficulty. Also, the reverse procedure (blowing) can be very helpful in uncovering beetles in flour or frass. The basic idea for this was picked up at the Purdue laboratory from Dr. Grady Martin.



THE TRIBOLIUM INFORMATION BULLETIN

The Tribolium Information Bulletin is prepared and published at irregular intervals by the staff of the Scientific Research Division, The William H. Miner Agricultural Research Institute as a service to agriculture.

Laboratories and individuals using this insect in any type of research are encouraged to submit information for publication in the bulletin.

Information appearing in the bulletin should not be used for publication without the consent of the author unless the note, "Reference Authorized," accompanies the information.

Suggestions concerning the bulletin, including the mailing list, will be welcome and given honest consideration.

* * *

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TRIBOLIUM INFORMATION BULLETIN

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Appreciation for printing the covers for this issue is expressed to the Industrial Arts Department, Mr. Robert Rathbun, Department Head, and Mr. William J. Transue, Principal, of the Chazy Central Rural School, Chazy, New York.

EDITOR'S NOTES

In the passing of Mr. Albert P. Withall, President of The William H. Miner Agricultural Research Institute and Chairman of the Board of Trustees of The William H. Miner Foundation, the Research Staff has lost a loyal friend and staunch supporter.

The accomplishments of the Research Staff of this Institute during the short time it has been in existence is gratifying. Of special importance and not reported elsewhere in this issue, is the invitational paper on "The Problem of Genetic Control," presented before the American Society of Animal Production by Dr. Earl L. Lasley on 27 November 1959. As this issue of the Tribolium Information Bulletin goes to press, Dr. Lasley is busy preparing an invitational paper on "Lessons from Experiments with Control Populations of Laboratory Organisms" to be presented to the National Poultry Breeders' Roundtable on 1 May 1960. A formal report on this important work will be published in the near future.

The longer we work with Tribolium, the more enthusiastic we become over its potential as a laboratory organism in pilot research in animal breeding and quantitative genetics. Properly designed experiments using this beetle can answer in a few months questions that would require many years with poultry and more than a human lifetime with cattle. As a laboratory aid in teaching basic and applied genetics, the possibilities of this beetle are unlimited.

Because this is so, we believe that more animal breeders will turn to pilot research with Tribolium to solve many of their vexing problems. With this in mind, the current issue of this publication presents a few general suggestions for establishing and operating such a laboratory.

If this publication encourages the establishment of only a few pilot research projects with Tribolium, major breakthroughs in animal breeding and genetic theory with practical application would be possible, and it would add to the value of this issue of TIB as our final tribute to the memory of Mr. Withall and the man he so loyally served for so many years, Mr. William H. Miner.

THE USEFULNESS OF TRIBOLIUM AS A LABORATORY ANIMAL

Several species of the flour beetle Tribolium possess many favorable characteristics as laboratory animals in studies of ecology and population genetics. Dr. Thomas Park at the University of Chicago, Dr. John Stanley at McGill University, and others have used this beetle extensively in fundamental studies of the ecology of animals. In recent years interest has been shown in Tribolium as an experimental animal for pilot research in the field of population genetics and animal breeding. The use of this beetle in the latter studies was initiated on a large scale by Dr. Don C. Warren at the United States Department of Agriculture's North Central Poultry Testing Station at Purdue University, Lafayette, Indiana. This work is now being continued by Dr. A. E. Bell and his associates at The Population Genetics Institute, Purdue University, and, in addition, Tribolium is being similarly used at the University of Kansas; the University of California; Dickinson College; Kansas State College; and The William H. Miner Agricultural Research Institute.

These insects are of value in such studies because they are easy to obtain, simple to raise, require an uncomplicated diet, have a high rate of reproduction, short life cycle, and a long life span. Each stage of the life cycle can be isolated and characterized, and the sex of individuals can be determined prior to sexual maturity. There are nine pairs of autosomes, and there is evidence that crossing over between members of pairs of autosomes occurs with equal frequency in males and females. Several autosomal and sex-linked genes are now available as marker genes. The small Y chromosome appears inert.

One of the chief disadvantages of Tribolium is that they have cannibalistic tendencies. Adults will eat eggs, and larvae will eat smaller larvae, eggs and pupae. This vice can be controlled to a considerable degree by removing parents from a culture as soon as it is believed sufficient eggs have been produced, by removing pupae from the culture at frequent intervals and by frequent transfer of the population to fresh medium.

Another disadvantage is that the beetles are subject to parasitism, especially protozoan, more fully discussed under Diseases. Furthermore, adult beetles, under certain conditions, produce quinones which may cause aberrant forms to appear in larvae, pupae and adults or completely destroy a culture. Occasionally these quinones produce allergic symptoms in humans. Ventilation of the culture containers will help in holding the quinones to a low concentration.

In some stocks there is a wide variation in the time required for pupation and emergence. This is a problem when a complete census is required of the offspring of a specific mating or period of egg production.

LIFE CYCLE

The following brief description of the life cycle of Tribolium is based on information gleaned from the references appended hereto and the activities in our own laboratory. Only a few references are actually cited as this is not a review paper. Its primary purpose is to establish a few guide lines and to present a few pertinent observations which may be of value to students of this beetle.

The life cycle of this family of beetles is made up of the egg, larva, pupa, immature adult, and mature adult.

Length of Life Cycle (Egg to Imago) of T. confusum and T. castaneum after Gray (1948) (G) and Park and Frank (1948) (P). Rounded off to full days.

	T. confusum					
Temp. (F)	P 75.2°	G 77°	G 81°	P 84.2°	G 91°	P 93.2°
Egg	9	8	6	5	4	4
Larval	34	29	24	16	16	17
Pupal	11	10	8	6	5	5
Total	54	47	37	27	25	26
	T. castaneum					
Egg	7	6	5	4	3	3
Larval	33	34	24	17	16	16
Pupal	10	10	8	5	4	4
Total	50	50	37	26	23	23

Note: Is it possible that there is a threshold temperature between 81°F and 84.2°F for larval development? The major difference between these two sets of data is in this area for both species. To the totals given here should be added the 5-8 days required for the imago to reach sexual maturity.

On an inadequate diet such as starch, females will live but not produce eggs. On an adequate diet, the females will begin to lay from five to eight days after emergence. The number of eggs produced is the highest immediately after the start of oviposition. This high rate is maintained for some time and then gradually declines. Fertility follows a similar course. Females lay very few fertile eggs after they are one year of age, but some males will retain their procreative power for as long as three years. The average daily egg production per female is in the neighborhood of fifteen, and there is considerable individual variation.

Ordinarily, eggs are deposited only on a suitable nutrient material such as ground cereals, grain dust, powdered milk, and like products. They are seldom deposited on such substances as wood, steel, concrete, glass, sawdust, absorbent cotton, or soil.

The eggs are about 0.6 mm long and 0.4 mm in diameter and weigh from 0.04 mg to 0.06 mg, depending on the species. These semiflexible eggs consist largely of a fluid material and do not increase in size during embryonic development. The shell is relatively smooth and pearly and covered with a mucilaginous material which probably acts as a lubricant in the egg-laying process. The eggs are quite viable so that 85 to 95 per cent of them hatch under suitable environment.

In addition to the age of the female, the number of eggs laid is greatly influenced by the temperature of the medium. The optimal temperature is in the neighborhood of 90°F, but fair production is obtained at a temperature as low as 77°F. T. castaneum is more fecund at these temperatures than T. confusum. At 63°F the egg stage is greatly prolonged and the resulting larvae do not complete their development. Above 95°F egg mortality increases so that at 105°F or above all eggs are killed within a short time.

Relative humidity has little effect on the length of the egg stage except that above 85 per cent R.H., a strong fungus growth develops and the eggs do not hatch.

