Literature cited

Chapman, R. N. 1933. The cause of fluctuations of populations of insects. Proc. Hawaii Ent. Soc. 8:279-297.

Ghent, A. W. 1963. Studies of behavior of the Tribolium flour beetles. I. Contrasting responses of T. castaneum and T. confusum to fresh and conditioned flours. Ecology 44:269-283.

Howe, R. W. 1956. The effect of temperature and humidity on the rate of development and mortality of Tribolium castaneum (Herbst) (Coleoptera, Tenebrionidae). Ann. Appl. Biol. 44:356-368.

Landahl, H. D. 1955. A mathematical model for the temporal pattern of a population structure, with particular reference to the flour beetle. Bull. Math. Biophysics 17:63-140.

Lloyd, M. 1957. Productivity, cannibalism, and population size in two laboratory strains of Tribolium castaneum. Unpublished Ph.D. thesis, University of Chicago.

_____. 1959. Population fitness and population density. Fifteenth Int. Congr. Zool., London, 1958: 796-797.

_____, and Park, T. 1962. Mortality resulting from interactions between adult flour beetles in laboratory cultures. Physiol. Zool. 35:330-347.

Park, T. 1934. Observations on the general biology of the flour beetle, Tribolium confusum Duval. Quart. Rev. Biol. 9:36-54.

_____. 1948. Experimental studies of interspecies competition. I. Competition between populations of flour beetles, Tribolium confusum Duval and Tribolium castaneum Herbst. Ecol. Monogr. 18:265-308.

_____. 1954. _____. II. Temperature, humidity, and competition in two species of Tribolium. Physiol. Zool. 27:177-238.

_____. 1957. _____. III. Relation of initial species proportion to competitive outcome in populations of Tribolium. Physiol. Zool. 30:22-40.

_____, and Frank, M. Burton. 1948. The fecundity and development of the flour beetles Tribolium confusum and Tribolium castaneum at three constant temperatures. Ecology 29:368-374.

_____, Leslie, P. H., and Mertz, D. B. 1964. Genetic strains and competition in populations of Tribolium. Physiol. Zool. 37:97-162.

_____, and Woollcott, N. 1937. Studies in population physiology. VII. The relation of environmental conditioning to the decline of Tribolium confusum populations. Physiol. Zool. 10:197-211.

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

Saunders, J. P., and Krueger, H. 1957. A rapid method for counting larvae of the confused flour beetle. J. Econ. Ent. 50:693.

Sonleitner, F. J. 1961. Factors affecting egg cannibalism and fecundity in populations of adult Tribolium castaneum Herbst. Physiol. Zool. 34:233-255.

Strawbridge, D. W. 1953. Population dynamics of the flour beetle Tribolium castaneum Herbst. Unpublished. Ph.D. thesis, University of Chicago.

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Feeding responses by stored-products insects to chemical stimuli from cereal products and microorganisms

Several workers have observed preferential feeding by stored-products insects confronted with a choice of several cereal products. Others have observed certain grain-infesting insects feeding preferentially on the germ end of wheat kernels. These observations suggest that insect feeding behavior has a chemosensory basis. This hypothesis was supported by recent findings that certain components in brewers' yeast, wheat flour, bran, and germ stimulated flour beetles to aggregate and feed on pith discs impregnated with these substances. In these experiments the number of insects aggregating in a given unit of time on treated and untreated discs in a "choice" type of experimental design, and the amount of feeding on discs were used as criteria of response. The amount of feeding was determined photometrically by measuring the difference in light transmitted through pith discs before and after a 24-hour period of exposure to 25 test insects.

Active components were defined as arrestants, feeding stimulants, or both depending on the types of behavior evoked by them. Amino compounds and sugars in yeast, and fatty acids in wheat germ elicited strong feeding responses from flour beetles. An ether-soluble fraction of yeast deterred feeding. Some active fractions were more stimulative in combination with 0.1M sucrose than either the sucrose or the fraction tested singly. Beetles fed but did not aggregate on sucrose-treated discs.

Experiments are currently in progress to determine the role of fungi in the feeding behavior of Tribolium confusum and Cryptolestes ferrugineus. Certain storage fungi associated with stored grain, and aqueous extracts of these fungi induce aggregating and feeding responses. Some fungi apparently deter feeding while others are chemotactically inert. Chemosensory

stimuli from fungi may influence the distribution of certain insects in stored grain.

If arrestants and feeding stimulants can be identified and produced commercially they may have useful applications. Food lures combined with toxic materials in baits may offer a promising approach to selective control of undesirable insects. Furthermore it may be possible to use these compounds to detect hidden insect infestations in granaries and food warehouses.

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*Activity differences in black and wild type strains of Tribolium confusum

During the course of numerous experiments with a mutant strain (probably McGill black) of Tribolium confusum, the impression was gained that McGb individuals were usually more "active" than wild type, moving about more rapidly and tending to stay on the surface of flour medium. Since inherited behavioral differences can influence fitness, a pilot experiment was designed to measure activity. A glass tube with an inside diameter of 13 mm. is bent 90° at one end and a mark made on the other end 45 cm. from the angle. Flour-yeast medium is poured in the other end, and with constant tapping 35 gms. will fill the tube to the 45 cm. mark. If the tube is then held horizontally, the plug can be removed with little loss of flour. Beetles can be put in the straight end which is then plugged, and the tube is laid horizontally on a rack in the incubator (30°C. and 50 - 60% R.H.) with the bent end projecting downward into a small beaker. When a beetle works its way through the 45 cm. column of flour it topples into the beaker and is trapped. Care must be exercised in filling the tube since small variations in packing and flourless gaps in the column probably influence the insects activity. Unmated males and females of McGb strain about three weeks old were tested separately by placing thirty adults in the tube and observing the number found in the beaker at the opposite end after various intervals of time. Some of the results of two experiments, each consisting of eight tubes, four male and four female, are given in the table. The only significant difference is between males and females ($F = 5.87$, $p < .05$). A more marked difference is found in the wild strain. The results of two experiments are given in the table and now females are clearly less active than males ($F = 146.25$, $P < .005$). Finally, when wild and mutant are compared (experiments A and C) there is a significant interaction ($F = 9.54$, $P < .01$) which reflects the difference in activity of the mutant and wild type females. Other experimental data suggest that mixing the sexes depresses the activity of the males. Also, younger beetles seem less active than older ones.

	Black Strain				Wild Strain			
Experiment	A		B		C		D	
Time	117		114		117		124	
Sex	M	F	M	F	M	F	M	F
	26	19	28	20	30	3	30	1
	21	19	28	21	18	4	20	4
Tube	27	9	28	26	25	4	29	3
	29	19	30	28	27	3	25	1
	103	66	114	95	100	14	104	9

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*Effects of sub-optimal temperatures on Tribolium castaneum

Pupae of less than 24 hours of age were exposed to constant temperatures of 10, 15, 17.5, 20, 25, 30, 35 and 40°C. Some pupae completed development at as low as 17.5°C, at which temperature workers have reported that both Tribolium castaneum and T. confusum pupae failed to complete development.

Pupae developed at sub-optimal temperatures of 17.5 and 20°C. resulted in deformed adults, visibly on the elytra. Pairing of deformed adults and deformed and normal adults produced fewer eggs which showed lower fertility.

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In nutritional studies with Tribolium confusum (Ebony-Chicago), a behavioral phenomenon was observed similar to that reported by Dawson in TIB 7 (p. 50). A comparison was being made of the survival of T. confusum adults on whole wheat flour versus a defined medium (Fraenkel et al., 1950). Ten adult T. confusum were placed in vials (75 × 23 mm) containing 2 grams of either whole wheat flour or Fraenkel's medium, and replicated 10 times. The vials were held at 24°C. and 35% R.H. for the duration of the observation. The T. confusum immediately burrowed into the whole wheat flour and remained there. When shaken to the surface or sifted out and replaced on the surface, they rapidly burrowed into the flour. Very few adults placed in the defined medium burrowed into it, most of them stayed on the surface

in constant motion. When shaken into the medium, they moved to the surface within 30 minutes or less. This condition remained constant for the 35-day duration of the observation. At the end of this time 25% of the adults in Fraenkel's medium were dead while 10% were dead in the whole wheat flour. Physically, the defined medium was somewhat coarser than the flour; both passed a 0.42 mm screen but only 50% of the defined medium passed a 0.21 mm screen while 100% of the flour passed through.

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*A new approach to data processing in selection experiments

The rapid development of high speed computers has been to the advantage of biologists. Data processing is fast, efficient and allows a complete analysis.

We have chosen to present a computer program developed in order to analyze genetic data in a selection experiment using Tribolium castaneum as the experimental organism. The program is written in the Fortran IV computer language and executed on the IBM 7094 with the IBM 1401 as input-output device.

The selection experiment is designed to compare the efficiency of tandem selection versus restricted index selection. The mating system is single pair matings. Five individuals are measured for each pair mating and information on the following traits is recorded: number of larvae per pair mating, 13 day larval weight, developmental time, and pupal weight within 24 hours after pupation. (Daily gain is also computed as a function of the last three traits.) There are seven populations each with fifteen pair matings. The experiment was replicated three times in each generation and has been run for eight generations of selection.

The program provides for an extensive analysis and estimation of population parameters. This is accomplished in a very simple fashion by generating four types of 5×5 cross product matrices, each type including four subtypes, and four 5×1 column vectors. All analyses follow from this full set of matrices.

The output includes the following parameters which are estimated by combining respective elements in the above matrices for a given set of traits:

1. Population mean

2. Standard error of population mean

3. One-way analysis of variance and covariance

4. Genetic variance - covariance matrix

5. Environmental variance - covariance matrix

6. Phenotypic variance - covariance matrix

7. Heritability estimates (from variance components and all possible regressions of offspring on parent or mid-parent value in addition to realized heritability)

8. Standard errors of the above heritability estimates

9. Genetic correlations and their associated standard error (from variance - covariance components and using Hazel's method--Genetics 28:1943)

10. Pooled estimates for parts 4 - 9.

11. Standardized and unstandardized selection differentials

12. Genetic gain

13. Restricted index selection

14. Relative efficiency of a given selection method

15. Correlated response to selection

The data can be presented in various arrangements on the page. A summary is available at the end of each population for within replication analysis and at the end of each generation for pooled estimates. The program will be further extended as soon as the seven dimension option is released by IBM.

This program is available upon request and can be easily modified to suit experiments of this type. The method allows extension of the "one way" to the "hierarchal" classification.

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*Methods of egg separation in Tribolium castaneum and their effect on hatchability

Two methods of separating eggs from flour medium are employed in our laboratory. One method involves hand sifting using a sifter with a No. 3 silk bolting cloth. The second method involves a metal sifter with the same sieve size placed over a vacuum system.

A 2^4 factorial experiment (replications, populations, methods and days) was set to determine the effect of hand method versus machine method used for separating 24 hours old eggs. The system of mating is single pair matings with ten observations per each cell.

Analyses have indicated that both methods give equal results when considering number of eggs laid, number of eggs hatched and per cent hatchability. Considering the time saved the machine method is to an advantage.

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*Pleiotropic effects of mutants affecting eye color in seven species of Coleoptera

Since the eye-color mutations in several flies, moths and cockroaches produce a lightening in the color of the malpighian tubules, testes and brain, several beetles homozygous for eye-color mutations have been examined for similar pleiotropic effects. Adult Dermestes maculatus (pearl), Carpophilus dimidiatus (pearl), Gnathocerus cornutus (pearl), Oryzaephilus surinamensis (pearl), Tribolium confusum (pearl), Tribolium castaneum (pearl and red) and Cryptolestes turcicus (red) were found to have pale or transparent malpighian tubules. These contrasted with the respective wild-types, in all of which the malpighian tubules were dark brown or black. The differences in the "pearl" and "normal" T. confusum were particularly marked and in this species the malpighian tubules could be seen in the living beetles through the abdominal tergites after removal of the elytra and wings. No marked differences in testes color were seen.

Studies on other mutants and feeding experiments with tryptophan derivatives are in progress.

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Development of Tribolium castaneum and T. confusum on seed-borne fungi

The life-history of T. castaneum and T. confusum were studied in the laboratory on 10 species of fungi isolated from deteriorating stored wheat and grown on potato-sucrose agar slants at 30° ± 1°C. and 70 ± 3% relative humidity, as a part of a project on the interrelation of insects, mites and fungi to deterioration of stored grain in Western Canada. T. castaneum Herbst. could complete its life cycle from egglaying to adult emergence, on Alternaria, Curvularia, Fusarium, Helminthosporium, Hormodendrum, Mucor, Nigrospora, Scopulariopsis, Stemphylium in 22 to 141 days. When reared on fungus-free agar slants, the insects died in the first instar.

T. confusum completed its life cycle, from egg to adult, on Absidia, Alternaria, Curvularia, Hormodendrum, Mucor, Nigrospora, and Stemphylium in 27 to 108 days. T. confusum did not develop on agar slant controls.

