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*Two homeotic mutants affecting the urogomphi and the genitalia

1. Tetra-urogomphi (Tu)

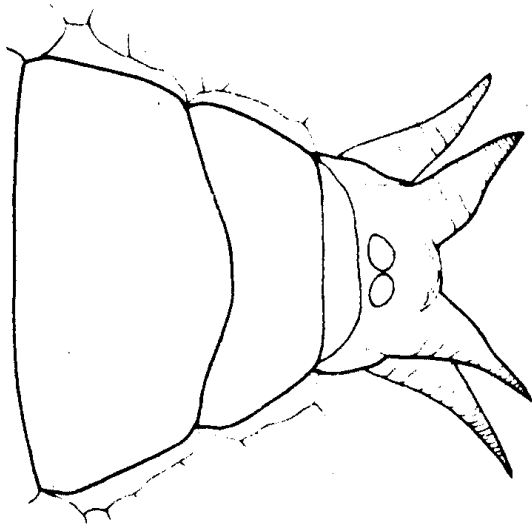
One ♀ pupa was discovered in a selection vial from a stock containing the genes ca, ju, ct that had four urogomphi. She was bred to synthetic +/+ ♂: her progeny yielded two larvae with extra urogomphi. These F₁ ♂ Tu were mated with normal sib ♀♀ to establish a stock that contained many Tu individuals with strong expression of the gene. Larvae, pupae and adults are affected. Larvae may have three or four urogomphi (two is the normal number). The new urogomphi are posterior to, and usually slightly smaller than, the normal urogomphi. (See Figure 1a, b, c, d.) Expression may vary yielding larvae with four large urogomphi, larvae with three large urogomphi plus a small "plate," larvae with two normal urogomphi plus two "plates" varying in size. The "plates" appear to be the new urogomphi in an incompletely everted state, i.e., like the uneverted finger of a glove. The plates are sclerotized and pigmented like the normal urogomphi.

Pupae are also affected variously: there may be four urogomphi, three urogomphi, or two plus small fleshy "lobes." The new urogomphi are again slightly smaller than the normal urogomphi. In addition, the genital region of the pupa may be involved. Male pupae are occasionally found with new fleshy "growths" posterior to the genital lobes. So far, however, no male pupae have been discovered without any genital lobes (compare with the mutant Eu). Female pupae may have abnormal genitalia: some pupae have been found with a single medial lobe posterior to the two normal genital lobes. In addition, a few female pupae have been discovered with four genital lobes; the new genital lobes are posterior to and comparable in size with the original genital lobes.

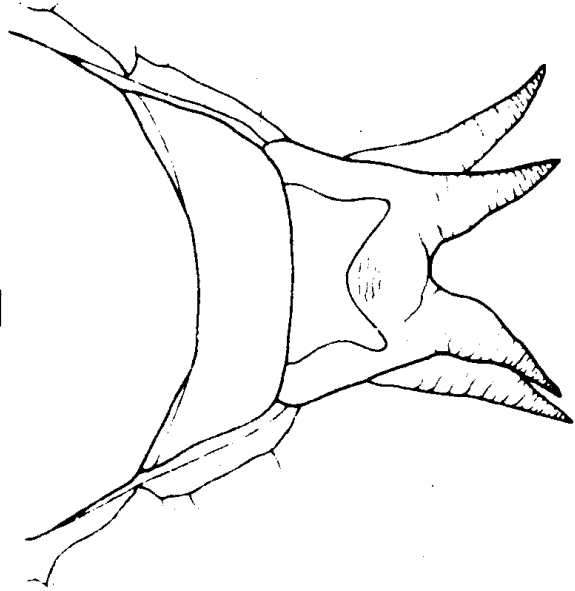
Adult Tu beetles may show a number of abnormalities. Tu males may be entirely normal as adults; they may have two aedeagi; they may have two aedeagi plus two fleshy lobes lateral to the anus. Males may have one aedeagus plus the fleshy lobes; or, occasionally they may have a "tumorous," hypertrophied aedeagus lacking almost completely in sclerotization and pigmentation. The latter males are, of course, sterile--as are the males with two aedeagi.