When embryonic development is complete the egg splits and the larva crawls out, a process requiring from 15 to 30 minutes. The larva grows in a series of steps or instars until development in this stage is completed. In the flour beetle the number and size of the instars varies so much that they cannot be used to denote larval size. They range in number from 5 to 11 with about 80 per cent having six and 20 per cent having seven in T. confusum and slightly over half of T. castaneum having six in a range of 5 to 8 instars.

The length of the larval stage is influenced by the temperature of the medium, the relative humidity and nutrition. At 77°F, this stage is completed in about 34 days by T. castaneum and 29 days by T. confusum. At 90°F these times are reduced to about 16 days in both species.

At 81°F and 25 per cent R.H. the larval period for T. castaneum is about 32 days and for T. confusum 27 days. At the same temperature and 50 per cent R.H. these periods are reduced to 29 and 26 days, and at 75 per cent to 26 and 23 days, respectively. The beetles are not dependent entirely on free moisture but are able to obtain the necessary water for metabolism by the breakdown of the food material.

Rapid growth is obtained in such favorable food materials as whole-wheat meal, whole-wheat flour, wheat middlings, malt flour, ground wheat

germ, laying mash, and long extraction flours. The flour beetle requires most, if not all, of the B vitamins, particularly B_1 and riboflavin. These beetles grow faster on coarsely ground material than on the same food finely ground. However, separation of the several stages of the beetle from the coarser material is much more difficult than from the fine media. Thus, most laboratories will find that the use of a medium made up of 95 per cent whole-wheat flour, from which the bran has been removed by sifting twice through a 66-mesh bolting cloth, and 5 per cent dried brewer's yeast is most satisfactory.

When the larva completes its development, it sheds its skin and enters the pupal stage during which the tissues are rebuilt to form the adult beetle. As this last larval skin is shed, the gut is evacuated. It is in the pupal stage that the sex of the beetles can most readily be ascertained.

The length of the pupal stage varies primarily with species and temperature. McNary in a preliminary trial at Purdue found that larvae grown on whole-wheat flour alone would pupate but not emerge. The pupa stage lasts from about 9.5 days at 77°F to 4.5 days at 90°F in T. castaneum while the corresponding figures for T. confusum are 10 days at 77°F to 5.5 days at 90°F.

Bray (1960) reported that body weight of beetles raised at 91°F and 70 per cent R.H. was about 10 per cent greater than that of beetles raised at the same temperature but 40 per cent R.H.

Little if any courtship activity takes place before copulation which may last from a few minutes to as long as fifteen minutes. Records obtained in our laboratory on groups of ten males and ten females indicate that individual beetles copulate about three times per hour and that sex-starved beetles do not exceed this frequency. We have also found that there is no tendency for preferential matings and this validates the frequent assumption of random mating within a culture.

Furthermore, by the use of marker genes, we have learned that in double matings of one female to two males, the offspring of the female immediately following the second mating are predominantly, but not wholly, sired by the second male. Thus, in experiments using multiple matings, males carrying marker genes must be used.

It has also been established that a single male can produce fertility in twenty females when left with them for a period of four days.

It has long been known that adult Tribolii produce an odoriferous gas when they are disturbed. This is most pronounced when the beetles are confined in large numbers and without food. This gas may reach a sufficient concentration to kill all adults in the container and produces abnormalities of form and function in larvae and pupae. Loconti and Roth (1953) found three different quinones in the odorous secretion of

T. castaneum. Of these, 80-90 per cent was 2-ethyl-1, 4,-benzoquinone; 10-20 per cent was 2-methyl-1, 4-benzoquinone and there was a trace of 2-methoxy-1, 4-benzoquinone. Roth (1944) obtained abnormalities in development similar to those produced by the odorous gas by subjecting the beetles to vapors of acetic acid and by application of glacial acetic acid and various solutions of hydrochloric acid to the several stages.

The average size of male Tribolium is smaller in the pupal and adult stages than the average size of females. In our laboratory, the following results for T. castaneum pupae were obtained:

	8878	♂♂	ave	2591.59 micrograms
	9076	♀♀	ave	2719.70 micrograms
Diff.	♀♀	♂♂	=	128.11 micrograms

However, there is enough overlapping that size cannot be used as a criterion for separating the sexes.

Both T. confusum and T. castaneum can and will fly. This is especially true when either species is tapped onto a plate that has just been removed from the sterilizing oven or on hot, humid days.

When the ventral posterior of the male and female pupae are examined under 7 to 15 × magnification, it can be seen that on the terminal segment the female has a pair of prominent genital lobes anterior to the cerci which are reduced to indistinct elevations in the male.

If it is necessary to sex adults, two procedures may be used. In both cases the beetles should be lightly anesthetized. The first is to examine the inner surface of the femur of the forelegs for the secondary "sex spot" which is present in males but not in females. The second method is to grasp the beetle with very fine pointed tweezers so that gentle pressure can be exerted on the elytras and abdomen until the genitalia are exposed. The identity of each sex is obvious from the nature of the structures thus exposed.

DISEASES

Adelina tribolii is a coccidian parasite which may invade a culture of flour beetles. In light infections the protozoa may be confined to fat bodies in the vicinity of the gut, and the larvae will show little if any symptoms. In heavily infected larvae, the parasite may be in all parts of the body. Such larvae are slow and sluggish and exhibit a decrease in response to stimuli. Similar symptoms may be observed in infected adults. Colonies in which the infection is heavy lose their vitality, decrease in numbers and may die out altogether. The only mode of transmission appears to be by uninfected individuals eating portions of the carcasses of dead beetles or larvae that were infected.

Three members of the Protozoan family Ophryocystidae (Laird 1959), Mattesia dispora, Farinocystis tribolii and Triboliocystis garnhami are parasitic to Tribolium and involve the fat body, haemocoele and muscles of their host. Since these parasites are not localized in the gut, the evacuation of the gut at pupation is of no help in controlling them in laobratory stocks. The clinical symptoms are similar to those of Adelina.

Strict laboratory sanitation and the frequent transfer of colonies to fresh medium after removing all frass and dead carcasses or parts thereof are the most effective methods for controlling these parasites. If a valuable stock becomes highly infected, a collection of eggs should be made and subjected to the washing technique described by Park (1948). Alcohol, Zepharin Chloride or sodium hypochlorite may be used as the disinfectant. In order to make effective use of this technique, it may be necessary to make daily transfers of the stocks to new medium for a period of a week before collecting the eggs.

The control of parasites localizing in the gut, such as Gregarina sp. (Laird 1959) may be accomplished by washing larvae in one of the disinfectants for a few seconds to a few minutes and transferring to fresh medium.

Molds may develop at relative humidities of 85 per cent or higher and destroy the usefulness of an experimental culture. Molds may also develop in crowded cultures. In this case, the use of fine mesh bolting cloth as a covering for the container may help.

The cereal psocid, Troctes divinatorius (Muller), is a small (1 mm long), wingless louse that may be found in large numbers in Tribolium cultures. They compete for the food supply and may be vectors of disease, although this is not indicated in the literature. Frequent transfers help in keeping the numbers of this pest to a minimum. In our laboratory they were extremely numerous during the summer and early fall of 1959. In February of 1960 we suddenly realized that we had not seen a single specimen for several weeks. Can anyone tell us why? In the literature available to us, the answer is not found.

SANITATION

As with all laboratory and commercial livestock the problems of sanitation are important in dealing with Tribolium as experimental animals. They are subject to diseases as are most any other plants or animals. From a geneticist's point of view sanitation has two definite purposes. The first is to prevent cross contamination of experimental stock and the second is to prevent the entrance of disease which would invalidate the results obtained from the experiment.

Controlling cross contamination of beetles is not too difficult, but it does require a real effort. Even though many precautions have been taken by the milling industry to eliminate any live forms from the flour as put on the retail market, it is quite possible that some eggs of Tribolium may be in the flour as received from the dealer. These eggs can be destroyed by subjecting the flour to a temperature of 48° C for a period of one hour. This can adequately be accomplished through the use of dry heat sterilizers, but it must be remembered that it takes some time for the heat to penetrate to the center of any large mass of flour. The length of time required to reach these conditions will be dependent, of course, on the mass of flour and the type of container. Thus, it would be well for each laboratory to make a rather careful check of the amount of time required under their conditions. Prolonged heating of the flour is not desirable since it causes the flour to cake and makes the separation of the live forms of the beetle from the medium difficult.