None of the Tribolium species developed on Penicillium or Aspergillus which are most commonly found in deteriorating stored grain. The results of the studies will be published in detail elsewhere.

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*Pleiotropic effects of sex-linked and autosomal genes in Tribolium

Among the mutants already described for T. castaneum it has been noted that Fused tarsi and antennae (Fta), an autosomal dominant with recessive lethal effects and the various autosomal dominant, semidominant or incompletely recessive alleles at the Sa locus (Sa and Sa-1 to Sa-4 and sa, sa-1 and sa-2) have effects on various appendages: Fta reduces the antennameres to as few as two or three, and the tarsus to two, more often to one tarsomere in all pairs of legs and causes the elytra to be split. The dominant alleles at the Sa locus cause mild to severe fusions of the antennae, these appendages often appearing curved, and some alleles (predominantly Sa-1 and Sa-2) cause the tibiae to be formed in a gnarled, knotted or twisted condition. The original Sa and other alleles, in strong expression, may also cause the proximal tarsomeres to fuse with the tibia. The sa alleles affect the antennae in a manner similar to that of Sa, but the effect on the legs is primarily on the femur (this podomere becoming short and thick), although not rarely the tibia may be formed in a curved shape.

The purpose of this note is to draw the attention to the fact that other genes (one already known for quite some time) also have pleiotropic effects.

Two sex-linked genes are known to affect the antennae differentially in the two sexes: Park and Frank (1951) described paddle (pd), a recessive which causes a fusion of the club segments. In the female (pd) the effect (with few exceptions which may resemble the male in expression) is confined to the antennal club, which may be variously fused. Usually fusion involves segments 9-10 or 10-11, but both antennae need to be examined since one may exhibit fusions and the other may be free of them. In the male the club segments are fused into a solid mass resembling a paddle; in addition, there is a loss of one or more funicular segments.

Park and Frank (l.c.) apparently were unaware that pd has effects on structures other than the antennae. The writer has recently found that the tarsus (primarily of the middle legs, but the tarsi of other legs may show the same defect) is variously modified: The penultimate tarsomere may be reduced in size and displaced off the longitudinal axis, lying more or less under the preceding tarsomere or the last two tarsomeres may be fused with few remaining hairs remaining as evidence of segmentation; or the segment may be completely missing. More proximal tarsomeres may also be affected. As in the case of the antennae, the effect on the tarsi is sex-influenced, being more frequent and pronounced in males. In a sample of the original pd strain recently provided by Dr. Thomas Park, University of Chicago, 83/243 males (34.1 per cent) and 4/126 females (3.2 per cent) exhibited tarsal fusions, malformations or elimination of segments. A sample of the abnormalities observed in the males can be seen in Table 1.

The four females found to have abnormal tarsi had these abnormalities:

1. Fourth tarsal segment of second right leg and third of left hind leg reduced.

2. Fusions of segments 3-4 of right hind leg; fourth of middle left reduced.

3. Symmetrical reduction of fourth tarsal segment of both middle legs.

4. Right legs normal; fourth segment of foreleg and third segment of hind leg reduced.

The pd gene also affects productivity: it has been found to be more productive (Phillips and McDonald, 1958) or less productive (Bartlett, Englert and Blair, 1962) than wild type.

Dawson (1964, TIB 7:39) gave a brief description of serrate (ser) and in preliminary studies noted that this sex-linked recessive expressed itself differently in the antennae of the two sexes. The writer, trying to establish further the location of ser noted that the tarsi were also affected,

and indeed the identification of this mutant is made more certain if the tarsi are examined since in certain cases the antennae appear entirely normal. In a survey of 50 females over 50 per cent showed antennal fusions in the funicle or in the club, 18 per cent showed fusions in both the funicle and club and 30 per cent showed no antennal fusions whatsoever (both antennae having been scored). Of 50 males examined, one male showed no antennal fusions; 80 per cent showed fusions of the club and funicle and the remaining 14 per cent were about equally divided: six per cent showed fusions of the funicle, eight per cent of the club. The same females, scored for tarsal fusions, showed a reduction or fusion of tarsi in at least one of the middle legs in at least 56 per cent of the cases; 30 per cent showed reductions or fusions of the middle and hind legs; the remaining 14 per cent had reductions in tarsomeres of at least one member of the front and middle legs, but only in two females did the effect extend to the hind legs. In the males, almost half had a reduction in tarsomeres of at least one member of all three pairs of legs. In the remaining beetles, the tarsus of the first pair of legs was not affected, but the tarsi of the middle legs were reduced by one or two segments; in only nine out of the 50 males examined there were no visible deformities of the hind legs. Thus, for both sexes, the tarsi of the middle legs provide the best criterion for classification of ser. A sample of 10 males and 10 females from the established stock were scored for antennal fusions and the numbers of tarsi. The results are given in Tables 2 and 3, respectively.

It is of interest to note that ser and pd are not allelic, but the two genes are very closely linked, since ser is located less than one unit to the right of r (unpublished data of P. S. Dawson) and pd is approximately one unit to the right of r.

Among the autosomal genes known for T. castaneum, two alleles of antennapedia (ap and ap^D) serve to identify linkage group VIII. Of the two alleles, perhaps ap^D is more strongly expressed (although ap has not been examined as often as ap^D) with the following parts of the body of the beetle being affected: The distal nine segments of the antenna are usually replaced by what appears to be a large irregular block of sclerotized material and a usually distinct tarsus. The large block presumably represents the first four leg segments. The "antennal tarsus," when well expressed, consists of five segments plus the typical tarsal claws at the end; thus, it is similar to the tarsus of the first two pairs of legs. In addition, the spurs normally found at the end of the tibia are usually present on the mutant antenna. (Figured in Sokoloff and Dawson, 1964, Linkage studies in T. castaneum IX.) Furthermore, in the adult, the metathorax is reduced in length to about two-thirds of the normal, and its medial surface is protuberant, giving the beetle a "humped" ventral appearance. The dorsal surface of the elytra appears more convex. In addition the two distal tarsomeres in all three pairs of legs fail to separate completely.

In this issue several autosomal mutants designated as "fused antennal segments" are described. Two of these are of interest because they affect the antennae and the tarsi: fas-3a is a stronger allele of fas-3. The latter, when first discovered, had only a mild effect on the antennae, and

no effect on the tarsi (see TIB 6:24). The effects of the new allele can be seen in Tables 4 and 5. In many cases the antennameres are fused into whole blocks with no identifiable segmentation, but generally the antennae do not appear excessively reduced in length. In the case of the tarsi, similar long blocks are formed, sometimes the only evidence of segmentation are a few hairs which develop in their corresponding position when the tarsus is divided into tarsomeres. It is clear that, although there is no apparent difference in the fusion of antennameres, the effect on the tarsomeres is more pronounced in the males. (It may be pointed out that fas-3, has now been kept in stock for at least three years, and still exhibits only mild fusions of the antennal segments. A recent re-examination of this stock yielded two beetles out of 20 scored which exhibited mild fusions of the tarsal segments.)

The last mutant to be considered for T. castaneum is Fas-5. Details on its mode of inheritance can be found in the section on new mutants. The effect on the antennae in the two sexes is summarized in Table 6, and it appears from this limited sample that males are more strongly affected than females. The effect on the tarsi is summarized in Table 7, and it is clear that this gene does not affect the tarsi very often, but it is of interest that in some cases the first tarsus fuses with the tibia. (A similar condition has recently been observed in a stock of Sa obtained from Purdue.)

Finally, attention is drawn to one mutation in T. confusum: stilted legs (stl) was described in TIB 5:18. It was noted that the effect of stl was a shortening of the femur and a variable fusion of the tarsi. In addition, as can be seen in Table 8, the antennae can become variously fused.

It is clear from the present survey that a number of mutants now are known for Tribolium which exhibit pleiotropic effects. (A number of other mutants such as aer and ptll also have effects on a number of body structures, but they have not been included here for the sake of brevity.) A fairly large proportion affect major taxonomic characters.

It is also noteworthy that there are now available a number of genes which are classified as alleles, but they produce a different phenotype. The most striking ones perhaps are those included in the ap locus. The effect of ap^D has been described above. By way of comparison, the reader is referred to the description of ap^S in the New Mutant section. This allele increases the number of segments in the antenna in some and produces a club-like structure. Further differences between ap^D and ap^S can be found in the effect on the metathorax and the effect on the tarsi. It is quite possible that these instances may represent examples of pseudoalleles, but it is clear that the proof that they are indeed pseudoalleles will require the examination of very large numbers of beetles.

(The writer is indebted to Mrs. Marjorie Hoy for scoring fusions of tarsi and antennae in the fas-3a and Fas-5 mutants.)

Table 1

Tarsal abnormalities observed in paddle males

(The numbers indicate tarsomeres numbered from proximal to claw-bearing segment. Numbers joined by a dash indicate a fusion of those segments. The letter "s" indicates a tarsus reduced in size.)

	Right leg			Left leg		
No.	I	II	III	I	II	III
1		4s			1-2	3s
2					4s	3s
3		4s	3s		4-5	3s
4			3s		1-2; 4s	3s
5			3s		4s	2-3
6		4s			4s	3s
7	4s	1-2; 4s	3s		4s	3s
8	4s	1-2; 4s	2-3		4s	2-3
9	4s	4s	2-3	4s	4s	2-3
10	4s	4s			4s	3-4
11						3s
12		4s	3s		4s	3s
13		4s	3s		4s	3s
14	4s	4s	2s; 3-4	4s	1-2; 4s	3s
15	4s	1-2; 4s	3s	4s	4s	3s
16	4s	4s	3s	4s	4s	3s
17	4s	4s			4s	3s
18	4s	4s	3s	4s	4s	3s
19		4s	3s		4s	3s
20		4s			4s	

Table 2

Fusions in the serrate mutant

(Numbers represent the antennameres numbered from proximal to distal. Fusions of adjoining segments are represented by a dash. Where more than two segments are fused to each other the dash connects the proximal and the most distal antennamere of the fused block.)

	Males		Females	
No.	Right	Left	Right	Left
1	4-5, 10-11	4-5, 10-11	4-5, 9-11	4-5, 9-10
2	4-5, 6-7, 8-9, 10-11	4-5, 6-7, 8-9	10-11	9-10
3	4-5	4-5, 6-7	0	0
4	9-10	9-10	0	0
5	4-5, 10-11	6-7, 10-11	8-9	0
6	4-5, 6-7, 10-11	4-5, 9-11	0	0
7	4-7	4-8, 9-11	0	0
8	3-5, 9-10	3-5, 9-10	0	10-11
9	3-7, 8-9	4-5, 6-7, 9-10	9-11	10-11
10	4-5	4-5	10-11	10-11

Table 3

Numbers of tarsomeres in 10 males and 10 females derived from the serrate stock. A figure followed by a decimal indicates partial fusion of adjacent tarsomeres.

	Males						Females					
	Leg 1		Leg 2		Leg 3		Leg 1		Leg 2		Leg 3	
No.	R	L	R	L	R	L	R	L	R	L	R	L
1	5	5	4	3.5	4	3.5	4.5	5	4	4	3.5	4
2	5	5	4	4	4	4	5	5	4	4	4	4
3	5	5	4	4	4	4	5	5	4.5	4	4	3.5
4	4	4	3.5	3.5	3	3.5	5	5	4.5	4	4	3.5
5	4.5	4.5	4.5	4	3.5	4	5	5	4	4.5	4	4
6	5	5	3.5	3.5	3.5	3.5	5	5	4	4	4	4
7	4	4	3	3	3	3	5	5	4	4	4	3.5
8	5	5	4	4	4	4	5	5	4	4	3.5	4
9	4.5	5	3.5	3.5	3.5	3.5	4.5	4.5	4	4	3.5	4
10	5	5	4	4	3.5	3.5	5	5	4	3.5	4	4

Table 4

Antennal fusions in the fas-3a mutant

	Males		Females	
No.	Right	Left	Right	Left
1	3-5, 6-7, 8-9, 10-11	3-7, 8-9, 10-11	3-5, 6-7, 8-11	3-5, 6-7, 8-11
2	3-5, 6-7, 8-11	3-5, 6-7, 8-11	3-5, 6-7, 8-11	3-5, 6-7, 8-11
3	3-11	3-11	3-5, 6-7, 8-11	3-5, 6-7, 8-11
4	3-5, 6-7, 8-11	3-5, 6-7, 8-11	3-7, 8-11	3-7, 9-11
5	3-7, 8-11	3-11	3-5, 6-7, 10-11	3-5, 6-7, 8-9, 10-11
6	3-7, 8-11	3-11	3-5, 6-7, 9-11	3-5, 6-7, 9-11
7	3-7, 8-11	3-7, 8-11	3-5, 6-7, 10-11	3-5, 6-7, 9-10
8	3-5, 6-7, 10-11	3-5, 6-7, 10-11	3-5, 6-7, 10-11	3-5, 6-7, 9-11
9	(1-2)(3-4)(5-8)(9-11)	3-5, 6-7, 8-11	3-5, 6-7, 8-11	3-5, 6-7, 8-11
10	3-5, 6-7, 8-11	(3-5) 6-7 (8-11)	(3-7)(8-11)	(3-11)

Table 5

Tarsal fusions in the mutant fas-3a in T. castaneum. The numbers in parenthesis show the total numbers of tarsomeres observed.