Adult Tu females may appear entirely normal; they may have fleshy lobes lateral to the ovipositor; they may have partly duplicated ovipositors (if they had three or four genital lobes). Females with partially duplicated ovipositors are capable of reproduction.

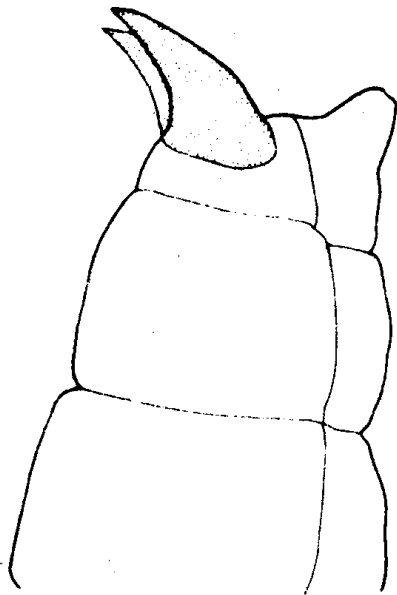
The gene Tu is an autosomal semi-dominant with recessive lethal effects. The Tu/+ heterozygote shows up only about 10-15% of the expected



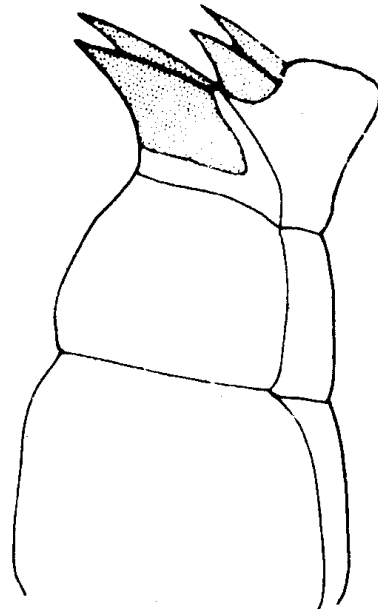
c. Tu ♂ pupa



d. Tu ♀ pupa



a. normal larva



b. Tu larva

Fig. 1

frequency due to its lethality. The eggs and first instar larvae appear to be the primary sources of deaths due to the Tu gene, but all stadia are less viable than normal--in part due to their difficulty during ecdysis to rid themselves of the exuvium from their terminal segments.

2. Extra urogomphi (Eu)

Eight larvae were found that had extra urogomphi in a cross of two ppas mgt-like ♂ × 5 F₁ ♀ virgins (ppas/+, mgt-like/+). A stock was started. Larvae have four large urogomphi in strong expression of the gene. Pupae also have four large urogomphi in the strongest expression of Eu; in addition, the pupae sometimes have abnormal genital lobes. In some female pupae there are three or four genital lobes visible. Male pupae may appear abnormal in the genital region in one of two ways: (1) the genital lobes may be missing completely, (2) the genital lobes may be hypertrophied into a large fleshy mass of tissue protruding from the surface of the pupa at an angle of approximately 90°. Adult beetles may show several results of the gene also. All males that were Eu as larvae and pupae have no aedeagus. They are therefore effectively sterile. A stock of Eu can be maintained only by mating Eu females to +/+ males. Approximately 20-30% of the progeny, both ♂ and ♀ are Eu, indicating that Eu is a dominant gene with some lethality. Penetrance and expression appear to be good in the background in which it is at the present time.

My special thanks to Mrs. Barbara B. Daly who kindly prepared the illustrations.