The storage room for the flour should be entirely separate from the research area, and the heating of the sifted flour should take place as soon as it is brought into the research area. If facilities are so set up that the flour can be weighed and the yeast added before being brought into the research area, it would eliminate one heat treatment. If, however, it is necessary to add the yeast after the flour has been brought into the area of activity, then the flour and yeast mixture should be given a second treatment bringing about the same conditions as indicated for the flour alone.

While this treatment may eliminate some stages of some disease organisms pertinent to the beetle, it is not sufficient to destroy spores and similar types of organisms. Other sanitary precautions must be taken. The entire working surfaces of the research area should be thoroughly swabbed down at the start of each day and more often if necessary with some disinfectant. The ones most commonly used in Tribolium research are 95% ethyl alcohol, 1 to 1000 solution of zephiran chloride, or a 0.5% solution of sodium hypochlorite. None of these materials are particularly effective against spore-bearing organisms. Zephiran chloride is ineffective in the presence of soaps or other detergents, and sodium hypochlorite rapidly loses its effectiveness in the presence of organic material.

All flour in the research area should be kept in covered containers at all times. This applies regardless of the size of the container. The medium should not be kept any appreciable length of time lest it become rancid or contaminated. In all transfer operations, dust should be held to a minimum to avoid feeding escapees and also because some people can develop an allergic reaction to the whole-wheat flour.

The instruments used in manipulating the beetles including the glassware should be autoclaved or placed in boiling water for 15 to 20 minutes in order to destroy as many spores and other types of organisms as possible.

One of the most effective ways of minimizing disease risk is to schedule a periodic transfer of all stocks at least once a month. In this transfer all frass and dead forms should be removed before the live forms are introduced into the new medium. New cultures of beetles being introduced into the laboratory should be held in isolation for some little time in order to determine that they are free of disease and in the holding period should be run through several medium transfers.

In the actual handling of the beetles all plates, construction paper, brushes, probes, and other equipment should be given heat sterilization before use and should be replaced in the sterilizing oven as soon as a particular culture or series of cultures has been completed. They should be so set in the sterilizer that any other worker will know that they have been used during that particular period. At regular intervals, all equipment should be autoclaved for 15 minutes at 15 pounds pressure. Live forms of beetles should not be left uncovered in the laboratory at any time lest there be cross contamination of cultures.

As a general rule, the cultures will be sifted and the live forms and frass tapped onto a plate. The frass is then gently blown off into some large container such as a garbage pail for which a tight fitting lid is available and used. A chemical hood, when available, is most satisfactory for this operation. See Naylor's note on page 19 of this issue.

All frass and used flour should be collected in heavy paper bags (garbage bags are excellent) and placed in garbage pails with tight fitting lids until it can be incinerated. Live forms of the beetle to be discarded should be placed in a closed container and then into the sterilizing oven until dead. They can then be added to the discarded flour for burning.

The sifting and mixing equipment should be kept clean at all times. All cracks or other places where beetles could hide should be vacuumed thoroughly at frequent intervals.

EQUIPMENT

Sifter. There are several mechanical flour sifters on the market, but most of them are expensive unless a large volume of flour is to be used. A description of a small, hand-made sifter is given by Schlager on page 20 in this issue. (B. F. Gump Co., 1325 South Cicero Avenue, Chicago 50, Illinois) (Sprout-Waldron, Muncy, Pennsylvania)

Incubators. Cabinet type, forced draft poultry incubators and hatchers are satisfactory. Most have arrangements for controlling temperature and supplying moisture. Humidity control will probably not be adequate in poultry incubators and should be given additional consideration if quantitative genetic research is contemplated.

Instruments which record temperature and relative humidity are also essential. (Petersime Incubator Co., Gettysburg, Ohio) (Leahy Manufacturing Co., Higginsville, Missouri) (Buckeye Incubator Co., P.O. Box 420, Springfield, Ohio)

Sterilizers. Hot air. Standard electrically heated hot air sterilizers with accurate thermostats are needed. Steam. A steam sterilizer (autoclave) is almost a must to control disease.

Flour mixer. Standard kitchen mixers, modified ball type grinders and small cement mixers are used for this purpose.

Microscopes. Stereoscopic (dissecting) microscopes with magnification from 10x to 25x and with some of the available accessories are necessary. A good source of reflecting light should be included.

Balances, Flour. Laboratory torsion balances are needed to weigh the flour and yeast for mixing and for measuring standard amounts of medium for various containers.

Balances, Beetles. An electrobalance has proven satisfactory for weighing various live forms. These can be obtained with ranges of 0-1 mg to 0-100 mg, a corresponding precision of 1 μg to 25 μg and an accuracy of 1 μg to 100 μg. Such balances are not subject to errors due to temperature, vibrations, air currents or leveling. (Cahn Electrobalance. Through your laboratory supply house.)

Blood cell calculator. Five key blood cell calculators are useful in making phenotypic counts.

Thermo-humidigraphs. These are useful aids for monitoring environment. (The Bristol Co., Waterbury 20, Connecticut)

Glassware washer. An automatic glassware washer saves considerable time and is very efficient when large quantities of glassware must be cleaned.

Culture containers. 3/4 ounce coffee creamers with cardboard caps are adequate for the progeny which one female will produce in a two-day period and even for single pair matings. Identification can be written on the cap. Some workers believe the cap should be perforated, while others hold this to be unnecessary. Shell vials, 25 × 50 mm, with plastic caps serve a similar purpose but are less durable and convenient. Identification can be made with a wax pencil or by small strips of paper placed inside the vial. Shell vials, 15 × 45 mm, with plastic caps can be used in studies concerning the daily production of one to five females and for the storage of small numbers of unmated adults. Four- and sixteen-ounce wide-mouth glass jars with metal screw caps are useful for larger populations. Their value is greatly increased by removing the center portion of the metal cap 1/4-inch from the rim. The liner can then be

perforated for ventilation and used for identification. Extra liners are inexpensive. Half-pint milk bottles can be used for the same purpose but are taller and take up more space in incubators. About 200 live forms per ounce (jar size designation) will create a competitive environment. Wide-mouth gallon jars are used in some laboratories for stock cultures.

Etherizers. A very effective etherizer, similar to Muller's, can be made from a half-pint milk bottle, a small wad of absorbent cotton, small aluminum funnel, two plastic vial caps and a 2-inch piece of glass tubing similar to a No. 4 shell vial. Make a round hole in one of the caps just slightly less than the diameter of the funnel spout. Force the funnel spout through the hole from the top of the cap and push the cap to the shoulder of the funnel. Using a dissecting needle, perforate the second cap so that there are 25 to 30 holes in it. Place this cap in one end of the glass tube and insert the funnel spout and its cap into the other end. Place the cotton in the milk bottle. Pour in a small amount of ether and place the funnel arrangement in the bottle. Drop the beetles into the funnel. Either cap can be removed to remove the anesthetized beetles. The cotton should not touch the lower cap of the funnel unit. When not in use, the funnel unit should be removed and the bottle capped.

Sieve rings. Two piece 5-inch heat treated brass or aluminum sieve rings are needed for separating the beetle forms from the medium. (Johnson Spinning Co., 2426 West 47th Street, Chicago 32, Illinois) Some of the sieves used for separating soil aggregates may also be used.

Silk bolting cloth.

No. 00 (29 meshes per inch) for retaining pupae and adults.
No. 2 (54 meshes per inch) for retaining pupae, adults and large larvae.
No. 5 (66 meshes per inch) for retaining all forms. (B. F. Gump Co., 1325 South Cicero Avenue, Chicago 50, Illinois.)