	Males						Females					
	Leg 1		Leg 2		Leg 3		Leg 1		Leg 2		Leg 3	
No.	R	L	R	L	R	L	R	L	R	L	R	L
1	4-5	4-5	(4)	(3)	3-4	(3)	0	0	0	0	0	0
2	(4)	(4)	(3)	(4)	(3)	(3)	(4)	(3)	(4)	(4)	(2)	(3)
3	(3)	(3)	(3)	(1)	(1)	(1)	0	0	0	(4)	(3)	(3)
4	(3)	(4)	(3)	(4)	(2)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
5	(4)	(4)	(4)	(3)	(3)	(3)	(4)	(4)	(4)	(4)	(3)	(3)
6	(4)	(4)	(3)	(4)	(3)	(3)	0	0	0	0	0	0
7	(3)	(3)	(3)	(3)	(2)	(3)	0	0	0	0	0	(3)
8	(4)	(3)	(4)	(4)	(3)	(3)	(3)	(3)	(4)	(3)	(3)	(2)
9	(3)	(3)	(3)	(3)	(2)	(3)	(1)	(2)	(2)	(2)	(2)	(2)
10	(4)	(4)	(4)	(4)	(3)	(3)	0	0	0	0	0	0

Table 6

Antennal fusions in the Fas-5 mutation in T. castaneum

	Males		Females	
No.	Right	Left	Right	Left
1	3-8	3-8	2-8	3-7, 8-9, 10-11
2	5-8	4-5	3-8	3-8
3	5-8	6-8	4-5, 7-8	4-5, 7-8
4	3-8	3-8	3-4, 6-8, 9-10	4-5, 6-8, 9-10
5	5-8	3-8	4-5, 6-8	4-5, 6-8
6	5-8	4-8	4-5, 6-8	4-5, 6-8
7	5-8	5-8	4-5, 6-8, 9-10	4-5, 6-8
8	6-8	5-8	4-5, 6-8	4-5, 6-8
9	4-5	4-5	4-5, 6-8	4-5, 7-8
10	4-8, 9-10	5-6, 7-8, 9-10	4-5, 6-8	4-5, 7-8

Table 7

Tarsal fusions in *Fas-5* in *T. castaneum* (t = tibia).

	Males						Females					
	Leg 1		Leg 2		Leg 3		Leg 1		Leg 2		Leg 3	
No.	R	L	R	L	R	L	R	L	R	L	R	L
1	3-4	0	0	1-2	0	0	t-1-2	t-1, 3-4	t-2	t-2	(t-1)	(t-1)
2	0	0	0	0	0	0	0	0	0	0	t-1	0
3	3-4	3-4	1-2	(4) 1-2	0	0	3-4	3-4	t-1	0	t-1	0
4	(t-1)	(t-1) 3-4	(t-1) 4-5	(t-1) 2-3, 4-5	(t-1) 2-4	(2-1) 3-4	3-4	3-4	0	1-2, 3-4	0	0
5	0	0	0	0	0	0	3-4	3-4	0	0	0	2-3
6	0	0	0	0	0	0	t-1, 3-4	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	t-1	t-1
8	0	0	2-3	1-2	0	0	1-2	0	0	0	0	0
9	0	0	0	0	0	0	t-1	t-2, 3-4	3-4	3-4	t-1	0
10	0	0	0	0	0	0	0	0	t-1	t-1	t-1	t-1

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*Revised linkage maps in Tribolium castaneum and T. confusum

Linkage maps in Tribolium castaneum.--The linkage relationships of a number of genes have been determined. They are summarized in Fig. 1. Gene symbols given in parenthesis have been identified with that particular linkage group, but their map position is yet to be ascertained from three point crosses. The maps are based on the data given below. In certain cases the data have not yet been published. In other cases indicated in brackets, the experiments need to be or are being, repeated, particularly for certain genes which have incomplete penetrance or marked reduction in viability, and for which the data appear inconsistent.

I

pd - l_1 = 61
pd - dpm = 40
r - M^r = 16
r - te = 11
pd - r = 1
pd - ser = 0
ser - r = < 1
pd - ma = 13
ma - py = 2
py - l_3 = 5
py - dve = 11
py - rs = 15†
py - l_2 = 23
py - l_4 = 25
pd - sp = 46
pok - dve = 2

II

p - pg = 30
p - p^{Pk} = 0

III

b - lod = 24
b - msg = 44
b - sc = 28-33

IV

Be - s = 25
s - ims = 11-23
Be - ims = 43
Be - ju = 44
Be - ct = 29
ju - ct = 7
s - sti = 41
sti - ims = 35
[Be - dfl = 27]
[s - dfl = 24]
[dfl - ims = 50]
[Be - fas-2 = 27]
[fas-2 - s = 25]
[rju - s = 13]
[rju - fas-2 = 38]
[eju - fas-2 = 38]

V

j - spl = 29
j - mc = 25
spl - mc = 41
m - j^E = 22‡
m - mc = 40‡
j^E - mc = 18‡

VI

Mo

VII

ble - c = 42-44
ble - Fta = 3-6
ble - Sa = 4
Fta - sa = 18
Fta - c = 34-45
sa - c = 38
Sa-l - c = 40
Sa - sa = 0

VIII

elb - sh^S = 23
sh^S - sq = 28
ap^D - sq = 7
ap - ap^D = 0
sh^S - $sh^{H\ \&\ D}$ = 0

IX

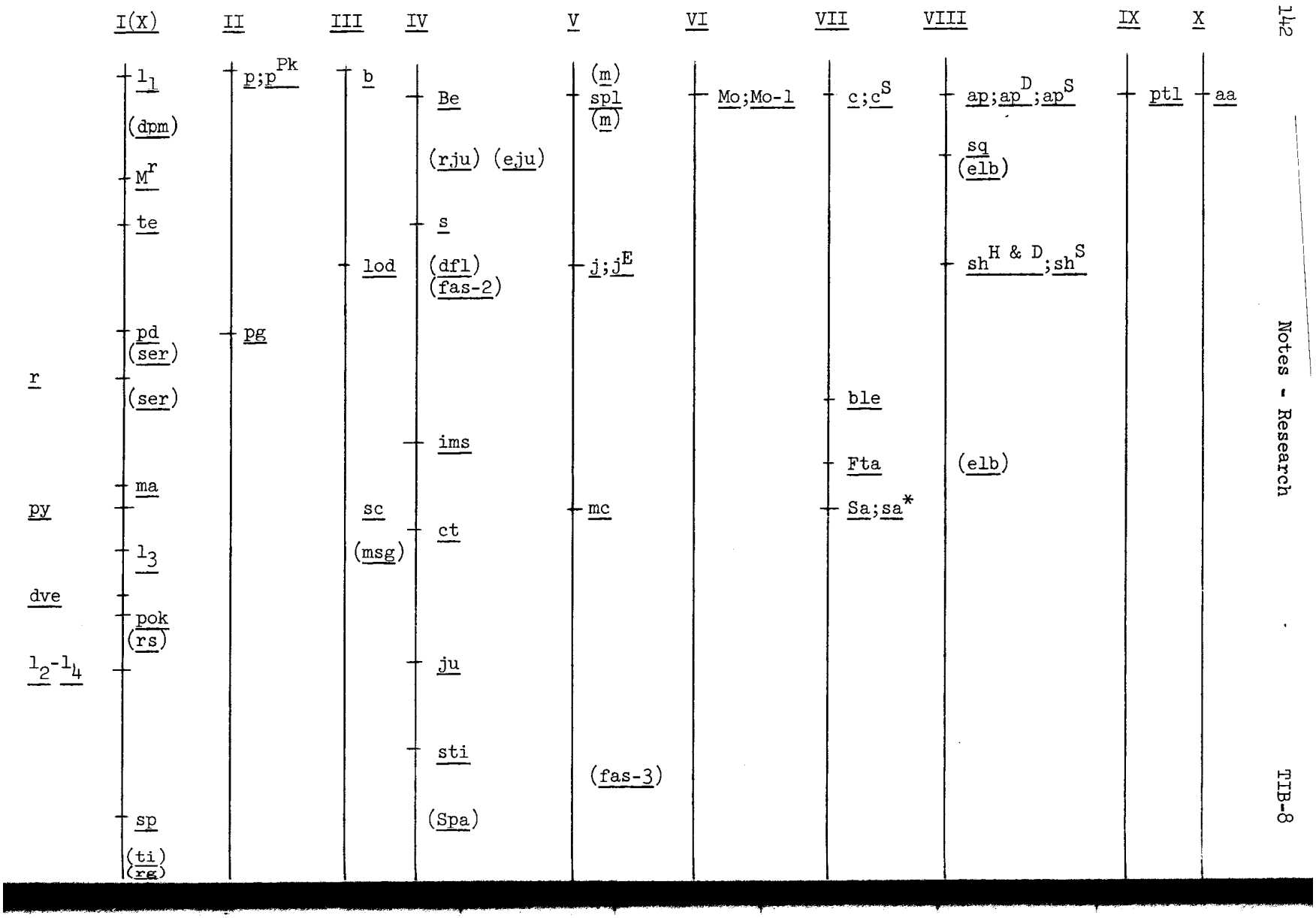
ptl

X

aa

† data of Reynolds (1964, TIB 7:32).
‡ data of Eddleman (1964, TIB 7:31).

Fig. 1. Linkage maps in Tribolium castaneum



Linkage maps in Tribolium confusum.--The maps given in Fig. 2 are based on the following linkage data some of which has been published (Sokoloff, 1964).

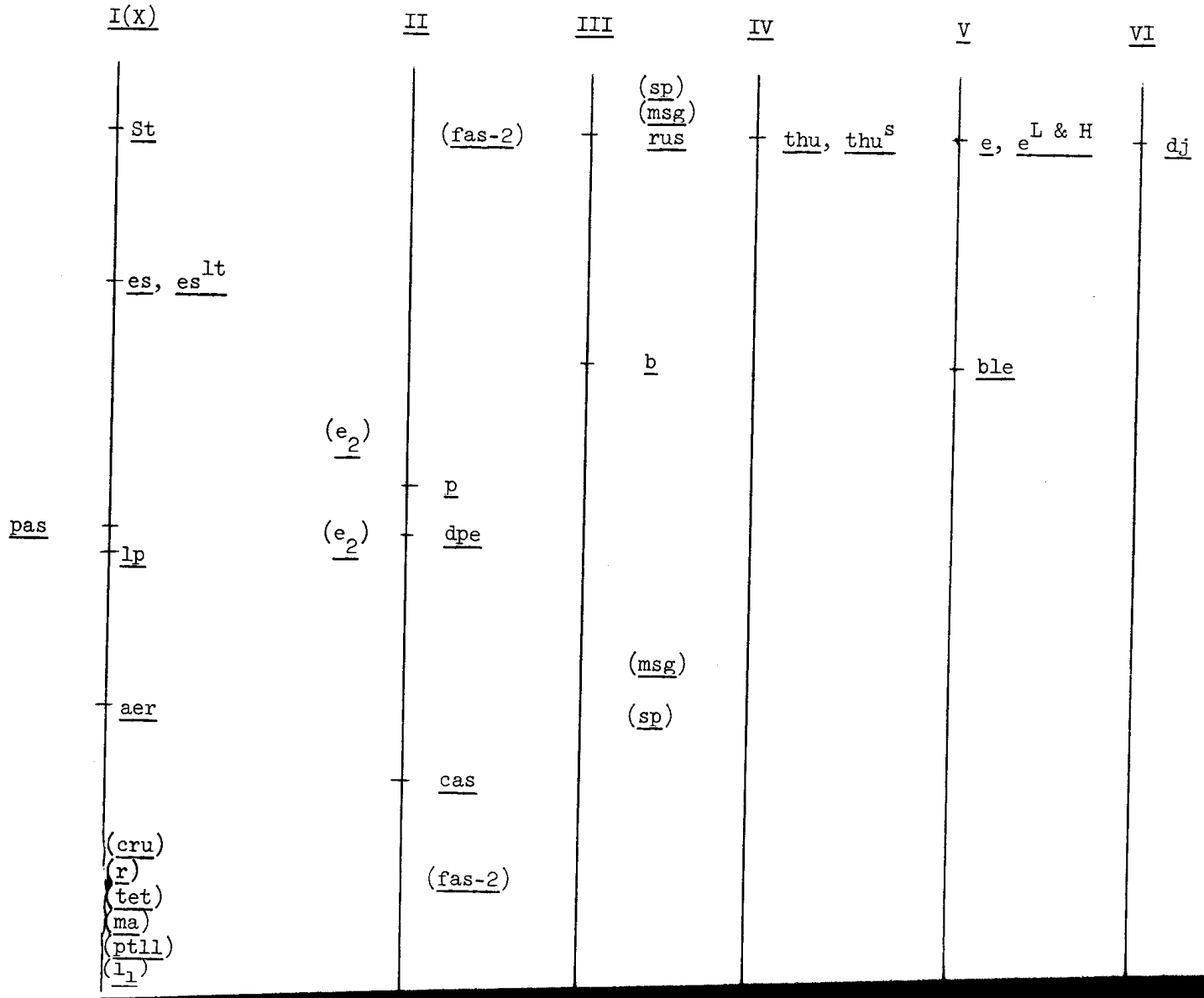
I	II	III	V
es - es^{lt} = 0	p - dpe = 5	b - rus = 41	e - ble = 30-40
es - St = 38	p - cas = 38	b - msg = 42	
es - lp = 47	dpe - cas = 30	b - sp = 42-44†	
pas - lp = 3	p - e_2 = 2.5		VI
lp - aer = 24	p - fas-2 = 42-44	IV	dj‡
es - tet = 53		thu, thu^{S}‡	
r - lp = 48			
es - l_1 - 40			

In addition, the following have been tested and proved to segregate independently: b from lod, fas-2; e from cas, ele, fas-1, fas-2, lod, p, rsy, sti, and stl; fas-2 from ele and lod; rus from ble; p from umb.