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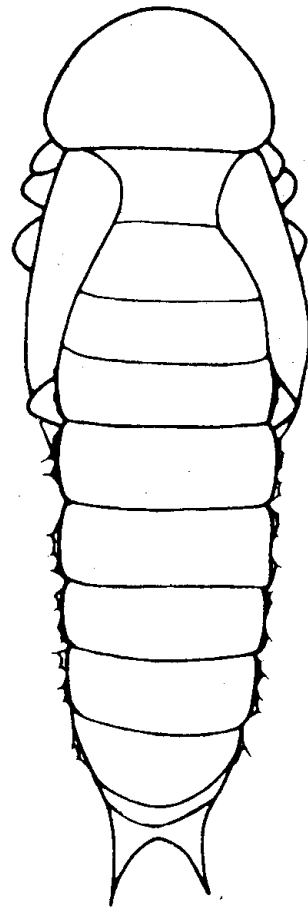
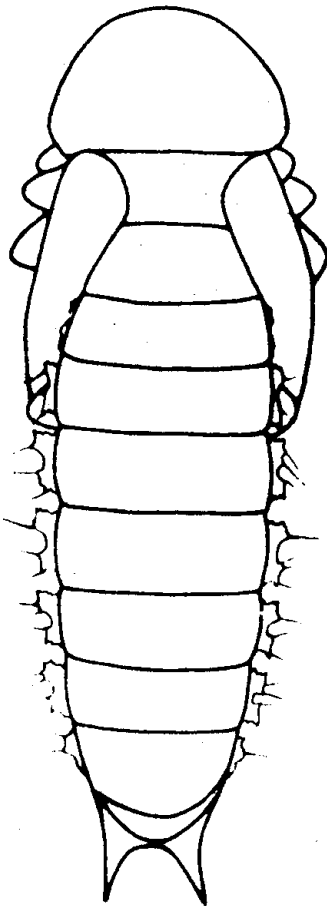
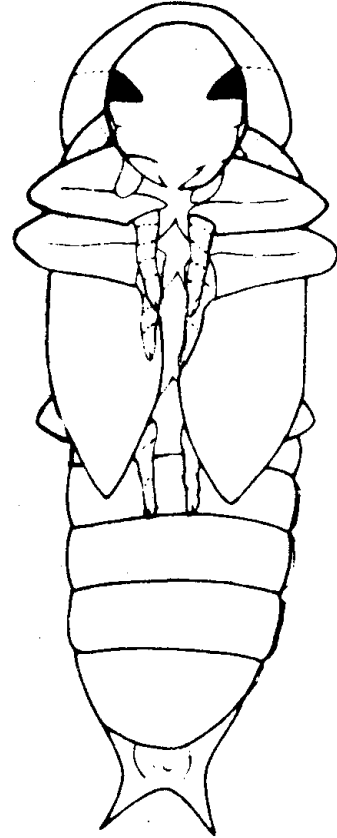
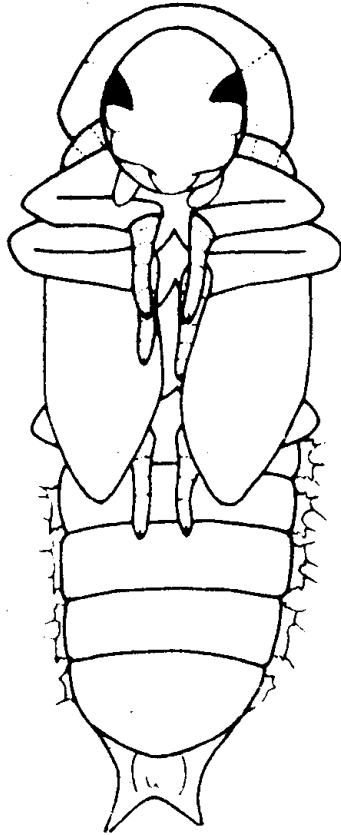
*"Reduced gin traps" (rgt)--A gene affecting only the pupal stadium in Tribolium castaneum

A stock containing spl^{PR}, fas-3, pdp, col, and ro yielded 30 pupae, including males and females, that had gin traps so reduced that only a little of the pigmented, heavily sclerotized "teeth" remained. The gin traps are reduced along the entire length of the abdomen; usually both sides are affected. Reciprocal crosses of rgt × +/+ failed. Two crosses of rgt × rgt failed. A selection jar containing the rest of the original rgt pupae produced only pupae with reduced gin traps. The reductions varied from very strong to moderate. No pupae had normal gin traps.

Pupae with the strongest expression have been selected for stocks, and pure stocks are available. The mode of inheritance has not been completely worked out. However, it is interesting to note that here we have a gene(s) that affect only one stadium, the pupa, since gin traps are a

+

rgt



purely pupal characteristic.

If the condition reduced gin traps is heritable in a simple manner, and can be isolated from the other genes (probably deleterious) in the stock we would have the means to test Hinton's (1946) assertion that the gin traps of Tenebrionidae are used as protection against cannibalism by larvae (and adults?). (Even if the condition rgt cannot be separated from the accompanying genes, there are stocks which contain pdp, fas-3, ro which do not have reduced gin traps and could be used to compare the effectiveness of gin traps in the survival of pupae subject to cannibalism.)