Pie plates. 9-inch with scalloped edge, sloping rims, sharp slope to shallow well are most satisfactory for receiving the beetles from the sieves.

Syracuse watch glasses with ground edge are useful in sorting the forms from the pie plates and for holding beetles for short periods.

Biological specimen jars. Nesting jars, 112 mm in diameter and 50 mm high, are useful as finger bowls, storage of bottle caps and collecting large numbers of beetles and pupae, while the larger size, 200 mm × 80 mm, are useful in collecting used medium.

Storage jars (9-3/4" × 5-1/2") with glass lids are useful in heating and storing the medium. So are round aluminum roasters of about 1 gallon capacity.

Jeweler's forceps sizes 3, 3c and 5 are useful in sexing adult beetles and other manipulations. (Swartchild and Co., 15 West 47th Street, New York 36, New York.)

Other useful equipment includes camel's hair brushes, dissecting needles and scissors, blow pipe, rubber or plastic tubing, black construction paper, vacuum cleaner, cheese cloth, sponges, thermometers, labels, adjustable magnifying lamps, laboratory carts, counter brushes, dust pans, garbage cans with tight fitting lids and regular housekeeping supplies and equipment.

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RESEARCH NOTES

Lerner, I. Michael and Frank K. Ho. Black mutant of T. confusum. (Reference authorized)

In August, 1958, we received a stock originating from a Canadian mill which was identified by the sender as T. madens. Its appearance suggested that it may be a black body color mutant of T. confusum. Accordingly, a breeding test was carried out. The tested beetles crossed readily with T. confusum, the reciprocal F_1's being brown in color. 50 single pair F_2 cultures were set up (ten from each of five single-pair F_1 matings) for each of the reciprocal crosses. The observed ratios of adults with brown and black pigmentation indicate that the variant is an autosomal recessive mutant. The F_2 from the cross +/+ × bl/bl gave a total ratio of 643 brown to 204 black; the reciprocal cross: 870 brown to 297 black. There is no indication of lower viability of the mutant as compared with a synthetic stock (derived from crosses between several sources) of the wild T. confusum used in the test. The goodness-of-fit tests based on combined F_2 counts within each F_1 mating were as follows:

χ^2	d.f.	+/+ × bl/bl	bl/bl × +/+
Total	5	0.662	4.026
Deviation from 3:1 ratio	1	0.379	0.225
Heterogeneity	4	0.283	3.701

We shall maintain the mutant stock for the time being. It is available for distribution to those interested.

Department of Genetics, University of California

Lasley, Earl L. Evidence for equality of recombination between split and jet in both sexes of Tribolium castaneum. (Reference authorized)

Preliminary data suggested that split and jet (a mutant made available through the courtesy of Dr. Thomas Park of the University of Chicago) are linked autosomally. To prove this suggestion the following F_2 results were obtained.

Parental gamete	Phenotype				Recombination value
	Wild	Split	Jet	Split-jet	
repulsion	416	158	194	4	0.16 ± 0.024
coupling	652	31	70	146	0.12 ± 0.008

Lack of independence of split and jet is clearly evident for both types of parental gametes. There is a tendency (not statistically significant) for genetic split to be misclassified as nonsplits. Adjustment for such misclassification reduces the estimated recombination value for repulsion gametes less than 0.01 and that for coupling gametes about 0.03. It is not clear, therefore, that recombination occurs or does not occur with equal frequency in both cases.

An attempt was made to estimate recombination in the two sexes by producing four kinds of backcross progeny. The results are presented in the following table where each value is expressed as the average per viable mating. Four cells in which unexpectedly low values were obtained for jet classifications are indicated by enclosing the observed results in parentheses.

Linkage in heterozygous parent	Heterozygous parent	Phenotype				Recombination value
		Wild	Split	Jet	Split-jet	
repulsion	male	3.8	55	64	3.5	0.06 ± 0.004
	female	3.1	49	(28)	(1.7)	0.06 ± 0.006
coupling	male	45	6.5	(3.8)	(19)	0.16 ± 0.007
	female	49	7.9	9.1	42	0.17 ± 0.006

It appears that two different recombination values are generated depending upon repulsion or coupling association of split and jet in the heterozygous parent. Furthermore, it appears that about 50 per cent of the expected jet individuals are missing from the second and third rows of the table. Neither of these unusual results has a bearing on the interpretation of sex differences in recombination, however. Since the average recombination value for heterozygous male parents is essentially the same as that for heterozygous female parents, it is concluded that equal recombination occurs in this species.

No explanation is offered for the apparent shortage of jet individuals or for the observance of two recombination values. Reciprocal matings (with regard to sex) between repulsion and coupling double heterozygotes may shed some light on the situation, but eventual clarification may require additional marker genes.

The William H. Miner Agricultural Research Institute

Shrode, R. R. Evidence that mating is random in T. castaneum. (Reference authorized)

Since the assumption that mating is random is a basic one in so much population genetics theory, it seemed desirable, if possible, to secure experimental evidence as to the validity of this assumption. The data tabulated herewith are the observed total numbers of copulations

engaged in by individual females in ten samples, each consisting of five females randomly taken from a stock culture of Chicago wild type T. castaneum. The members of each sample were marked in the following manner for individual identification. The left antenna was removed from one female, the right antenna from another, half the left antenna from another, half the right antenna from another, and the antennæ were left intact on another. These identifications are designated in the table as L, R, HL, HR and I, respectively. One male was placed with each group of five females in a Syracuse watch glass containing a thin layer of flour for five one-hour periods of observation. Copulations engaged in by each female during the hour were recorded.

	Females					
Sample	L	R	HL	HR	I	χ^2
1	5	4	6	4	3	1.18
2	4	5	4	3	4	0.50
3	2	5	3	4	3	1.53
4	4	2	1	3	3	2.00
5	2	3	4	2	2	1.23
6	3	5	5	4	3	1.00
7	1	4	3	2	3	2.00
8	4	5	3	2	4	1.44
9	2	1	1	2	3	1.56
10	5	4	3	4	2	1.44
					Total χ^2	= 13.88

The only meaningful chi-squares which can be computed from the data are the individual sample chi-squares, each based on four degrees of freedom, and the total chi-square based on forty degrees of freedom with hypothetical frequencies being one-fifth of the total number of copulations recorded for each sample. The smallest probability of a greater chi-square, considering any of the eleven values computed is about 0.75. These data certainly validate the assumption that mating is random if one is willing to concede that the marked differences between the conditions under which these observations were made and those prevailing in a culture of many males and many females would have no influence on the randomness of mating.

The William H. Miner Agricultural Research Institute

Sokoloff, A., E. L. Lasley, and R. R. Shrode. A map of the X chromosome in T. castaneum. (Reference authorized)

Five new sex-linked recessive mutations have appeared spontaneously in various laboratory stocks:

1. red (r.). Lasley. A mutation of excellent viability, variable expressivity, and complete penetrance. Black pigment eliminated from all ommatidia, but retained in ocular diaphragm. Hence, the compound eye appears "spectacled" like pearl. The central facets appear pink to Bordeaux red almost indistinguishable from black. Young beetles' eyes may not show any pigment, and may be confused with pearl. Pearl is epistatic to red. Found in a stock derived from Chicago wild type and subsequently in a mixed culture containing pearl and wild type.

2. pygmy (py). Lasley. Originally found in a stock derived from Chicago wild type. Later rediscovered in a mating between a heterozygous red, miniature-appendaged paddle (+ r +/pd + ma) female and a red male. Length reduced to about two-thirds the normal. Weight reduced to about one-half. Viability good. Fecundity greatly reduced.

3. miniature-appendaged (ma). Sokoloff. Found in a stock derived from Chicago wild type and subsequently in the progeny of a female heterozygous for pearl. Gene has pleiotropic effect, i.e., body is generally shorter and "stouter." Elytra and membranous wings reduced to about two-thirds the normal, and podomeres of all legs (except perhaps the coxa) shorter and thicker. Semilethal. A portion of the ma beetles die in the larval stage. The adults may live for two months, but most die when they are a few days old.