† Some crosses give these recombination values, but others give values nearer 50 per cent. Three point crosses have not been carried out.

‡ Dawson, personal communication, has determined that thu and dj are not linked to b, p and e. They are here, therefore, assigned as markers of linkage groups IV and VI, respectively.

Fig. 2. Linkage maps in Tribolium confusum



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*Further remarks on sta (spikes on trochanters and antennae).

This homeotic mutant has been found three times in this laboratory. Two of these occurrences were found about two years apart in crosses involving squint, and it is possible that the genes, owing to their incomplete penetrance, may have been overlooked. But the third occurred in descendants of an irradiated normal male mated with an unirradiated female, both kindly provided by Dr. Howard E. Erdman of the Biological Laboratory, Hanford Laboratories, General Electric Company, Richland, Washington, from a Brazil strain which was not available at this laboratory (and which also yielded the rt, akb and jac mutants described in the mutant section. The following description is from a well-established stock which was founded from beetles found in the course of determining the frequency of crossing over between ap and sq. (Some ap and sq beetles occasionally appear, indicating these genes are present in a few heterozygotes.)

The history of sta is as follows: several males and females were found among the progeny of a single pair mating which attracted the writer's attention because the dead or dying imagoes had failed to shed the pupal skin. When the pupal skin was removed, the trochanters of all the legs and the second antennal segment exhibited very large spike-like growths. Their parents were transferred repeatedly to fresh food and they produced a number of additional sta beetles with less marked effects, sometimes the spikes being confined to the antennae (but not necessarily arising from the second antennamere) or to the trochanters. Mated inter se for several generations sta beetles produced but a few individuals resembling their parents. A chance examination of the larvae in the stock jar by my assistant Mrs. Marjorie Hoy revealed that sta has a distinctly recognizable effect on the larvae: the legs may exhibit fleshy growths, apparently originating from the trochanters, which may be almost as large as the whole leg, they may be modified in various ways, sometimes resembling the antennae of prothetelous individuals, and occasionally being completely duplicated. While the incidence of abnormal larvae now approaches about 80 per cent in the stock, the incidence of imagoes drops to a very low level. The leg growths apparently are resorbed at metamorphosis, and imagoes emerging from larvae known to have leg or antennal abnormalities may appear normal. The range of expression of the antennae in affected sta adults has become quite variable: spikes may originate from different antennameres even in one individual. The spikes may be small and pointed, or very long, and filamentous. Some spikes may exhibit various degrees of segmentation. In many individuals instead of spikes there may be segmented branches arising at any point of the antenna, or the antenna may be completely duplicated. Examples of these various phenotypes are shown in the accompanying figure.

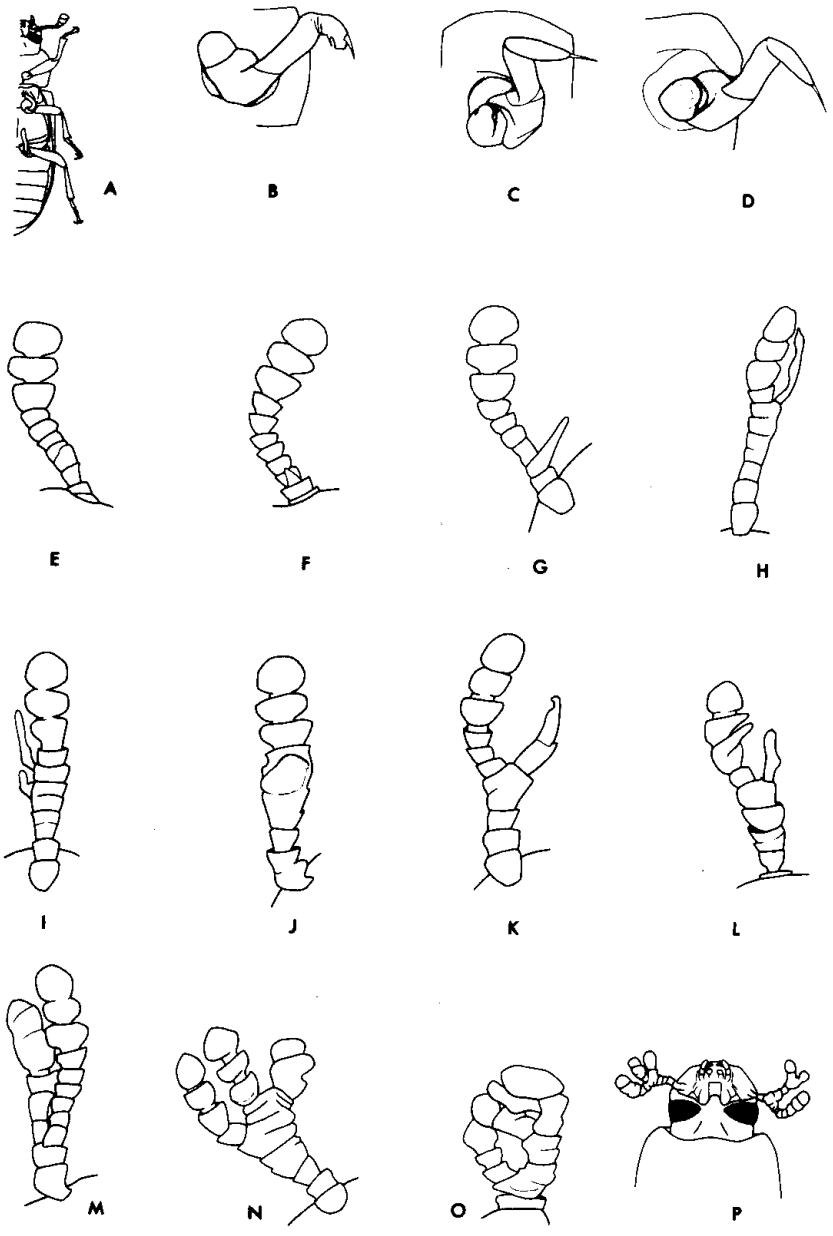
It is clear that some of the sta beetles, judging by the appearance of the antennae, resemble the bra phenodeviant described by Dawson (compare

with Fig. P). Unfortunately bra was no longer available, having been lost owing to infertility following intensive inbreeding and selection. Hence, it was not possible to compare bra with sta larvae. A stock was available of the earlier sta-like mutation found in this laboratory in other crosses involving sq. When first found, this mutant had a small process arising from the second antennal segment and it was designated horned antennae (ha). In crosses carried out by Mr. Richard Paige, one F_3 were obtained with fused tarsi and the tibia of the left middle leg swollen and segments 3-5 of the right antenna fused. In another creamer a male was found with segments 2-3 of both antennae fused; the tibia of the left middle leg had a spike-like branch and the left hind leg was badly deformed. In other F_3 creamers were found rare individuals with branched antennae. Tests of allelism between ha and sta revealed that they are the same gene.

Thus, in ha some individuals possessed supernumerary growths in the tibiae, while in sta these growths arose from the trochanter.

In Balazuc's (1948) monograph on the teratology of Coleoptera the following species of beetles are cited and illustrated whose antennae are two-branched or three-branched: Ibidion sp., Dihammus musivus, Rhamnusium bicolor, Magdalis barbicornis, Collops tricolor, Zabrus ovalis, Arthrodactylus elongatus, Lucanus cervus, Julodis Clovei, Cantharis pellucida, Carabus obsoletus var euchromus, Macrotoma sp. prope senegalensis, Neodocardion egregium, Odontolabis Stevensi. Jayne (1880) reported a Prionus californicus with all three pairs of legs duplicated entirely beyond the femur (cited and illustrated in Balazuc, p. 170). Balazuc himself described and figured (p. 172) a Lagria hirta which in addition to fusions of segments 3-4 and 5-6 of the right antenna, had spike-like processes arising from the femur of all three legs, and from segment eight of the left and from segment ten of the right antenna. I believe that the sta mutation may provide a genetic explanation for such monsters as have been cited in the teratological literature.

The writer is indebted to Mrs. Barbara Daly for drawings A-O and to Mr. Roger St. Hilaire for drawing P. All the references cited above can be found in Balazuc, J. 1948. Mem. du Mus. Nat'l. d'Histoire Naturelle 25:1-293.


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P

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*Further comments on mutants with juvenile-urogomphi-like appendages in the adult Tribolium castaneum

Three independent occurrences of mutations producing urogomphi-like or setigerous-like appendages near the anal opening have been recorded for this beetle by the writer: juvenile urogomphi, ju (TIB 6:25); elongated juvenile urogomphi, eju (TIB 7:35) and reduced juvenile urogomphi, rju (TIB 7:36). The first two are recessive and might be recurrences of the same mutation, although they appeared in unrelated stocks; the third is undoubtedly a different mutation, since it overlaps wildtype in dominance in certain crosses. Since its discovery eju has become closer to ju in phenotype; rju on the other hand, primarily produces very tiny setigerous structures. Preliminary crosses suggested that none of these mutants was allelic, but preliminary results from linkage tests indicate eju and rju may be in the same linkage group (IV) as ju.

A few months ago through the courtesy of Dr. Howell V. Daly, Department of Entomology and Acarology, of this University, the ju material was examined by phase contrast microscopy, and photographs taken of ju males and females and of the urogomphi in the normal larva and normal pupa. The photographs, too large and too costly to reproduce in TIB, will be published elsewhere, but sketches from them, considerably reduced, are given in Figs. 1-4. In the larva (Fig. 1) the urogomphi are pointed structures which are triangular and very broad at the base. There is no evidence of folds. In the pupa (Fig. 2) the urogomphi also appear triangular, but much more narrow. The distal third appears smooth, but the proximal two-thirds of each urogomphus appears creased or thrown into folds. The ju adults studied exhibited large urogomphi-like appendages. The photograph of the male appendages reveals the same characteristics exhibited by the pupal urogomphi: the tips are smooth but the bases exhibit the same type of folds (Fig. 3). In the female (Fig. 4) these folds extend more distally than in the male, and the entire appendages appear less heavily sclerotized. The only observable differences between the structures in the adult and the pupa appear to be at the base. While in the pupa the urogomphi are narrow at the tip and gradually broader toward the base, in the male adult they appear broadest somewhat distally from the point of attachment, and they become narrow at the point of origin. There is some evidence of a sclerotized double "articulation" lying within the posterior segment. In the female there is also a decrease in size of the urogomphi at the base, but the double "articulation" seems to be missing, the urogomphi being attached to the body (lateral to the anal opening) by a membranous cuticle.

The photographs, hence, reveal that in the ju mutant the structures appearing near the anal opening are indeed urogomphi, and the term "juvenile urogomphi" is not at all misleading.