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Preliminary observations on the incidence of Hymenolepis diminuta in the flour beetle, Tribolium confusum, as a function of age

All stages of the beetles were maintained in a medium consisting of 95% Gold Medal Wondra bleached white flour and 5% National Active Dry yeast. Eggs randomly selected from a stock culture were used to establish a population of beetles of known ages. When the adults of these cultures were 18 to 28 days old, eggs were removed and were placed in individual 12 x 35 mm. patent lip vials containing approximately 5 mm. of the medium. In this manner, selection of the first eggs laid by the beetles of known age was avoided. The individually isolated beetles were put into fresh vials and medium every two weeks. All cultures were kept at a temperature of 77°F, a relative humidity of 71% and under constant light.

For the experimental work, T. confusum was considered to be "young" at 4-5 weeks following eclosion, "middle-aged" at 23-24 weeks and "old" when 47-51 weeks.¹ Virgin adult beetles obtained from young parents and maintained in individual vials throughout the experimental periods were used in the experiments reported here.

The beetles were divided into two groups, those starved 5-6 days and those starved 7-8 days. Following the starvation period, all beetles were

¹ Adult Tribolium confusum was considered to be "old" on the basis of parental age studies by Raychaudhuri and Butz, both in published work (Raychaudhuri, A. and A. Butz, 1965. Aging. I. Effects of parental age on the life cycle of Tribolium confusum (Coleoptera, Tenebrionidae), Ann. Entomol. Soc. Amer. 58:535-542) and in a personal communication with Raychaudhuri.

allowed to feed for a 24-hour interval on 3-4 freshly obtained gravid segments of *H. diminuta*. The male Sprague-Dawley rats from which the tapeworms were removed were inoculated per os at 5 weeks of age with 3-5 cysticercoids dissected from infected meal beetles, *Tenebrio molitor* (Carolina Biological Supply Co.). The infections in the rats were 5-15 weeks post-inoculation. Only those *Tribolium* which were observed to have fed on proglottids were used in the accumulation and analysis of the data recorded below. After their exposure to the proglottids, the beetles were returned to vials containing fresh medium for a period of at least 14 days prior to being preserved in 10% formalin. The preserved beetles were dissected and examined for cysticercoids. The sex of the beetle, which was initially determined in the pupal stage, was checked at the time of dissection.

The results of this investigation to date are summarized in Table 1. The data show that there was no pronounced change in the incidence of cysticercoids in young and middle-aged females. However, it appears that there was a decrease when the females were old. This decrease was not observed in males but rather an increase in the incidence occurred in the middle-aged group of males.

Table 1.--The percentage of *Tribolium confusum* adults infected with *Hymenolepis diminuta*.

		No. of days starved					
		5 to 6		7 to 8		Total (5 to 8)	
		Total number studied	Percent infected	Total number studied	Percent infected	Total number studied	Percent infected
Young:							
4 to 5	Females	33	78.8	41	82.9	74	81.0
weeks	Males	39	71.8	30	50.0	69	62.3
Middle-aged:							
23 to 24	Females	42	78.6	42	83.3	84	81.0
weeks	Males	30	80.0	33	87.9	63	84.1
Old:							
47 to 51	Females	31	6.0	26	46.2	57	24.6
weeks	Males	32	68.8	42	52.4	74	59.5

The duration of the starvation appears to be inversely related to the incidence of cysticercoids in young and old males. The incidence in old

females is presently under further investigation but apparently there is a pronounced decrease in all old females when compared to those of young and middle ages.