4. spotted (sp). Sokoloff. Incompletely recessive. Appeared in a pearl stock. Mutant beetles have a light spot on the elytra. Expressivity variable. The spot may be small and limited to the tips of the elytra, it may extend as more or less symmetrical stripes to the axillary margin of the elytra, or may be absent. Therefore, penetrance is not complete and the gene overlaps wild type. Viability good.

5. truncated elytra (te). Sokoloff. Lethal. Appeared in a red stock. In the pupa the elytra look cut off at various levels. In the adult the terminal portions of the elytra seem truncated or depressed. If viewed from the side, the elytral tips are seen to be folded under the rest of the elytra. May not be possible to maintain as a stock. Linkage studies are under way.

The genetic data giving the distances between the pd, r, ma, py, and sp genes will appear shortly in the Canadian Journal of Genetics and Cytology. The preliminary data indicating that the above-listed genes are sex-linked are given in thefollowing tables.

I.

Mating type	Males		Females		N
	Wild	red	Wild	red	
+/+ × r/	123	0	138	0	7
r/r × +/	0	475	463	0	21
+/r × +/	492	491	994	0	30
+/r × r/	385	383	388	371	25

II.	Males		Females		
Mating type	Wild	pygmy	Wild	pygmy	N
+/+ × py/	69	0	62	0	6
py/py × +/	0	235	233	0	9
+/py × +/	217	218	437	0	7
+/py × py/	264	241	250	218	7

III.					
Mating type	Wild	spotted	Wild	spotted	
+/+ × sp/	445	2	453	0	13
sp/sp × +/	93	329	381	4	9
+/sp × +/	27	18	43	0	1
+/sp × sp/	26	25	27	31	2

IV.					
Mating type	Wild	min. app.	Wild	min. app.	
+/+ × ma/	88	0	90	0	2
ma/ma × +/	0	3	3	0	1
+/ma × +/	977	381	2396	0	25
+/ma × ma/	32	26	19	14	1

The linkage relationships of the above genes and pd is as in the following diagram.

pd-r about one crossover unit
pd-ma about 12 crossover units
ma-py about 2 crossover units
pd-sp about 50 crossover units

(The position of sp is uncertain because of its overlap with wild type in expression.)

The William H. Miner Agricultural Research Institute

Sokoloff, A. and R. R. Shrode. Techniques for handling beetles for space flight. (Reference authorized)

On December 4, 1959, some 11,000 T. castaneum beetles of all stages of the life cycle were included in the biopack carried by the NASA's Little Joe rocket 55 miles out into space. Because of the ability of these beetles to withstand high and low temperatures and prolonged periods of starvation, they appear to be ideal organisms for such an experiment. The following techniques may help other investigators in setting up similar experiments.

Among the beetles sent on the flight were 400 male and 400 female adults, 400 pupae of each sex, 100 larvae approaching the pupal stage, 400 large active larvae, and 400 small larvae. The last two types of larvae were included (along with one-half sheet of toilet tissue rolled into a loose wad to increase the surface) in one-dram vials, closed with a plastic cap with a small hole for ventilation. The other stages were enclosed in plastic containers which are commonly used to contain jelly dispensed in restaurants. These containers have a flange which is very useful if the container has to be taped in place. A single sheet of tissue was rolled into a loose wad and introduced with the beetles. The plastic cover was resealed with scotch tape, and the sides of the container were punctured with a sewing needle to permit free exchange of gases.

At the suggestion of Major Cloid Green, School of Aviation Medicine, Brooks Air Force Base, Texas, a number of eggs were affixed to two 2" × 3" pieces of graph paper (200 squares to the square inch) which were subsequently taped down on a track plate. The graph paper was covered with double gummed scotch tape, and one-quarter inch of the margin covered with masking tape to provide a lip by which the track plate could be taped to a metal bracket affixed to a rectangular plate firmly screwed to the outside of the container carrying the monkey. The eggs were sprinkled on the sticky scotch tape left exposed by the masking tape. After all the eggs were sprinkled on the two plates, the plates were tapped on the edges to dislodge any eggs loosely glued to the sticky tape. The eggs were again sprinkled, and the process repeated over and over until all the eggs remained attached after three taps. Of the 8,000+ eggs about 2,000 were lost through jarring of the capsule in flight or as it plunged into the ocean. Some eggs were lost through squashing. Of the remaining eggs, larvae emerged from more than 90 per cent. After the track plate was recovered, the graph paper was cut into squares 1/2 inch to the side. Each square was placed in a vial, making sure that the sticky surface between the eggs became covered with flour. Because of this precaution very few larvae died as a result of becoming stuck to the paper.

The track plates played a dual function, i.e., they recorded any hits of primary or secondary cosmic particles and also gave indication as to which eggs might possibly have been hit. Also, the plates were suspended over the plastic containers holding the adults, pupae and large larvae. Thus, if any of these stages were in the path of a cosmic particle, the track plate would record the fact, and breeding efforts could be concentrated on that group.

The William H. Miner Agricultural Research Institute

Naylor, Alfred F. Transferring beetles to fresh medium.

Dr. Monte Lloyd has suggested that when transferring to fresh medium, and when seed imagoes in moderate numbers are adequate, quick and dustless transfers are possible. Fold a narrow strip of paper to stiffen and

thrust it into the medium. Imagoes will crawl up on the paper from which they can be shaken loose into a fresh stock jar.

The University of Oklahoma

Schlager, G. Efficient, dust-free flour sifter. (Reference authorized)

A simple efficient method to sift flour was devised which would be useful for small Tribolium laboratories. It consists of a motor capable of turning at 50 rpm, a three-jaw chuck, a tripod, a 5-cup flour sifter (dime store variety), and a large jar over which the sifter fits fairly snugly. The motor is clamped on the tripod so that the chuck is level with the shaft of the sifter resting on the jar (the turning mechanism of the sifter can be easily modified into a straight shaft). Bolting cloth of the desired size can be stapled to the sieve in the sifter. With this mechanism there is no flour dust problem and approximately 50 pounds of flour are sifted each month by the University of Kansas Tribolium Laboratory.

The University of Kansas

McDonald, Daniel J. A population cage for Tribolium confusum.

Populations of T. confusum have been continuously maintained for over fourteen months in population cages with the following structural characteristics. Each cage consists of a 1-gallon stainless steel tank, 13.0 cm wide, 18.5 cm long, and 18 cm deep, with a 1 cm overhanging rim projecting outward around the top. A strip of 1/4-inch foam rubber weather stripping is applied to this rim, and a plywood cover is cut to fit over the top of the tank. A rectangular opening about 5 × 15 cm is cut in the top cover and over this a piece of fine mesh cloth is glued. Holes drilled through the cover and the rim are fitted with bolts and wing nuts for secure fastening. Each cage has eight removable food compartments, 4.5 cm wide, 6.5 cm long and 3.3 cm deep, made of do-it-yourself aluminum sheeting with circular perforations in it. Four hundred grams of flour-yeast medium is placed in the cage, which fills the cage up to the level of the compartment tops. The diagram below shows the arrangement of the compartments and the changing schedule.

A	D	E	C
F	H	B	G

Week	Schedule 1	2	3	4	5	6	7 etc.
Compartment	AB	C	D	EF	G	H	AB

Each compartment remains in the cage for six weeks. The beetles can move from compartment to compartment through the holes in the sides. The population reaches a maximum size of eight to ten thousand adults and a minimum of less than a thousand.

Dickinson College, Carlisle, Pa.