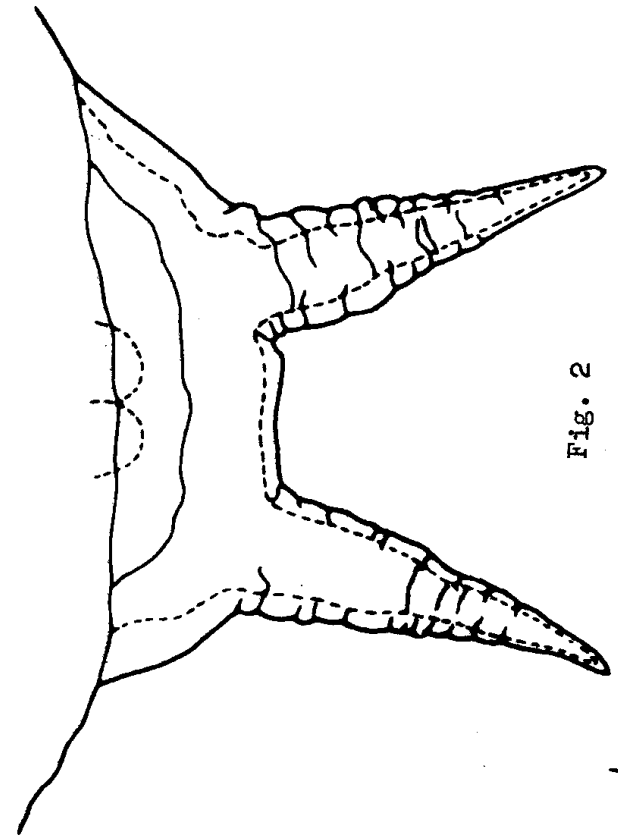

Fig. 2

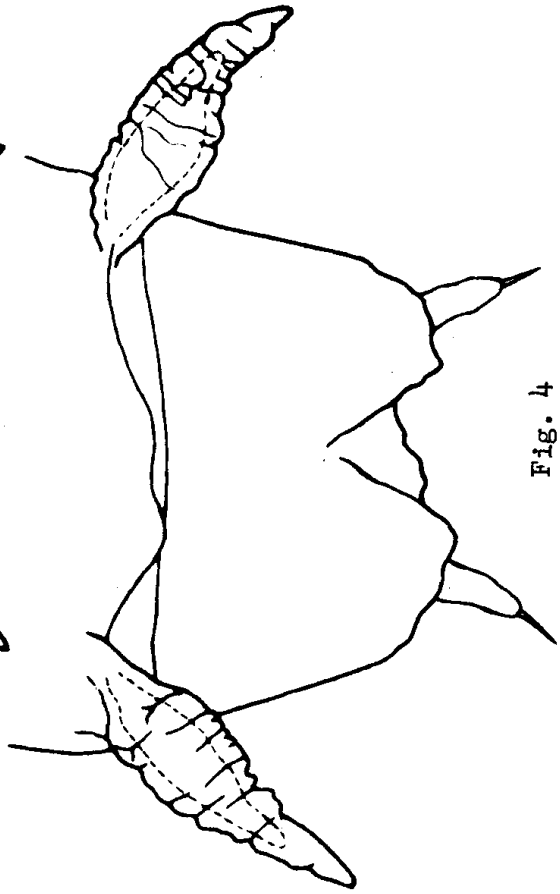

Fig. 4

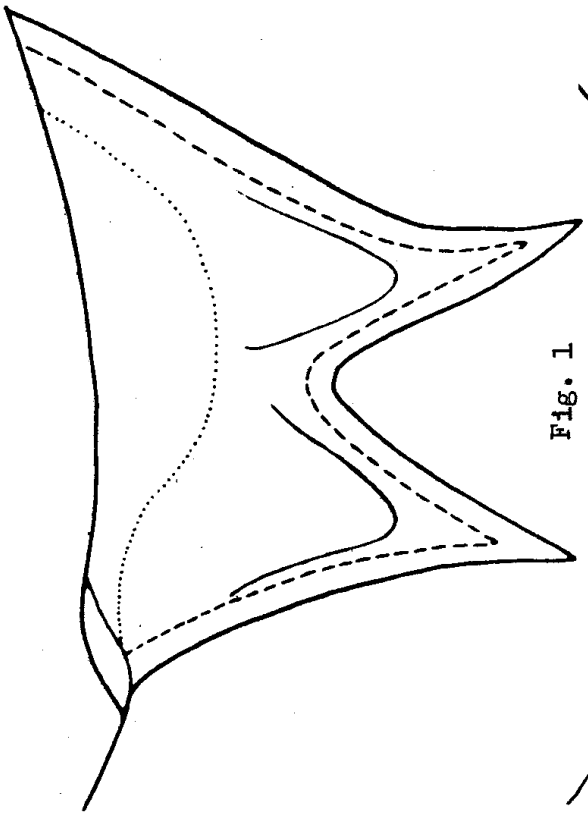

Fig. 1

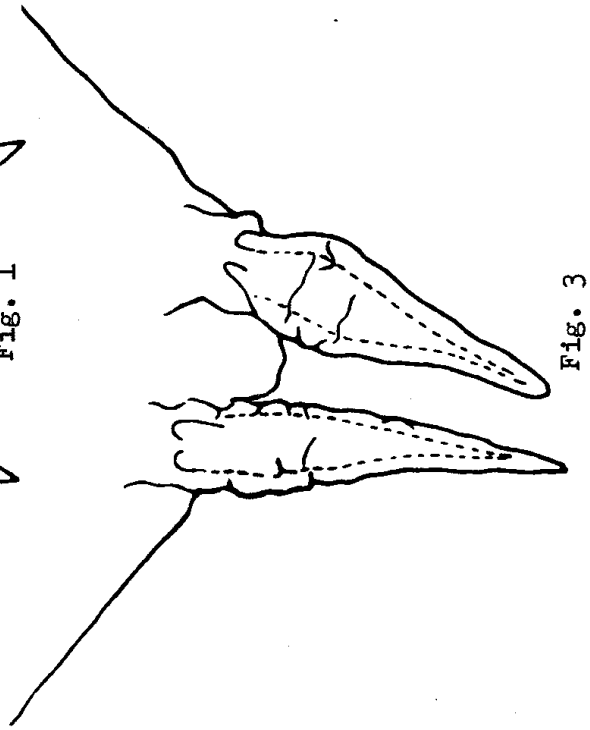

Fig. 3

It would seem that the mutation is an example of paedomorphosis, although the term was originally used by Garstang (1898) and later developed by De Beer for vertebrates (1930) (see review by Gregory, W. K., 1946, Quarterly Rev. Biol. 21:354) to describe the retention of embryonic or larval characters in the adult.

(See comments by Crowson above. Ed.)

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*Possible genetic basis for prothetely in Tribolium castaneum and Latheticus oryzae

In holometabolous insects in which the larva has external legs the imaginal legs form within the larval legs and grow within the larval cuticle. The elytra and membranous wings form within pockets of the larval epidermis. Adult legs and both types of wings are not extended until the moulting of the last larval skin in the pre-pupa. In prothetely the imaginal legs and wings are prematurely extended in the larva. In Tribolium this may occur when larvae are exposed to high concentrations of quinones or other gases. A number of such cases have been recorded (see, for example, R. N. Chapman, 1926, J. Exp. Zool. 45:293-299; L. M. Roth and R. B. Howland, 1941, Ann. Ent. Soc. Amer. 34:151-175). Recent observations of two mutant strains in T. castaneum and L. oryzae suggest that prothetely might have a genetic basis. The strains referred to are sta (spikes on trochanters and antennae) in the first species and ele (elongated elytra) in the second. The rather high incidence of prothetelous individuals in sta was first observed by one of us (M. A. H.) in the course of selecting for the sta character. Since we were aware that quinones have this effect, we collected eggs from sta beetles and reared these in the absence of imagoes (odoriferous glands are formed in the pupal stage and teneral adults if excited are capable of secreting quinones from the thoracic glands). In this culture were found a number of larvae exhibiting prothetely. Unfortunately, these individuals failed to metamorphose as is usual for these precocious individuals.

In ele one of us (A. S.) noticed a number of prothetelous individuals. In this culture there were 30 adults and over 100 larvae, and 15 of these had either partial or complete eversions of the elytra and/or membranous wings. It may be argued that these individuals may have been exposed to quinones, since the parents and their progeny were in the same culture. This is undeniable, but in the senior author's experience in working with these species such an event may usually happen when the whole contents of a culture are removed from the flour, and left in a container for a few hours, and then only one or two larvae exhibit these abnormalities. Never have the larvae appeared prothetelous as in these cases immediately after removal of the beetles from the flour.

At this writing, it is not known whether prothetely in these two strains is genetically or environmentally induced, but material now has become available to test whether this phenomenon has genetic basis, and further tests are under way.

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*Sudden elimination of T. confusum by T. castaneum in different media

The experiments reported here were designed to study the outcome of competition between T. castaneum (henceforth referred to as CS) and T. confusum (CF) in diets different from the standard whole wheat flour plus yeast used in previous studies (see, for example, Park, Leslie and Mertz, 1964 and Lerner and Ho, 1961).

The experiments were initiated with 10 pairs each of ten-day old CS and CF derived from our synthetic strains in each of 10 vials containing rice flour plus five per cent brewer's yeast. This experiment was repeated in its entirety. Subsequently, a third experiment, of the same experimental design, was performed with soy flour plus five per cent brewer's yeast. All three experiments were carried out in a converted Jamesway incubator maintained at 29°C. and 70% relative humidity. The food was renewed every month, the populations were continued with the preadult stages, and the adults found at each census scored and discarded.

The results, reported in Tables 1 and 2 as fractions give the numbers of CF in the numerator over the total numbers of CF and CS observed at that census. It is evident that CF, in rice plus yeast, fails to show up in most vials beyond the second census period, and in soy plus yeast it failed to appear in half of the vials at the third census. It may be pointed out that this phenomenon has also been occasionally observed when these two species are introduced in whole wheat flour plus yeast (Dawson, personal communication).

The early elimination of CF cannot in any way be attributed to competition. Rather, it must be explained by an inhibition of reproduction of CF by CS. These instances then may represent further examples of an analogous situation, previously reported by Lloyd and Park (1962, Physiolog. Zoology, 35:330), of mortality resulting from interactions between the adults of these two species.

Table 1. Numbers of adults of T. castaneum (CS) and T. confusum (CF) observed at different transfer periods in rice plus yeast. (Fractions represent numbers of CF over the total CS and CF observed at that census. Upper block, first experiment; lower block, repeat experiment.

Replicate	0	1	2	3	4	5	6
1	20/40	22/41	0/67	0/46	0/102	0/51	0/62
2	20/40	19/40	0/114	0/23	0/58	0/50	0/75
3	20/40	18/38	0/82	0/40	0/54	0/33	0/44
4	20/40	20/40	0/71	0/19	0/53	0/27	0/56
5	20/40	20/38	0/82	0/31	0/44	0/61	0/35
6	20/40	20/40	0/80	0/27	0/54	0/70	0/47
7	20/40	20/40	0/99	0/18	0/68	0/45	0/49
8	20/40	20/40	0/91	0/37	0/66	0/52	0/58
9	20/40	20/40	1/113	0/29	0/67	0/53	0/58
10	20/40	20/40	0/88	0/21	0/75	0/28	0/61

An additional set of 10 cultures set up to repeat the above:

Replicate	0	1	2	3	4	5	6
11	20/40	20/47	1/71	1/60	0/36	0/42	0/46
12	20/40	20/35	0/53	0/74	0/42	-	-
13	20/40	20/42	0/43	0/94	0/64	-	-
14	20/40	20/50	0/78	0/66	0/42	-	-
15	20/40	20/56	0/83	0/52	0/57	-	-
16	20/40	20/48	2/50	0/67	0/34	0/40	-
17	20/40	19/46	0/75	0/68	0/33	-	-
18	20/40	20/42	1/69	0/52	0/38	0/33	-
19	20/40	20/52	3/81	0/74	0/59	0/45	0/39
20	20/40	20/47	0/56	0/71	0/44	-	-

Table 2. Numbers of adults of T. castaneum (CS) and T. confusum (CF) observed at different transfer periods in soy plus yeast. (Fractions represent numbers of CF over the total CS and CF observed at that census. Upper block, first experiment; lower block, repeat experiment.

Replicate	0	1	2	3	4	5	6
1	20/40	20/40	2/23	0/22	0/16	2/27	-
2	20/40	20/40	2/21	2/20	0/20	0/10	0/21
3	20/40	20/39	4/17	2/23	0/15	0/37	0/11
4	20/40	19/37	0/17	0/14	0/12	-	-
5	20/40	19/39	0/27	0/13	0/17	-	-
6	20/40	20/40	2/17	2/26	0/24	0/15	0/19
7	20/40	19/37	2/22	1/16	0/15	0/18	0/15
8	20/40	20/40	9/27	1/17	1/31	0/17	0/31
9	20/40	19/38	2/28	0/20	0/19	0/23	-
10	20/40	19/39	2/21	0/19	0/10	0/31	-

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*Effects of hypothermy and tarsus-clipping on fecundity and longevity of Tribolium castaneum

Hypothermy has been described before (TIB 5:45) as a means of immobilizing beetles. Tarsus-clipping has recently been investigated as a method of marking adult beetles. While immobilized on a cold plate, part of the tarsus of a particular leg can be removed by the use of a tool made from a fragment of razor blade welded to the tip of a glass rod. The following experiments were performed to reveal any effects of these treatments on the biological properties of the adults.