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*Genotype-environment interactions in the outcome of "competition"
in Tribolium. I. Population cage studies

The evidence (Sokoloff, Lerner and Ho, 1965. Am. Nat.; Inouye and Lerner, 1965. J. Stored Prod. Res., and others) is rapidly accumulating in favor of the interpretation that the interspecies behavior of T. castaneum and T. confusum introduced into a common environment does not illustrate competition as technically defined by Birch (1957. Am. Nat.). Rather the phenomenon observed is a mutual predator-prey relationship in which each species acts both as predator and prey. We use the term "competition" to describe this situation for two reasons: (1) this term has been applied by Elton and Miller (1954. J. Ecol.) in a very broad sense to situations in which one species affects the population of another by a process of interference, that is, by reducing the reproductive efficiency or increasing the mortality of its competitor; (2) although we recognize that the phenomenon being studied is more properly predation than competition, it is the most useful single-word term with which we are familiar which describes an interspecies relationship leading to the extinction of one species and the survival of another.

The results presented here were gathered for a somewhat different purpose but, pending the completion of body measurements of beetles reared in different media and environmental conditions, we wish to bring these data to the attention of others, since they serve very well to illustrate the genotype-environment interactions in the outcome of "competition." In addition, the data show in part certain food preferences of T. castaneum and T. confusum.

The data were obtained from population cages designed by Inouye (see Technical Note in this issue of TIB). The single-species cages were initiated with 200 pairs of beetles, and the mixed-species cages with 100 pairs of each species. In each cage the central chamber contained a mixture of corn (C), rice (R), soy (S) and whole wheat (W) of uniform particle size with 5% brewer's yeast added. Attached to the central chamber were eight side chambers each containing only one medium. In a clockwise direction the side chambers contained C, R, S, W and C, R, S, W, respectively, each medium with the brewer's yeast additive.

Different strains were kept in different cages, as it appears in the heading of the various tables. The first four of them summarize the data

Table I. Distribution of CF in the food chambers containing various media at 29° C. and 60% R.H.

Transfer	Mixture N	Corn N	Rice N	Soy N	Wheat N	Total
1	130	53	107	12	97	399
2	737	373	838	154	699	2,801
3	229	132	38	8	89	496
4	516	259	457	65	368	1,665
5	672	50	104	22	110	958
6	1,115	187	371	75	241	1,989
7	1,230	165	225	195	173	1,988
8	1,790	157	248	90	165	2,450
9	1,484	197	178	95	133	2,087
10	2,750	298	234	141	252	3,675
11	2,207	142	204	46	206	2,805
12	2,453	183	336	116	140	3,228
13	3,096	169	148	161	185	3,759
14	3,229	166	104	78	278	3,855
15	1,972	146	95	107	233	2,553
16	2,043	150	69	7	85	2,354
17	2,680	187	105	78	149	3,199
18	2,427	220	85	140	91	2,963
19	2,754	225	112	87	129	3,307
20	1,516	177	87	105	109	1,994
21	2,426	114	33	61	138	2,772
22	2,052	164	56	75	130	2,477
23	1,851	119	46	58	113	2,187
24	<u>2,228</u>	<u>209</u>	<u>75</u>	<u>61</u>	<u>85</u>	<u>2,658</u>
Total	43,587	4,242	4,355	2,037	4,398	58,619

Table II. Distribution of CS in the food chambers containing various media at 29° C. and 60% R.H.

<u>Transfer</u>	<u>Mixture</u>	<u>Corn</u>	<u>Rice</u>	<u>Soy</u>	<u>Wheat</u>	<u>Total</u>
1	332	68	48	45	38	531
2	4,090	332	178	171	204	4,975
3	1,574	185	128	49	191	2,127
4	1,498	136	146	87	212	2,079
5	1,141	77	90	39	67	1,414
6	1,673	260	168	151	234	2,486
7	1,436	244	123	91	106	2,000
8	2,063	162	195	92	129	2,641
9	1,675	200	147	107	145	2,274
10	1,782	196	164	127	189	2,458
11	1,305	101	42	51	70	1,569
12	1,920	127	98	64	71	2,280
13	1,760	149	131	61	78	2,179
14	1,450	135	81	28	38	1,732
15	1,668	108	70	40	64	1,950
16	1,411	51	28	43	53	1,586
17	2,067	124	90	33	48	2,362
Total	28,845	2,655	1,927	1,279	1,937	36,643

Table III. Distribution of CF in the food chambers containing various media at 29° C. and 40% R.H.