Lasley, Earl L. The incompletely recessive effect of the sex-linked gene, pygmy, on pupa weight in Tribolium castaneum. (Reference authorized)

Sokoloff, Lasley and Shrode (Canadian Journal of Genetics and Cytology, 1960) described a sex-linked gene in T. castaneum which reduces pupae weight about one-half. Data available at the time the paper was written pertained only to progeny of heterozygous females mated to pygmy males and are presented as matings one and two in the accompanying table. These results suggest that the presence of one pygmy gene causes some reduction in weight since the average pupa weight of heterozygous females was 0.186 mg less than that of their brothers. This is contrary to the knowledge that wild type female pupae weigh about 0.128 mg more than wild type males. The incompletely recessive effect of the pygmy gene was confirmed by subjecting female progeny of matings 3, 4, 5 and 6 to breeding tests.

Pupa Weight in Milligrams

		♂♂				♀♀					
		+/		py/		+/+		+/py		py/py	
	Mating	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.
1.	+/py × py/	26	2.732	20	1.326			27	2.510	20	1.362
2.	+/py × py/	29	2.706	34	1.397			31	2.555	25	1.417
3.	+/py × +/	21	2.583	19	1.168	13	2.765	17	2.445		
4.	+/py × +/	5	2.742	15	1.357	21	2.824	14	2.524		
5.	+/py × +/	27	2.564	18	1.163	20	2.746	11	2.476		
6.	+/py × +/	16	2.669	25	1.281	16	2.805	15	2.487		

The average difference (unweighted) between +/+ females and +/ males, 0.145 ± 0.045 mg, is in good agreement with the sex difference, 0.128, based on weights of thousands of wild type pupae. The average difference between +/+ females and +/py females, 0.302 ± 0.043 mg, confirms the suggestion that pupa weight is reduced for carrier females. Some carrier females were probably misclassified as noncarriers owing to the nature of the breeding test even though decisions were based on thirty or more progeny (not classified for sex) in almost all cases. Bias introduced in this way should not seriously affect the basic conclusion that the pygmy gene is incompletely recessive in its effect on pupa weight.

Although data are not presented, it should be pointed out that the standard deviations are proportional to mean weight for each genotype. Transformation to common logarithms is necessary to produce independence of variance and mean. It is concluded that the mode of action of background genes is multiplicative.

The William H. Miner Agricultural Research Institute

NEW MUTANTS

Prothoraxless (ptl). Lasley and Sokoloff. Spontaneous in several strains of T. castaneum derived from Chicago wild type and maintained in isolation. Autosomal, semi-dominant of variable expressivity overlapping wild type, and incomplete penetrance. Lethal in a homozygous state. Identifiable in the earliest larval instars. In the heterozygote the prothoracic segment in the larva or the prothorax in the pupa or adult, or the prothoracic legs may be affected: If only the prothorax is affected, the protergum may exhibit a deep groove at right angles to the midline, or it may have various indentations at one or the other anterior corners. In the extreme cases the protergum is almost completely absent, and only half of the prosternum remains. If the prothorax is not affected, either leg, both legs, or no legs may be affected. If they are affected, the tibia and the femur will be considerably shorter and thicker and sometimes the leg is paralyzed.

The homozygote mutant is more severely and uniformly affected. The prothorax is almost completely gone, leaving the "neck" exposed. Only a small portion of the prosternum remains, and to this is attached a pair of vestigial legs (in the larva, pupa or adult). Most of the homozygous mutant larvae pupate, but many pupae die before becoming imagoes. The adults walk poorly on four legs, the first pair being nonfunctional, and generally they remain on the surface of the flour, attesting to the importance of the first pair of legs in tunneling. The only stock of this mutant established failed after about three months. The stock must be maintained by selecting the heterozygote.

Droopy elytra (dre). Sokoloff and Lasley. Spontaneous in Chicago wild type T. castaneum. Autosomal recessive with poor penetrance overlapping in expression with wild type so that only 10 per cent of F_2 progeny exhibit the character. Mutants mated inter se will produce phenotypically wild type progeny. Elytra fail to meet at the midline over the whole length of the abdomen and present a variable degree of droopiness. Beetles may die at an early age because of dehydration.

Fused antennal segments (fas). Sokoloff. Spontaneous in Chicago wild type T. castaneum stock. Autosomal recessive. Penetrance poor. The ninth and tenth distal segments of one or both antennae fused. Frequency in a stock can be increased by selection so nearly all beetles exhibit the character, but on outcrossing the frequency of the mutant drops, in some crosses to a very low level (3-5 per cent).

Microphthalmic (Mo). Sokoloff. Appeared spontaneously in a mixed culture of wild type and pearl T. castaneum. Autosomal dominant. Variable expressivity and incomplete penetrance in the minority of individuals examined, the heterozygous Mo/+ beetles resembling the wild type. Facet size may be reduced if there is no reduction in number, and the eye is displaced ventrally. If the facets are reduced in number, the dorsal facets are most often eliminated. Less often the dorsal and lateral portions of the eye are eliminated, the eye being confined to the ventral surface of the head. In its extreme form the cranium behind the genae is reduced, and the beetle has a microcephalic appearance. In some of these beetles the eye may consist of three or four facets lying on the ventral surface of the head. The head in these beetles is usually retracted into the prothorax up to the level of the eyes. Matings with pearl (to which Mo is not linked) reveal that the ocular diaphragm is present, even when the eye is split into two widely separated components, as it has happened in one case.

Warped elytra (we). Sokoloff. Spontaneous in a fused antennal segments stock of T. castaneum. Autosomal recessive. Poor penetrance. Generally the elytra meet producing a continuous line from the posterior tips to just posterior to the mesonotal scutellum. However, in we the elytra are displaced either to the right or to the left of the dorsal midline of the abdomen, one or both elytra are lifted away from the abdomen, and a shallow wave produced by both elytra may be present. Frequency of beetles expressing the character can be increased by selection, the frequency dropping to a low level on outcrossing. Adult viability reduced, as the dorsal part of the abdomen is exposed permitting evaporation of moisture and accumulation of flour grains.

Deformed legs (dfl). Sokoloff. Autosomal recessive, penetrance poor. Discovered as a female pupa of a Chicago wild type T. castaneum stock which, in the adult, had the following abnormalities: right and left foreleg tibia short and curved outward. Left middle leg normal; right middle leg with short tibia and thin femur. Right hind leg normal; left with tibia curved inwardly or posteriorly. F_2 progeny of this female reveal that the expressivity of this gene is quite variable. Any leg may be affected, usually one of a pair only, and the tibia is the podomere most frequently affected.

Split elytra (spl). Lasley. "Split" was isolated from a mass culture of Tribolium castaneum. This mutation is located on the "Jet" chromosome and inherited as an autosomal recessive. The elytra of beetles which are homozygous for "split" are usually separated at the posterior extreme. The femurs are also somewhat shortened and broadened. Phenotypic expression is quite variable and overlaps wild type so that about 80 per cent of genetically split beetles exhibit a recognizable separation of the elytra. In some genic backgrounds, both the elytra

and legs may be shortened. A majority of the split pupae can be identified since the elytra tend to separate and curve away from the abdomen. Mortality is high among emerging imagoes and productive life is reduced markedly in the adult female.

Pink (p^{Pk}). Lasley. This eye mutation originated in a carefully pedigreed stock of Tribolium castaneum derived from Chicago wild type. Pink is completely recessive to wild type and is allelic to pearl. The pink eye phenotype strongly resembles the pearl eye phenotype; the two are practically indistinguishable in newly emerged imagoes. The center portion of the eye which is devoid of pigment in pearl imagoes develops a pinkish tinge in pink imagoes as they become older. Imagoes which are heterozygous for both pearl and pink also develop the pink phentotype; therefore, pink is dominant to pearl in the allelic series. Fedundity and viability are apparently not affected by the presence of one or two genes causing the pink eye phenotype.

Squint (sq). Bywaters. In examining a mixed culture of T. castaneum and T. confusum, six specimen of T. castaneum, three males and three females, were found in which the eyes appeared to be squinting. Closer examination revealed no facets or ommatidia. The pigmented ocular diaphragm appears broadened and when viewed from the ventral surface appears as a rough triangle with the apex forward. This diaphragm extends around the head, constricting at the side and expanding on the dorsal surface in another rough triangle, smaller than the ventral one. The inner edges of the diaphragm come very close to each other producing the squint appearance.