Hypothermy treatment (HP) involved exposing adults on a cold plate for 10 minutes. Tarsus-clipping (TC) involved the amputation of a tarsus while the beetle was immobilized on the cold table. Control (C) beetles received neither treatment. Equal numbers were used for amputation of each of the six legs. All beetles used were young (a few days after emergence) and were of two strains, wild type and bb. They were kept at 85°F., 70% relative humidity in a medium of fine sifted whole wheat flour plus 5% yeast.

Survival.--Within each treatment, 100 beetles (sex ratio 1:1) were kept in a small jar of medium. Observations were taken on mortality each week. The accompanying table shows no differential survival between the treatments. The stars (*) indicate that adults were observed to have lost additional tarsi.

Survival of wild type beetles:

	weeks											
treatment	1	2	3	4	5	6	7	8	9	10	11	12
C	100	99	99	98	97	97	97	96	94	92		86
HP	100	98	98	98	96	95	94	93	92	91		86
TC (2 jars)	100	100	100	100	100	100	100	99	98	96*		93*
	100	100	100	100	100	100	100	100	99	99		94*

Survival of bb beetles:

	weeks											
treatment	1	2	3	4	5	6	7	8	9	10	11	12
C	100	99	99	99	97	97	97	97	96	91		86
HP	100	100	100	99	98	98	97	97	96	94		90
TC (2 jars)	100	99	99	99	98	98	98	97	96	95		92*
	100	100	100	100	100	99	98	97	96	95		92*

Fecundity and fertility.--These were measured on single pairs set up in shell vials with 4 gms of medium. Net fecundity was measured at 3-day intervals; fertility (% hatchability of eggs) at 6-day intervals. There were 25 control, 25 hypothermy and 30 tarsus-clipped pairs (i.e., 5 for each leg; both beetles of a pair had the same type of mutilation). The experiment was divided into 2 blocks; each being continued for 6 census periods.

Eggs/female/3 days ± standard error:

	day					
treatment	3	6	9	12	15	18
Fecundity wild type block 1						
C	52.2±5.1	63.1±3.0	65.4±2.2	59.6±3.9	69.5±1.9	63.7±2.4
HP	38.3±3.0	52.8±3.2	61.1±5.3	64.7±2.3	69.2±2.7	63.6±2.0
TC	41.6±3.0	56.1±3.3	63.9±3.5	60.7±3.5	68.7±3.6	64.6±3.3
Fecundity bb block 1						
C	43.5±3.1	58.7±3.0	57.1±3.3	59.2±2.5	61.9±2.4	56.8±2.4
HP	51.7±1.5	61.3±1.6	63.1±2.2	63.3±1.8	64.6±2.2	58.8±1.4
TC	50.9±2.4	55.1±3.1	66.7±2.4	64.1±1.9	67.7±1.9	64.3±2.1
Fecundity wild type block 2						
C	58.2±5.5	64.3±2.9	67.5±3.2	65.3±3.9	62.1±4.0	63.2±2.6
HP	41.5±4.4	53.5±4.2	57.7±4.0	56.5±3.2	57.8±3.0	61.5±3.0
TC	14.4±3.1	55.3±4.1	66.6±2.1	66.1±2.7	64.1±4.0	61.5±3.1
Fecundity bb block 2						
C	59.3±3.6	71.5±4.7	66.2±5.4	60.8±5.5	61.9±3.3	60.1±3.1
HP	62.7±2.5	59.7±2.5	67.4±2.5	58.3±3.5	62.7±2.6	57.3±5.3
TC	25.7±3.0	62.5±2.6	64.6±1.5	60.5±1.7	61.9±2.2	58.1±2.3

The above tables show that there tends to be a depression of fecundity for the first six days following either HP or TC treatments. This is most pronounced in block 2 (bb HP is the exception), probably because the cold plate had a temperature closer to 0°C. on that occasion. The effects of hypothermy are not as great as the combined treatment of hypothermy and tarsus-clipping. The bb strain seems to have been affected less than the wild type.

Although there were one or two exceptional replicates in all three treatments with consistently low fertility, in the majority of cases 90% or more of the eggs hatched. (Larvae were removed each day to prevent their cannibalism of the remaining eggs.) There was no corresponding depression of fertility immediately following the treatments and evidently mounting and copulation is not impaired by the lack of one tarsus on the male.

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*Dechorionation and in vitro culture of Tribolium eggs

As a preliminary step in obtaining organ and embryo cultures of Tribolium confusum it has been necessary to devise a method of sterilizing and removing the chorion. Sterilization of the surface has been achieved by placing the eggs in Bouin's fixative for five minutes. The eggs are then rinsed in four changes of sterile distilled water and transferred aseptically to a strip of mending tape[2] mounted sticky side up on a slide. Quarter-inch masking tape is used to bind the mending tape to the slide. After the water has evaporated, a fine needle or pair of jeweler's forceps is used to dislodge the egg. The chorion adheres to the tape and is thus broken. As the egg is rolled on the tape, the remainder of the chorion is removed. This technique is satisfactory for all eggs which are in the latter half of embryonic development. At earlier stages the vitelline membrane is apparently too fragile to withstand such treatment; only a small portion of the younger eggs will survive dechorionation by this method.

Eggs dechorionated in this manner have been cultured in a variety of media, both solid and liquid (see Table 1). Five to ten eggs were placed in about 5 ml. of medium in a tightly closed 4 dram screw-top vial. Under these conditions a significant portion of the embryos complete their development and hatch. It is a startling sight to see flour beetle larvae wiggling in the surface tension of saline or struggling on an agar slant. Larvae hatched in this manner have been transferred to dry media and have completed their life cycle normally. Hatching success under varying conditions is shown in Table 1. Discarded cultures were tested for sterility by addition of an equal quantity of double strength Na thioglycollate broth and incubation for 48 hours at 37°C. The majority of the cultures showed no growth. All media contained streptomycin sulfate, penicillin "G" sodium and nystatin.

Although eggs normally hatch equally well at 25.5 and 33°C., marked differences in the per cent hatch occurs when dechorionated eggs are simultaneously exposed to high temperature and high saline concentrations. Although all the figures are not statistically significant, the values obtained with equal milliequivalents of different ions suggest definite ion effects. Thus it appears that the vitelline membrane is permeable to certain ions. That it is permeable to water is demonstrated by the fact that

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[1] This study was supported in part by NSF Fellowship No. 65087 and in part by a University of Utah Research Fellowship.

[2] Scotch Magic Mending Tape, manufactured by Minnesota Mining and Manufacturing Co., St. Paul 6, Minn.

Table 1

Hatching Success of Eggs of Tribolium confusum

Treatment	Medium	Temp.	Total Eggs	Number Hatched	Percent Hatched
none	dry	33°C	300	230	76.5
tape dechorionated	.18M v.s.†	"	49	19	38.8
"	.27M v.s.	"	55	10	18.4
"	.13M NaCl	"	48	17	35.4
"	.26M NaCl	"	53	6	13.3
"	.39M NaCl	"	51	2	3.9
"	.26M KCl	"	52	2	3.8
"	.13M $CaCl_2$	"	50	8	16.0
"	.13M $MgCl_2$	"	50	12	24.0
"	.13M Na_2SO_4	"	57	9	15.8
	distilled H_2O	"	51	17	33.3
Na hypochlorite	.18M v.s.	"	39	0	0.0
none	dry	29°C	255	195	76.5
tape dechorionated	.18M v.s.	"	28	14	50.0
"	.18M v.s. agar‡	"	30	20	66.7
"	.27M v.s.	"	29	17	58.7
"	.27M v.s. agar	"	30	14	46.7
"	.36M v.s.	"	29	8	27.6
"	.36M v.s. agar	"	30	17	56.7
"	.45M v.s.	"	29	4	13.8
"	.45M v.s. agar	"	29	9	31.0
	distilled H_2O	"	20	7	35.3
none	dry	25.5°C	120	91	76.0
tape dechorionated	.13M NaCl	"	51	26	51.0
"	.26M NaCl	"	45	7	15.5
"	.39M NaCl	"	50	2	4.0

† v.s. = vertebrate saline; Oxoid Ringer's Solution tablets manufactured by Consolidated Laboratories, Inc., Chicago Heights, Illinois. Contain 9.0 parts NaCl, 0.42 parts KCl, 0.48 parts $CaCl_2$ and 0.20 parts $NaHCO_3$.

‡ 2% Difco agar added to the saline.

dechorionated eggs rapidly shrivel if left on a dry surface in the air. The relatively high per cent hatched in distilled water suggests that the vitelline membrane possesses some regulative ability. The somewhat higher values for agars compared to salines of the same ionic composition may be due to the certain availability of a gas phase or to reduced contact with the medium. It should be noted that some of the eggs in the liquid cultures float. In no case was the per cent hatch obtained in culture as high as that for the controls.

It is assumed that the per cent hatch reflects the compatibility of the medium and the embryonic tissues. On this basis, this technique is suggested for testing and comparing the ionic components of possible tissue culture media.

It is interesting to note that Na hypochlorite sterilization (Park 1948), although working well on eggs allowed to hatch on a dry surface, is not successful for invitro culture. Eggs were placed in a 5% solution of commercial bleach for 5 minutes, rinsed four times with distilled water and the swollen chorion removed with fine forceps. None of these hatched.

References

Park, Thomas. 1948. Experimental studies of interspecies competition. I. Competition between populations of the flour beetles, Tribolium confusum Duv. and T. castaneum Herbst. Ecol. Mon. 18:265-308.

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*Teratology in Tribolium confusum

During attempts to select for the cas mutant, an abnormality was found in the abdominal sternites of a single male. In this specimen the third and fourth sternites appeared crossed as shown in Fig. 1. The abnormality was not found among numerous individuals of the F_1 or F_2 generation. The affected male appeared robust when dissected at nine months of age. At that time it was demonstrated that there was one complete sternite and two incomplete portions filling the remaining spaces; the internal organs appeared normal.

[1] Observations were made during tenure of NSF Fellowship No. 63087.

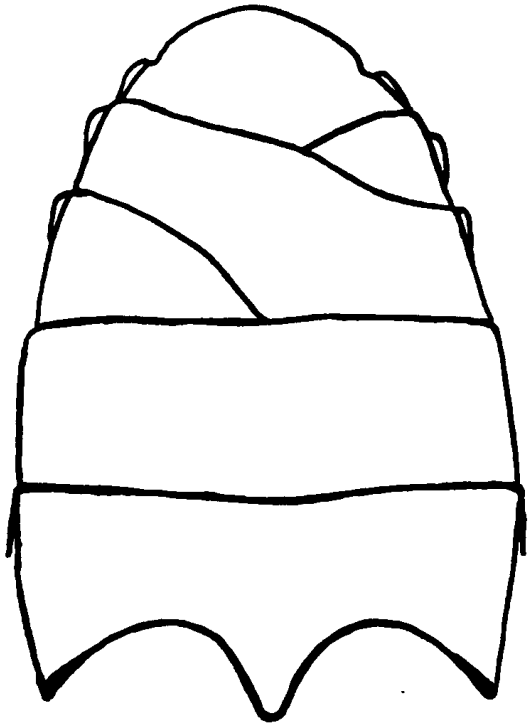

Fig. 1

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*The effect of isolated pockets of damp and mouldy grain on the behavior of the flat grain beetle, Cryptolestes ferrugineus

Adult C. ferrugineus accumulated with equal intensity in pockets of non-mouldy and mouldy grain of 18 per cent moisture content placed within larger bulks of 14 per cent moisture. Insects in a preferendum arena, however, were more abundant on the side at 70 per cent relative humidity than on the side at 85 per cent relative humidity. The underlying mechanism of this response was klinokinetic. Further analysis of behavior showed that hygrokinetic response to damp grain was subordinated to trophic and oviposition patterns of behavior. Adults feed more easily and lay more eggs in the softer, damper grain.

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*The effects of isolated pockets of damp and mouldy grain on the behavior of the saw-toothed grain beetle, Oryzaephilus surinamensis

Pockets of non-mouldy and mouldy grain of 18 per cent moisture content were placed in larger bulks of grain of 14 per cent moisture. Using adults bred at 70 per cent relative humidity, approximately 30 per cent of a population accumulated in the isolated pocket of damp, non-mouldy grain after one week. Accumulation was intensified if adults were previously kept at 85 per cent relative humidity for 14 days. Accumulation of unconditioned adults in damp grain was due to a klinokinetic response, while in conditioned adults it was due to an orthokinetic one. Adults did not accumulate in a pocket of damp grain supporting sporulating Aspergillus candidus, but accumulated in the nearest damp, non-mouldy grain around it.