<u>Transfer</u>	<u>Mixture</u>	<u>Corn</u>	<u>Rice</u>	<u>Soy</u>	<u>Wheat</u>	<u>Total</u>
1	223	35	72	6	55	391
2	6,567	923	693	222	520	8,925
3	631	64	32	25	272	1,024
4	2,361	712	614	228	218	4,133
5	3,612	249	135	397	652	5,045
6	1,547	427	200	129	222	2,525
7	1,883	442	382	104	181	2,992
8	1,924	489	221	144	258	3,036
9	2,080	491	327	234	193	3,325
10	1,652	78	193	71	104	2,098
11	2,181	343	158	135	185	3,002
12	1,433	110	75	81	61	1,760
13	1,674	104	72	57	81	1,988
14	1,476	65	66	81	109	1,797
15	944	41	36	31	113	1,165
16	1,136	62	36	35	85	1,354
17	1,137	96	51	30	45	1,359
18	939	59	51	49	83	1,181
19	956	62	58	16	23	1,115
Total	34,356	4,852	3,472	2,075	3,460	48,215

Table IV. Distribution of CS in the food chambers containing various media at 29° C. and 40% R.H.

<u>Transfer</u>	<u>Mixture</u>	<u>Corn</u>	<u>Rice</u>	<u>Soy</u>	<u>Wheat</u>	<u>Total</u>
1	155	53	96	15	66	385
2	657	212	340	205	263	1,677
3	658	96	120	47	156	1,077
4	726	118	163	16	149	1,172
5	1,696	140	342	38	339	2,555
6	2,496	206	250	136	331	3,419
7	2,160	484	274	51	399	3,368
8	2,180	403	193	70	251	3,097
9	1,565	299	341	21	181	2,407
10	2,425	430	237	116	319	3,527
11	2,394	215	227	33	109	2,978
12	3,087	323	148	54	242	3,854
13	3,025	213	167	43	206	3,654
14	2,147	138	113	26	138	2,562
15	2,008	216	156	30	69	2,479
16	1,783	307	209	82	138	2,519
17	2,443	188	176	161	144	3,112
18	1,729	206	101	146	70	2,252
Total	33,334	4,247	3,653	1,290	3,570	46,094

Table V. Distribution of synthetic CF and CS in the food chambers containing various media at 29° C. and 60% R.H.

Transfer	Mixture		Corn		Rice		Soy		Wheat		Total	
	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS
1	204	370	45	20	21	14	10	44	20	14	300	462
2	426	3,128	41	158	30	165	5	146	61	310	563	3,907
3	19	1,552	5	241	6	236	0	58	6	286	36	2,373
4	15	1,358	4	260	7	218	0	138	5	368	31	2,342
5	8	1,293	1	130	0	95	0	33	0	103	9	1,654
6	22	1,614	5	204	2	112	1	117	1	162	31	2,209
7	5	1,813	1	199	1	129	0	79	0	100	7	2,320
8	0	1,528	0	101	0	87	0	56	0	68	0	1,840
9	0	2,674	0	261	0	129	0	78	0	103	0	3,245
10	0	829	0	162	0	84	0	42	0	71	0	1,188
Total	699	16,159	102	1,736	67	1,269	16	791	93	1,585	977	21,540

Table VI. Distribution of synthetic CF and CS in the food chambers containing various media at 29° C. and 40% R.H.