Beetles possessing this abnormality have difficulty righting themselves when placed on their backs, are much slower in their movements than wild type individuals, and appear to be blind. The condition can be detected in pupae about to emerge by a crescent configuration in the eye area.

Three single pair matings were made from the specimen found, but only one produced offspring. Most were wild type as the female had been mated when found. Several small cultures were set up, and eventually a sizable stock of homozygous squint was developed. Difficulty was experienced in obtaining homozygous squint that would reproduce.

Matings involving wild type and pearl with squint have led to the conclusions that the lowered reproductive performance of squint is due to a marked decrease in production and viability of eggs, the gene is a semi-lethal autosomal recessive which is epistatic to pearl, and that pearl and squint are on separate chromosomes.

Stocks are available to those who wish them.

Red (r). Sokoloff. 1 An eye color mutation in Latheticus oryzae similar to that in T. castaneum (q.v.). Appeared in a cross of two pearl heterozygotes. Complete penetrance, although with variable expressivity, since the pigment may darken as the beetle ages. Pearl is epistatic to red.

Truncated elytra (te). Sokoloff. Found as pupae in a wild type stock of Latheticus oryzae. The mutant resembles the truncated elytra condition described for T. castaneum. The data so far are meager, but the mutation is probably, at worst, a semi-lethal, in contrast to the lethal character of this gene in T. castaneum. Matings are under way to determine the crossover values.

TRIBOLIUM STOCKS

Dickinson College, Carlisle, Pennsylvania

1959

Number	Species	Genotype	Source	Date
			♀ from feed bin -	
1	Confusum	Wild	Columbia	12/20/55
2	"	"	"	"
4	"	"	"	1/28/56
5	"	"	"	"
6	"	"	"	"
			♀ from feed bin -	
3	Castaneum	Wild	Columbia	12/20/55
7	"	"	"	1/28/56
9	"	"	"	"
10	Confusum	Wild	Smith - Canada	7/30/56
11	"	Black	"	"
13	"	Ebony	Park - Chicago	5/31/57
17	"	Wild	"	8/28/56
18	Castaneum	"	"	"
19	Castaneum	Paddle	Park-Chicago	2/24/57
20	"	Pearl	"	"
21	Confusum	Wild	Stanley - McGill	"
23	Castaneum	"	"	6/19/57
24	"	Black	"	"
25	Confusum	Split	McDonald	7/24/56
26	"	Striped	"	"
27	"	Striped-Black	"	8/7/57
28	"	Split - Black	"	"
29	"	Split - Ebony	"	"

Also: In confusum

One stock which has rough, deformed or blistered elytra. We call it blistered and are trying to work out inheritance.

One stock of an eye mutation, similar to "pearl" but not as obvious. Probably a sex-linked recessive. Found about three months ago in our number 1 wild strain of confusum.

Daniel J. McDonald

Population Genetics Institute
Purdue University

Foundation A - wild type base population formed without selection from a broad genetic base and maintained with a minimum of inbreeding.

Foundation B - base population unrelated to Foundation A and marked with McGill black (bb).

Foundation P - base population unrelated to A or B and marked with the pearl mutant (pp).

15 wild type laboratory stocks.

10 unrelated inbred lines with more than 50 per cent inbreeding.

Mutant lines - pd/pd (paddle); b/b (McGill black); e/e (Purdue ebony); p/p (pearl); pd/pd p/p; pd/pd e/e; p/p e/e; b/b e/e; pd/pd p/p e/e.

A. E. Bell

University of Kansas

T. castaneum

Wild type	Source
1. Kansas	University of Kansas
2. Minnesota	Purdue
3. Georgia	Purdue
4. "foundation" (outbred)	Purdue
5. CS - 4	Purdue
Sex-linked	
1. paddle	University of Chicago

University of Kansas--continued

T. castaneum--continued

Autosomal	Source
1. pearl	University of Chicago
2. black	Purdue

T. confusum

Wild type	
1. Kansas	University of Kansas
2. Chicago	University of Chicago

Autosomal	
1. ebony	University of Chicago

G. Schlager

Department of Genetics
University of California

I. T. castaneum

1. Synthetic. Prepared by incorporating the black mutant (no. 2) into each of several wild and laboratory stocks and combining them into a single population. Maintained as the laboratory standard in competition experiments.

2. Black mutant obtained from Purdue University.

3-10. Stocks entering the synthetic population, originating in California (two), Kansas, Georgia, Illinois, Washington, Indiana and Minnesota.

II. T. confusum.

11. Synthetic. Prepared by combining a series of wild and laboratory stocks into a single population. Maintained as the laboratory standard in competition experiments.

12. Black mutant (see note, page 14, of this publication).

13-18. Stocks entering the synthetic population, originating in California, Indiana, Kansas, Illinois, Washington, Minnesota.

III. T. destructor.

19. Stock from the University of Chicago.

IV. Gnathocerus cornutus.

20. Wild stock collected in California

* * *

In addition to these we are preparing brother × sister inbred lines of both T. castaneum and T. confusum, and are extracting various lines of these species from competition experiments.

I. Michael Lerner

The William H. Miner Agricultural
Research Institute

WILD TYPE

T. confusum
Chicago

T. castaneum
Chicago
Georgia
McGill
Texas
Virginia

Latheticus oryzae
Chicago

MUTANT	Symbol	Source
T. castaneum		
Sex linked		
paddle	pd	U. of Chicago
miniature appendaged	ma	Miner Institute
pygmy	py	Miner Institute
red	r	"
spotted	sp	"
truncated elytra	te	"
Autosomal		
pearl	p	U. of Chicago
Chicago black*	Cb	"
McGill black*	McGb	McGill University

MUTANT--continued	Symbol	Source
T. castaneum--continued		
Autosomal--continued		
synthetic black	Cb/McGb	Miner Institute
jet	j	U. of Chicago
prothoraxless*	ptl	Miner Institute
Microphthalmic	Mo	"
fused antennal segments	fas	"
warped elytra	we	"
deformed legs	dfl	"
squint	sq	"
pink	p^{Pk}	"
split	spl	"
Multigenic		
pd py		
pd sp		
pd r		
pd py sp		
r py		
j spl		
Multichromosomal		
b p		
b p sp		
j p		
Mo p		
b ma pd		
Latheticus oryzae		
Sex linked		
red	r	Miner Institute
truncated elytra	te	"
Autosomal		
pearl	p	U. of Chicago

*Semi-dominant

A. Sokoloff

Department of Zoology
University of Chicago

Below is a partial list of stocks maintained in the laboratory of Dr. Thomas Park. Those that are available may be obtained by properly qualified workers. (Department of Zoology, University of Chicago, Chicago 37, Illinois)

1. Tribolium castaneum (Herbst) "Chicago." The Chicago laboratory strain.
2. T. castaneum, "Paddle." A sex-linked recessive antennal mutant.
3. T. castaneum, "Pearl." An autosomal recessive eye mutant.
4. T. confusum duVal, "Chicago." The Chicago laboratory strain.
5. T. confusum, "Ebony." An autosomal recessive body color mutant.

In addition to these there is a body color mutant of Tribolium castaneum that may be similar to ebony in T. confusum. Apparently this is an autosomal recessive with complete penetrance. It is known as "jet" and the phenotype shows a uniform black body color. Publication about this mutant is being withheld until further genetical analysis is completed. It is not yet available for distribution.

Thomas Park

Sokoloff, A. Aberrations in Tribolium and Latheticus oryzae.

The following abnormalities have been observed by the staff of the Miner Institute.

The symbols used are: NH = Not genetic; U = genetics unknown; L = Latheticus; T.C. = Tribolium castaneum; T.cf. = T. confusum.