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*The influence of damp and mouldy grain on the oviposition behavior of Oryzaephilus surinamensis

When populations of O. surinamensis were allowed a choice of grain in which to lay eggs, more were laid in grain of 18 per cent moisture than in grain of 14 per cent moisture, and more eggs were laid in non-mouldy grain than in that supporting a flora of sporulating Aspergillus candidus. In the test where mouldy grain was used, overall egg production was depressed.

Number of eggs laid by Oryzaephilus surinamensis
(mean of 4 replicates)

Control		Test I		Test II	
Grain moisture content		Grain moisture content		Grain moisture content	
14%	14%	14%	18% (non-mouldy)	14%	18% (mouldy)
17.3	21.0	21.8	50.0	10.0	3.8

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*Differences in behavior of unmated and mated grain weevils, Sitophilus granarius

During the first five weeks of life, unmated females were found more frequently on the surface of grain samples than were mated ones. Unmated males were found on the surface on consecutive days, whereas mated males appeared more irregularly. After the fifth week of life, the behavior of mated adults changed and corresponded more closely with that of unmated ones. These patterns of behavior facilitate meeting between the sexes on the surface of the grain in the early weeks of life and also serve to sustain egg production during later life.

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*Movement of prepupation larvae and pupation site of Oryzaephilus surinamensis

During observations on the behavior of larvae of O. surinamensis, it was noted that before late larvae lost the power of movement, they moved to the bottom of the dishes in which they were kept. This response was therefore investigated in more detail. A metal tower, 15 cm. tall and 10 cm. diameter, in two sections, was filled with grain. When 10 late instar larvae were released, either at the top or the bottom of this tower, at least three-quarters of the pupae were recovered from the bottom section.

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*The response of adult Oryzaephilus surinamensis to living and inorganic substrata

In the course of an extensive study of the behavior of the saw-toothed grain beetle, the response to grain and non-living substrata has been investigated. Fifty adults have been released in glass dishes 12 cm. in diameter by 7 cm. deep at 25°C and 70 per cent relative humidity. Half of the floor of each dish used in the test was covered with either a layer of grain or glass balls or a piece of sacking. The numbers on these three substrata were counted every 30 minutes, from the time of release, up to 6 hours. Accumulation was as intense on the glass balls and sacking as on the grain. After about 2 hours, there were always significantly more insects on the test substrata than on the plain-glass side of the area. The

mechanism of this response was orthokinetic, the speed of individuals on plain glass being approximately 120 mm/min, while on the test substrata it was approximately 58 mm/min.

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*Differences in the locomotory behavior of susceptible and pyrethrin-resistant Sitophilus granarius

A technique developed for measuring the rate of movement, and other aspects of locomotory behavior, of insects in grain, has been used to study differences between the standard sock of Sitophilus granarius cultured at the laboratory and the pyrethrin-resistant strain produced by Lloyd and Parkin. At all stages of adult life, individuals of the susceptible strain moved significantly faster than those of the resistant strain. The rate of movement of susceptible individuals fell from about 80 mm/min. in the first week of life to about 40 mm/min. in the 10th week of life. There was no difference between males and females or between mated and unmated individuals. Over the same period of time, the rate of movement of pyrethrin-resistant individuals fell from about 40 mm/min. to 17 mm/min. At all times, more susceptible individuals were active than resistant ones.

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*The effect of grain size on the development of the grain weevil Sitophilus granarius

Using English wheat, it was found that heavier adults emerged from the heavier grains. With two weight classes of grain, 30-35 mgm and 50-55 mgm, the mean weight of emerging adults from each was 2.59 and 3.05 mgm at 20°C., 2.51 and 2.86 mgm at 25°C., and 1.99 and 2.41 mgm at 30°C. The mean developmental period (oviposition to emergence) decreased from 67 days at 20°C. to 33 days at 30°C.

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Request for information

A study is being undertaken of the structure and function of the femoral pit of T. castaneum. Information available to us is limited to the description given by Hinton (1942). Any information which TIB readers could provide would be welcomed and fully acknowledged.

NOTES - TECHNICAL

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Modifications of the Macdonald test kit for establishing the susceptibility of stored product insect pests to certain chemicals

The Macdonald test kit was developed at Macdonald College, Quebec, to provide a standard method which could be used on a world-wide basis for measuring variability of resistance of stored product insects to certain chemicals. The test kit consists of Dixie paper cups, insecticide-impregnated filter paper to line the interior of the cups, glass rings to prevent insect escape, and data report forms. Species of Tribolium, Cryptolestes, Sitophilus and Oryzaephilus are being treated for susceptibility to various concentrations of DDT, dieldrin, lindane, aldrin, and heptachlor. The insects were subjected to a pre-treatment period when they had no food, a treatment period when they were in contact with the impregnated papers, and a post-treatment or holding period. At the end of the holding period, dead, moribund, and live insects were counted and recorded. A control series of insects was set up in untreated cups.

During preliminary tests with the kit, it was noted that some insects escaped over the test rings. The use of vaseline as a barrier was suggested in the instructions supplied with the kit. However, insects became entangled in it, precluding its use. A modification of the test kit was made, i.e., replacement of the glass rings by 1/8-inch thick plexi glass discs. Three-quarter inch holes were made in the centers of the discs to provide aeration and finger grips. The discs were less fragile and easier to install than the glass rings. They prevented escape of all species tested except Sitophilus granarius L. which was able to walk on the discs. For tests with this species it was necessary to cover the holes with fine plastic mesh. Small plexi glass bars cemented on the discs provided finger grips.

Oryzaephilus surinamensis (L.), and particularly Cryptolestes ferrugineus (Stephens) escaped behind the treated papers lining the cups. This was prevented by placing a thin ring of melted paraffin around the lower edge of the paper lining the wall after it was fitted tightly to the bottom of the cup. The paper disc for lining the bottom of the cup was then quickly inserted and tamped around the outer edges to seal it against the paper lining the wall. Small cracks were filled with melted paraffin using a camel hair brush. The vertical juncture of the paper on the wall of the cup was similarly sealed.

The cups were transferred in groups to and from rearing cabinets on retainers to minimize handling of individual cups. The retainers consisted of rectangular pieces of 1/8-inch plywood, with holes to fit the cups,

mounted on 4-inch high end bases. Sufficient holes were made in each retainer to hold the number of cups required in each test. The retainers were stacked to fit the shelf spacings of the rearing cabinets.

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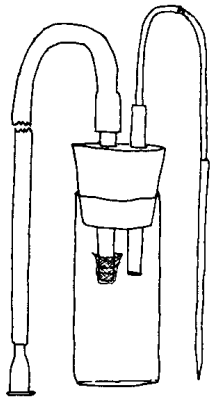
*A practical method for handling eggs and small larvae

A practical method for handling large numbers of eggs and small larvae has been worked out. Aspirators are commonly used by Drosophila workers and entomologists and are easily adapted for use with Tribolium.

An aspirator (sketch below) can easily be made with common laboratory supplies. A shell vial (about 2" high, 1" diameter) can be fitted with a rubber stopper. Glass tubing is especially useful as a tip when it is drawn out as fine as desired and the tip broken off until the diameter is a little larger than the eggs and/or larvae to be handled. A hematologist's rubber tube with a plastic mouthpiece is easy to hold on to. Very fine silk bolting cloth should be fitted over the bottom of the glass tube leading to the mouth to prevent inhalation of eggs or larvae. It would also be possible to use a vacuum line to provide suction if one is available.

Eggs and small larvae can be separated from flour, frass, etc. by methods outlined by Saunders and Krueger (1957, J. Econ. Ent. 50:693) and by Sokoloff (1959, TIB 2:36). A large number of eggs or tiny larvae can be quickly and easily picked up once separated from the flour. By using a desk or hand counter one can also count as the eggs are aspirated under a dissecting microscope. When the required number of eggs or larvae are present in the aspirator vial, the rubber stopper can be removed and fitted into a new, clean vial. It is easy to tap the eggs or larvae from the aspirator vial into the proper rearing container, especially if the rim of the glass vial is cleaned. Eggs and tiny larvae transferred in this manner are not damaged as they are if a brush or forceps are used.



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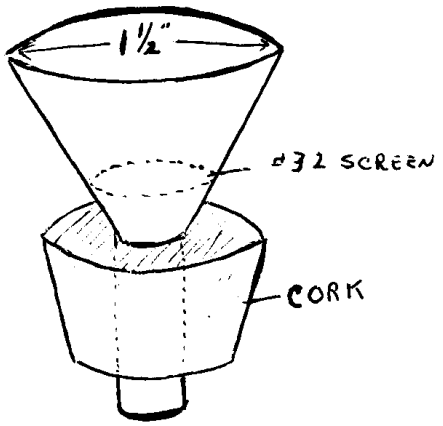
*A device for removing flour particles from Tribolium pupae for accurate pupal weights

Without a source of forced air, complete removal of flour particles from pupae with ordinary sifting could not be attained. Therefore, a brass funnel with a screen was constructed for this purpose. The funnel is 1 1/2" wide with a 1-inch stem. A #32 mesh/inch brass screen, 3/4" in diameter is then soldered to the inside surface of the funnel. This funnel is then glued into a bored-out cork which fits an eight-dram vial. Complete removal of flour particles could be achieved with a brush and slight blowing. This method is only adequate for small numbers of pupae.



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*Mazes for Tribolium

Several mazes were constructed for studies in behavior genetics of Tribolium similar to those of Hirsch and of Dobzhansky and Spassky on Drosophila.

I. Y-maze (Figure 1)

This maze was constructed from the following materials:

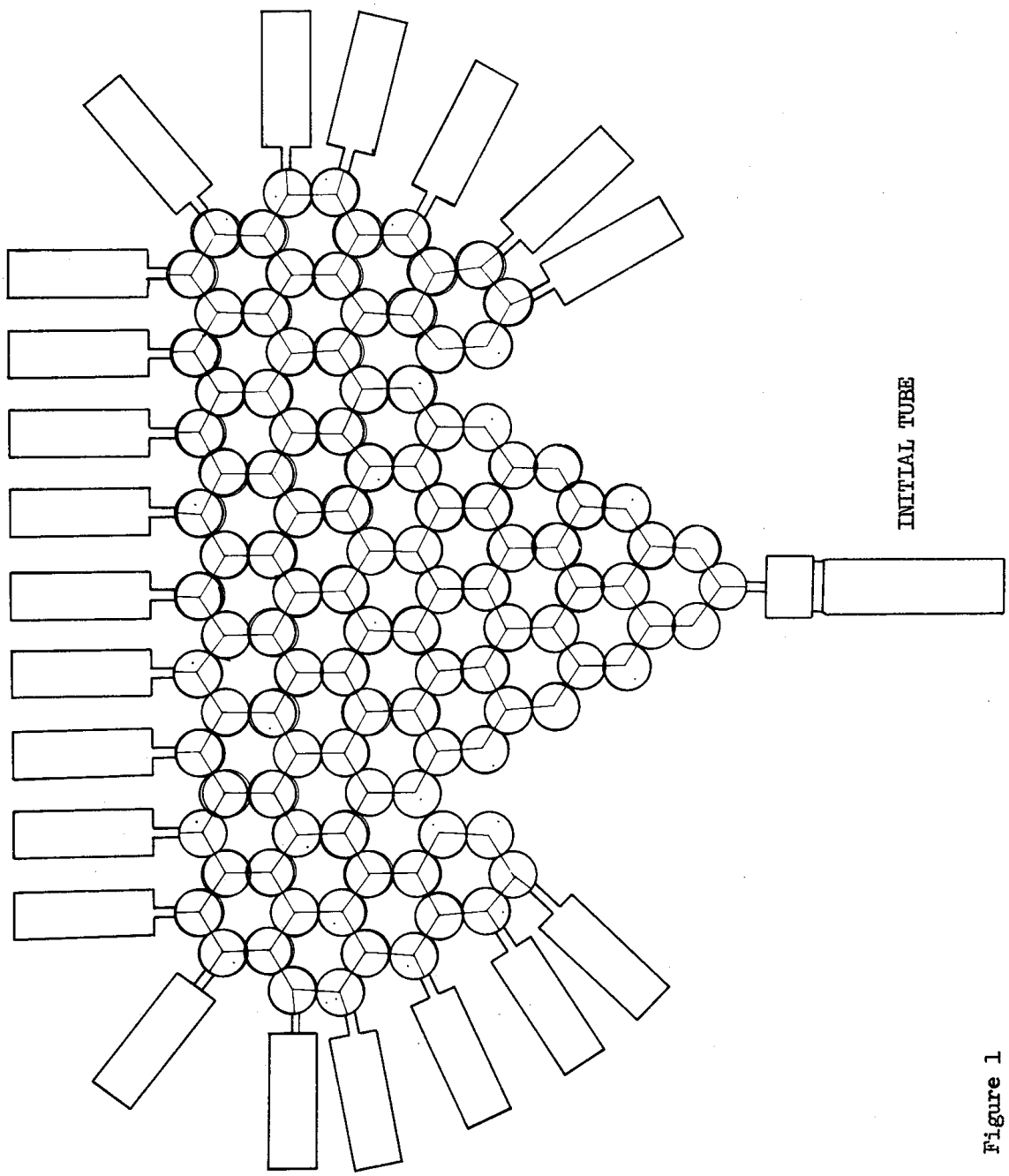

Figure 1

1. 3/4" extruded-acrylic-plastic rod
2. Polyethylene tubing with dimensions of: I.D. .085" - O.D. .128"
3. Twenty-one plastic vials (snap on cap) - 5 dram size.
4. One 50 ml - polypropylene - centrifuge tube
5. One 1-1/4" extruded-acrylic-plastic rod 1-1/2" long
6. 1/4" thick cork, 3/4" plywood, rubber bands, small eye hooks and straight pins.