Transfer	Mixture		Corn		Rice		Soy		Wheat		Total	
	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS
1	120	151	43	20	18	18	1	16	21	17	203	222
2	2,873	2,377	175	140	229	217	32	47	232	84	3,541	2,865
3	569	1,271	73	117	30	234	16	46	41	225	729	1,893
4	939	1,835	121	236	87	180	12	89	99	246	1,258	2,586
5	864	1,228	94	137	75	243	34	50	56	232	1,123	1,890
6	1,092	856	143	133	64	197	21	55	95	219	1,415	1,460
7	1,251	769	136	129	82	209	94	81	88	217	1,651	1,405
8	1,400	1,004	159	94	47	112	37	45	55	77	1,698	1,332
9	1,524	636	117	112	62	104	64	85	86	97	1,853	1,034
10	1,332	545	105	50	77	113	44	70	97	139	1,655	917
11	1,542	386	61	65	46	67	33	30	45	42	1,727	590
12	1,488	466	79	99	67	70	46	14	58	55	1,738	704
13	1,166	524	68	70	38	66	28	16	49	33	1,349	709
14	795	732	39	103	27	66	22	38	41	55	924	994
15	591	1,046	25	81	23	139	16	36	38	58	693	1,360
16	348	1,582	13	109	15	189	5	94	16	122	397	2,096
17	239	1,385	11	121	22	189	5	35	16	113	293	1,843
18	151	1,765	2	163	16	300	0	28	11	124	180	2,380
19	91	1,767	8	236	11	185	2	37	19	203	131	2,428
20	46	1,527	5	253	3	132	0	34	9	118	63	2,064
21	22	1,881	6	248	3	180	0	65	9	165	40	2,539
22	15	1,761	4	130	0	94	0	58	0	114	19	2,157
23	6	1,556	4	186	3	175	0	43	0	107	13	2,067
24	6	2,114	0	180	0	164	0	70	1	169	7	2,697
25	0	1,402	0	151	0	166	0	48	0	75	0	1,842
26	0	1,558	0	108	0	179	0	96	0	190	0	2,131
27	0	1,682	0	94	0	148	0	59	0	195	0	2,178
Total	18,470	33,806	1,491	3,565	1,045	4,136	512	1,385	1,182	3,491	22,700	46,383

Table VII. Distribution of inbred CFI-11a and CSI-2c in the food chambers containing various media at 29°C. and 60% R.H.

Transfer	Mixture		Corn		Rice		Soy		Wheat		Total	
	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS
1	216	243	33	65	36	32	14	10	104	50	403	400
2	330	322	54	58	37	51	13	24	99	26	533	481
3	79	237	21	57	14	31	9	14	27	22	150	361
4	104	314	28	55	24	38	6	18	25	10	187	435
5	60	267	9	42	8	19	2	17	6	29	85	374
6	34	307	8	71	2	44	3	24	19	29	66	475
7	23	401	17	41	3	57	0	18	7	32	50	549
8	10	303	3	51	1	38	0	15	3	27	17	434
9	7	378	3	60	5	49	0	20	10	41	25	548
10	3	331	2	54	4	52	0	38	3	48	12	523
11	1	288	2	65	2	48	0	32	1	111	6	544
12	1	490	2	64	1	33	0	24	4	27	8	638
13	2	398	0	49	0	29	0	24	1	63	3	563
14	0	399	1	85	0	44	1	26	0	24	2	578
15	0	316	0	85	0	44	0	16	0	47	0	508
16	0	441	0	54	0	33	0	16	0	34	0	578
17	0	649	0	40	0	54	0	39	0	66	0	848
Total	870	6,084	183	996	137	696	48	375	309	686	1,547	8,837

Table VIII. Distribution of inbred CFI-11a and CSI-2c in the food chambers containing various media at 29° C. and 40% R.H.

Transfer	Mixture		Corn		Rice		Soy		Wheat		Total	
	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS	CF	CS
1	109	96	29	55	19	33	4	5	38	15	199	204
2	901	66	111	7	64	11	45	2	103	3	1,224	89
3	1,740	41	176	17	93	26	58	7	155	12	2,222	103
4	3,173	21	396	2	418	0	277	0	365	2	4,629	25
5	1,542	24	302	6	214	5	129	1	245	2	2,432	38
6	4,547	1	538	0	432	0	53	0	222	0	5,792	1
7	3,607	8	530	1	480	8	61	1	667	2	5,345	20
8	2,985	0	435	0	372	0	70	0	351	0	4,213	0
9	2,374	3	433	0	242	0	23	0	320	0	3,392	3
10	1,722	0	272	0	427	0	19	0	323	0	2,763	0
11	2,296	0	334	0	166	0	24	0	248	1	3,068	1
12	1,384	0	261	0	247	0	30	0	341	0	2,263	0
13	2,345	0	377	0	137	0	25	0	442	0	3,326	0
14	1,190	0	123	0	136	0	3	0	143	0	1,595	0
15	2,081	0	225	0	84	0	19	0	182	0	2,591	0
Total	31,996	260	4,542	87	3,531	83	840	16	4,145	37	45,054	484

from the single species Berkeley synthetic strains of T. castaneum (CS) and T. confusum (CF) at 29° and 60 per cent R.H. and at 29°C. and 40 per cent R.H. Every month the medium was renewed, the adults scored and discarded, and the cages continued with the larvae and pupae present. The data in tables I-IV are self-explanatory: there are clear-cut differences in distribution of the two species, both CS and CF preferring the mixture to the single media, and both species generally avoiding the soya medium which appears to be toxic to both species.