Larval abnormalities

a. Split prothoracic tergite. NH, L and T.C. If present, normality is not restored in subsequent larval instars, the pupa or the adult. Tergite split along midline, each half may have a deep depression. May resemble a bow tie. Split in larval sternite must occur, since adults with this condition have been found.

b. "Bar" eye. U, T.C. Larvae, instead of bearing ocelli, had a nearly crescent-shaped eye. Behavior also affected--the larvae jerked constantly as if in response to a poking stimulus. Larvae failed to pupate.

c. Wing buds. NH, T.C. Results from larvae being subjected to high concentrations of quinones emitted by adults (see pages 3 and 6).

d. Incomplete abdominal sternites. U, T.C.

e. Vestigal forelegs. T.C. Gene responsible is ptl (prothorax-less). See page 22.

Pupal abnormalities

a. Fused cerci. NH, L and T.C. The position of the cerci is subject to much variation: they may originate wide apart or close together behind the genital pit or genital lobes. The extreme deviations

are those in which the cerci have fused into a single medial cercus. This medial cercus may have a bifurcated tip attesting to its dual origin, or may be single.

b. Cerciless. NH, T.C. No cerci at all.

c. Bar eye. U, T.C. Pupae failed to hatch.

d. Split thorax. NH, T.C. (Description in adult.)

Aberrations in the adult

a. Antennal abnormalities.

1. Antennaless. U, T.C. Antennae completely missing.

2. Reduced antennae. U, T.C. Antennae made up of 2-11 antennal segments. Sometimes one side affected more than the other. If normal number of segments is present, the whole antenna may be reduced in size.

3. Elongated antennae. U, T.C. The whole antenna appears as if the segments had been stretched. The antennae may appear very pale.

4. Fused antennal segments (fas). T.C. Recessive, overlapping wild type. Frequency of beetles showing the character increases with selection, but percentage drops on mating to wild type. Involves fusion of the 9th and 10th antennal segments in one antenna, both antennae or neither.

5. Bifurcated antennae. NH, T.C. Usually unilateral. Variable: from a two branched antenna (each consisting of 6 or 7 segments) to a normal antenna with a branch consisting of one segment.

6. Serrated antenna. NH, T.C. Fusion of 3 or 4 antennal segments (usually including the terminal ones) on one side of the antenna. There may be a reduction in the number of segments.

7. Fuscous antennae. U, T.C. Antennae look dark grey or black. Condition may be caused by the high concentrations of the quinone released by beetles in crowded vials without food.

b. Eye abnormalities.

1. Red (r). L and T.C. Sex-linked recessive. (See page 17.)

2. Squint (sq). Autosomal recessive, semilethal. All eye structures distal to the ocular diaphragm missing. Beetles are blind. (see page 24.)

3. Microphthalmic (Mo). Autosomal dominant. (See page 23.)

4. Beaded. Looks like squint, but pearl facets are present. Eye somewhat reduced in size. Blind?

5. Pink (p^{Pk}). T.C. Allelic to pearl. (See page 24.)

6. Reduced eye. U, L. The facets don't reach the outer border of the eye--as if outer ring of facets were missing.

7. Eyeless. U, L. One eye completely missing. Died before leaving progeny.

8. Shifted eye. U, T.C. One eye shifted mid-dorsally.

9. Bulging eye. U, T.C. One eye asymmetrical, bulging.

10. "Somatic" eye mutation (?) NH, L and T.C. In 4 T.C. heterozygous for pearl; in one L. heterozygous for pearl. Pearl area covering the whole, or part of one eye, or both eyes. One L and 1 T.C. had one eye pearl and the other eye black. The other somatic mutations in T.C. involved smaller pearl areas only in one or both eyes: in one case the ventral half of one eye was pearl, the dorsal half black; in one case 6 facets of one eye and 8 facets of the other eye were pearl. In two cases 8 facets of one eye and 12 facets of the other eye were pearl. The pearl gene is yet to be introduced in a different genotypic background to establish conclusively that what is being observed is a somatic mutation.

11. Triophthalmic. U, T.C. One pair of normal eyes, plus a third eye on top of the head consisting of about 12-14 ommatidia. The same beetle had a single horn in the midline just above the mandibles.

c. Abnormalities of the prothorax.

1. Split thorax (or hemithorax). NH, T.C. Prothorax is split along midline, sometimes resembling a bow tie. Usually causes ventral sternite to bulge downward. Condition may occur on sternite or tergite of prothorax.

2. Ventral abdominal split. NH, T.C. Involving meso- and metathorax.

3. Prothoraxless (ptl). T.C. Semidominant, lethal in homozygous condition (see page 22).

4. Bumps on thorax. T.C. Beetles look as if something had taken a bite out of the thorax, which otherwise looks normal. Heterozygote of mutation ptl. (Page 22)

5. Transverse prothoracic groove. U, L and T.C.

d. Abnormalities of the elytra.

1. Warped elytra (we). T.C. Autosomal recessive overlapping wild type. (See page 23.)

2. Wrinkled elytra. NH, T.C. May be caused by dehydration during metamorphosis from pupa to imago.

3. Miniature-appendaged (ma). T.C. Elytra and membranous wings about 2/3 normal length and legs affected. Sex-linked, semi-lethal, recessive. (See page 17 and 33.)

4. Split (spl). T.C. Tips of elytra diverge, exposing posterior one-third of abdomen. Recessive. (See page 14.)

5. Short elytra (se). U, T.C. Elytra are abbreviated, exposing the tip of the abdomen.

6. Spotted (sp.). T.C. Sex-linked recessive. (See page 17.)

7. Blistered elytra. U, L, T.C. and T.cf. A blister in one or both elytra. Condition semi-lethal since it permits rapid dehydration of the adult.

8. Curly elytra. U, T.C. About four waves on elytra.

9. Vestigial elytra. U, T.C. One elytron partially or completely missing. May be blistered if present.

10. Truncated elytra (te). L and T.C. Sex-linked recessive in L, sex-linked lethal in T.C. (See page 17.)

11. Droopy wings. U, T.C. Elytra diverge, exposing the unsclerotized abdomen.

12. Wavy elytra. U, T.cf. Elytra have large blisters and several waves.

e. Abnormalities of the membranous wings.

1. Notches. U, T.C.

f. Abnormalities of the legs.

1. Miniature-appendaged (ma). T.C. Podomeres of all legs shorter and thicker, elytra and membranous wings shorter. Sex-linked recessive. (See pages 17 and 32.)

2. Legs vestigial (ptl). T.C. One of the effects of the prothoraxless gene ptl. (See page 22.)

3. Deformed legs (dfl). T.C. Autosomal recessive overlapping wild type. Deformation may involve the tibia of one or both members of all pairs of legs, but the mesothoracic legs are most frequently affected. The extreme case, hatched from the pupa where abnormality was first detected, had following appearance: R and L foreleg with short tibia curving outwardly. Middle legs: R tibia short, femur thinner. L leg normal. Hind legs: R normal, L curved inwardly and posteriorly.

4. Shifted mid-legs. U, T.C. The second pair of legs is attached to the prothorax, just behind the forelegs, instead of attaching to the mesothorax. Coxa of one or both forelegs misplaced laterally, away from prosternum. Prosternum missing. Mesosternum seems absent.

5. Bow legs. U, T.C. Due to shortening and curving of the femur of forelegs.

6. Peg legs. U, T.C. General shortening of all legs. Tibia missing or almost so. Tarsi fail to develop. Claws present, attached to tibia or to femur.

7. Displaced femur. U, T.C. All legs affected: coxae normally placed with long trochanters directed ventrally. Rest of leg attached by proximal end of femur, laterally to the coxa, to its corresponding sternite.

g. Abnormalities of the abdomen.

1. Fused sternites. NH, T.C. Variable. Half of one sternite may be missing on one side, in which case adjoining sternites are broader to fill the space, or the abdomen may curve toward the side where the sternite is missing. Parts of sternites may be missing or fused in more than one segment.

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