The 3/4" plastic rod was first drilled with a 3/32" bit and later re-drilled larger with an 1/8" bit. The double drilling was necessary to prevent the plastic tubing from clogging the center of the discs, as is shown diagramatically in Figure 2. After drilling, the plastic rod was cut into approximately 3/8" thick disks which were then sanded and buffed. A minute hole was drilled in the center of each disc for air circulation.

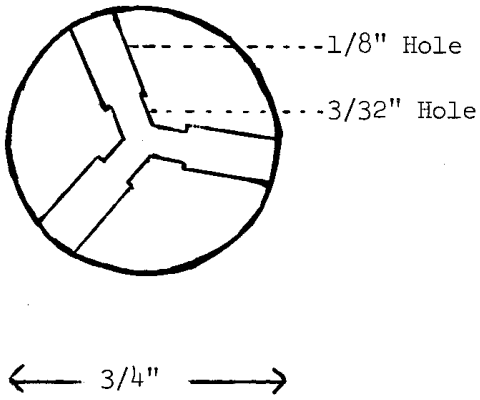

Figure 2

The polyethelene tubing was cut in lengths of approximately 1/2" and the pieces were inserted into the holes of the discs thus interlocking the plastic discs. The plastic (5 dram) vials were used as end-tubes connected to the terminal discs by the polyethylene tubing. The 50 ml centrifuge tube with a 1-1/4" plastic rod as a stopper was used as the entry to the maze. The stopper was funnelled inside to an opening of 1/8". With straight pins, the body of the maze (discs) was mounted on flat 1/4" cork which in turn was mounted on 3/4" plywood. Rubber bands with eye hooks were used to keep both the end initial tubes immobile.

II. The continuous T-maze (Figure 3)

This maze was made by using three acrylic-plexiglas sheets (9" × 15", 1/8", 3/8" and 1/4" thick respectively. The 1/8" sheet was permanently mounted on 18" × 18", 1/2" plywood. The 3/8" sheet was grooved with a router bit 5/64" wide and 1/16" deep and was then covered with the 1/4" sheet drilled with minute holes along the maze route at all T-unions for air circulation. The top two sheets, the cover and maze body were then screwed with set screws to the 1/8" sheet, permitting the components to be taken apart periodically for cleaning. The initial tube and polyethylene tubing were the same as in the Y-maze but the end tubes were of a smaller size (3 drams).

III. The straight T-maze (Figure 4)

This maze is similar in construction to II but does not have intermediate-joining grooves. The grooves are wider (1/8") and deeper (5/32"). Larger polyethylene tubing with dimensions of I.D. .148" O.D. .189" was used. The maze is completely constructed from sheets of acrylic plexiglas, the base being 13" × 19-3/4" × 3/8", the grooved body 2-5/8" × 12" × 1/2" and the top 2-5/8" × 12" × 3/8".

Results:

All maze runs were conducted in a converted Jamesway Poultry incubator in the dark at 29°C and 70% relative humidity. Preliminary test runs with the Y-maze showed that isolated females ran through the maze faster than isolated males which in turn performed better than groups of mixed sex. If the beetles were semi-starved for 72 hours (1/4 teaspoon of whole wheat flour with 5% yeast for approximately 300 beetles) and if a reward of flour (1/2 teaspoon) was provided in the end tubes, the runs were faster.

Selection for tendency to right and left turns, respectively attempted for three generations in the Y-maze was unsuccessful. Selection for geotaxis was also tried by placing the Y-maze on its side using only 15 end tubes. The results were erratic, but because of a tendency on the part of the beetles to aggregate in the lower end tubes, suggesting that the inner-tube walls were too smooth for upward movement. Similar discouraging results were also obtained in the continuous T-maze. It was felt that this maze could not give a valid demonstration of the tendency of the beetles to turn in one or the other direction because a beetle selected from the extreme right tube could have made all left turns up to the last horizontal groove. In both of these mazes frequent traffic jams occurred with the beetles going in circles, thus yielding erratic and inconsistent results. The straight T-maze was constructed in the hope of eliminating such inconclusive results. This maze is set in the incubator vertically on its right side and we are now selecting for speed of running and for geotaxis. The first generation of selection with T. castaneum indicates that running speed can be selected for successfully.

The assistance of Mrs. Barbara Strong in carrying out the experiments and in the preparation of the figures is gratefully acknowledged.

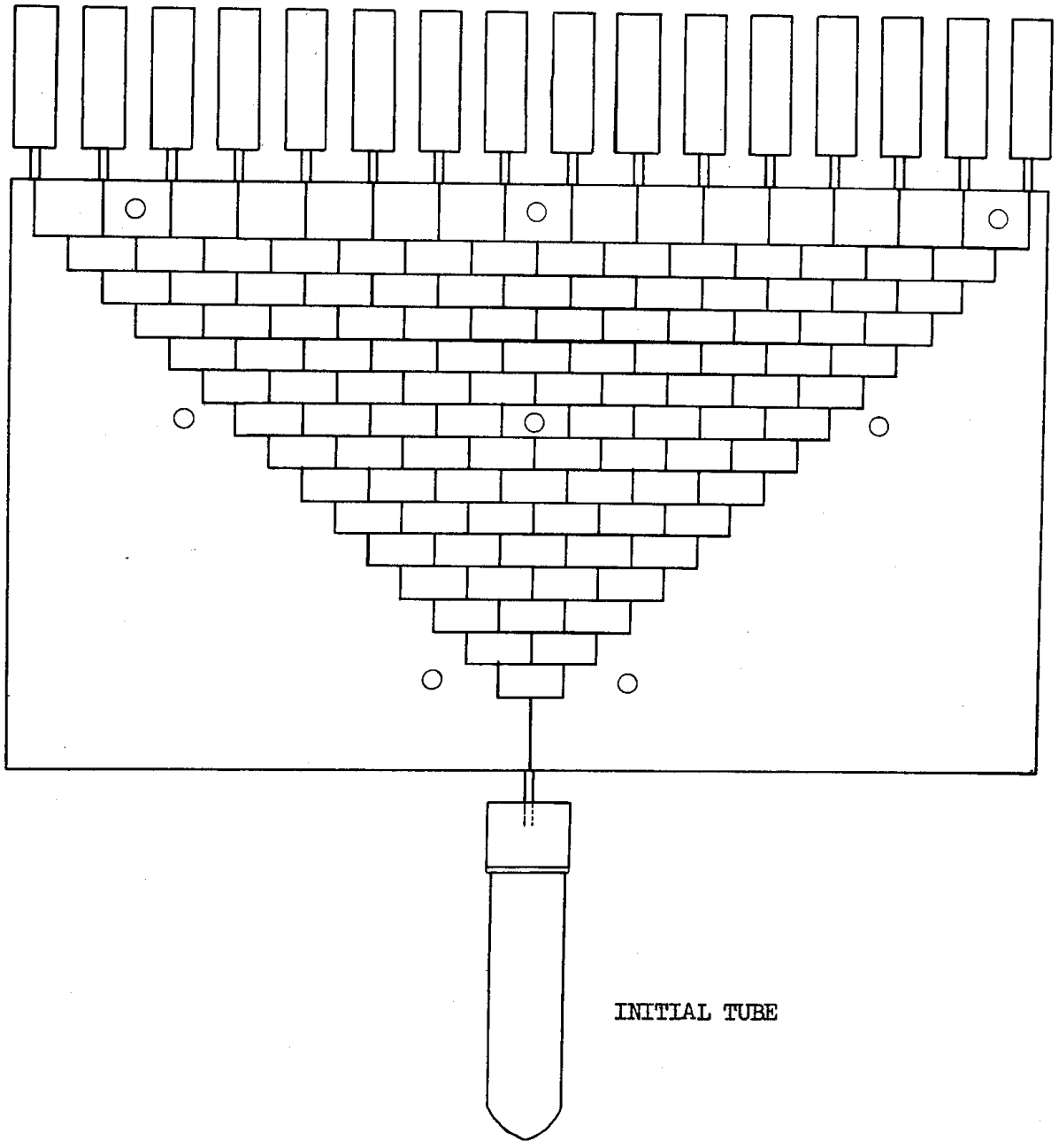

Figure 3

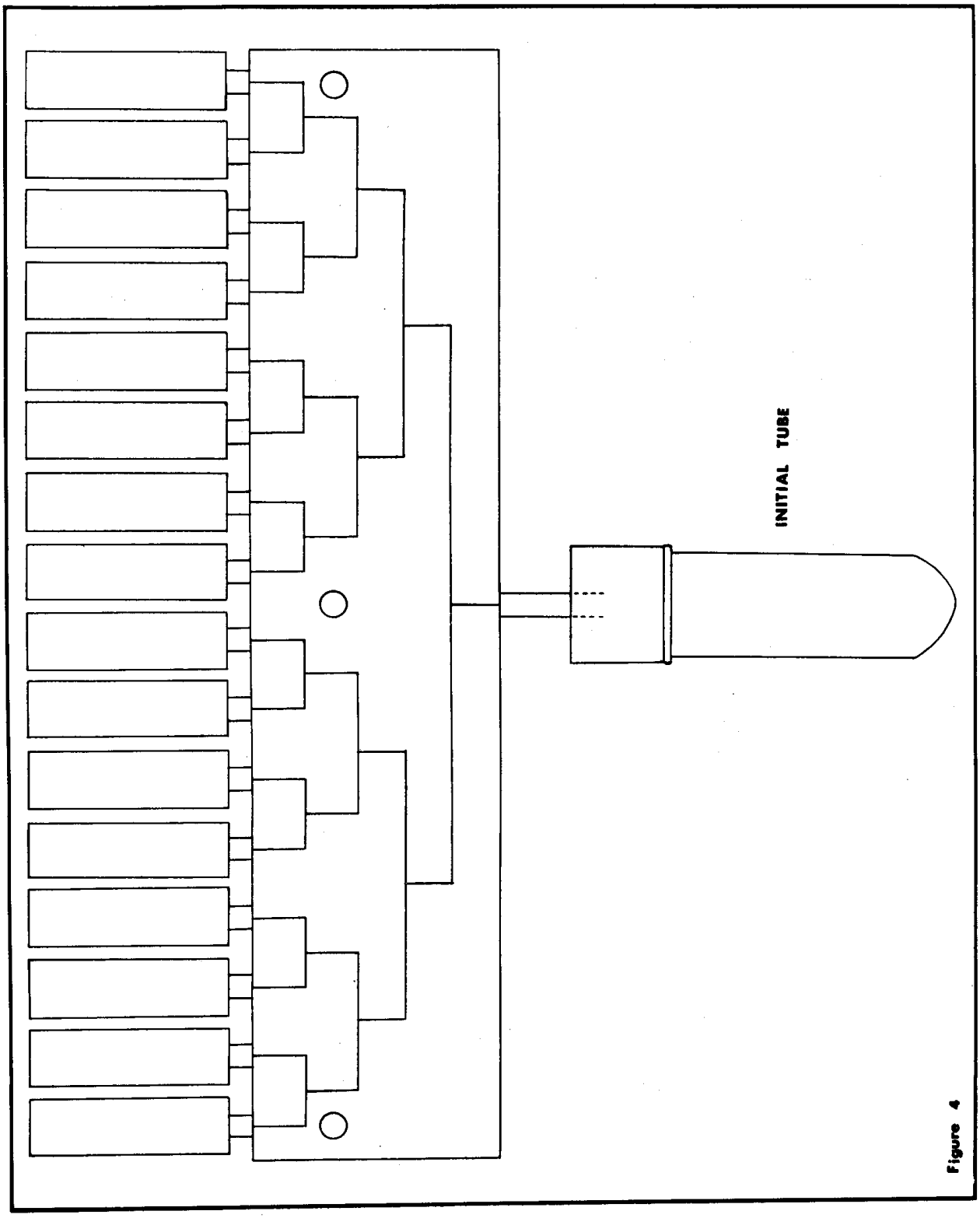

Figure 4