Once it was established that the two species could persist indefinitely under the conditions stated, competition experiments were initiated by placing 100 pairs of each species in a cage under the same conditions as in the single-species cages.

In the first of two competition experiments the synthetic strains (see Lerner and Ho, 1961. Am. Nat.) were used. The data in tables V-VI show that under both conditions CS eliminated CF, although under 29°C. and 40 per cent R.H. at one stage (Table VI Transfers 7-13) CF outnumbered CS. The second competition experiment involved two inbred strains of each species. In this case CF lost at the higher, but won at the lower humidity.

One of the purposes of this experiment was to determine whether or not the poorer competitor might coexist with the superior one in this closed environment by retreating to a niche in which it may have an advantage. Under the conditions of these experiments this proved not to be the case.

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*Some further observations on Tribolium confusum populations

Populations of McGill black and a wild red strain of Tribolium confusum were maintained in cages for over three years (see McDonald, American Naturalist 97:383-396 for cage details). The average number of adults in the black and red populations between weeks 13-62 was about 3800 and 3000 respectively. At a later period (weeks 80-179) the numbers were 4600 and 2500. During this latter period the adult population remained fairly constant in size in all cages. The number of dead adults recovered during the latter period provided a minimal estimate of weekly mortality. From this data, in conjunction with the live adult number, maximal estimates of adult longevity were calculated to be 16.3 and 12.6 weeks for the black and red strains. The average number of eggs was about 6500 in the black populations and 4800 in the red during the latter period. Since these differences in population characteristics must reside in the genetic constitution of the two strains, black and red strains were reisolated from other population cages where a mixture of the two forms had been maintained for over two years. Presumably, the genetic background of the strains was now similar and differences in

population characteristics are more likely the result of genic substitution at the black locus. Populations of reisolated black and red contained about 4000 and 5750 live adults respectively between weeks 80-129. The black strain does not appear very different from what it was before but the red strain does. Now this strain forms a much larger population. The gene pool created by the previous cage experience makes the attainment of greater population densities possible for the red individuals but fails to do this for the black ones. Possibly this difference in the response of the mutant and wild type genes may be a cause of the declining frequency of black in the mixed populations (See McDonald and Peer, Genetics 45:1317-1333).

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Studies on the effects of ionizing radiations
on *Tribolium confusum*

Sexed, adult, *Tribolium confusum* are employed in studies on the effects of ionizing radiations. Adult beetles are subjected to Cobalt-60, Cesium-137, gamma or 280 KVP X-radiation. Studies on the modification of radiation sensitivity by temperature, dose-rate, and oxygen tension are in progress. Median lethal dose, death frequency distribution, and lethal dose in 24 hours, following irradiation are employed as end-points. Sub-optimal pre and post-radiation temperatures markedly increase median lethal dose values. Super-optimal temperatures enhance the lethal effects of gamma irradiation.

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Studies on the effects of methyl bromide fumigation on
mycetomal microorganisms of *Sitophilus granarius*

Effects of methyl bromide fumigation for several generations on the mycetomal microorganisms of *Sitophilus granarius* (which are maternally inherited in the cytoplasm) have been studied. Results have been published (see bibliography). Other aspects of this work are being investigated.

The ultrastructure of the mycetomal microorganisms and their peripheral membranes have been studied. Two papers are in press--one has been published (see bibliography).

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Individual effect of the male on the egg laying rate of
fecundated females in *Tribolium castaneum**

It is well known that *Tribolium castaneum* virgin females lay eggs at a very low rate as compared with fecundated ones.

In our genetic research we are working with egg production as the character selected. We have defined a measure for that character as follows: number of eggs which a female lays during the period of four days between the 7th and the 11th day after adult emergence.

From several experiments investigating the influence of different agents on egg production, we have obtained quite variable figures; but we can give an average for egg number during those four days:

Virgin females: 18 ± 7 eggs
Fecundated females: 67 ± 11 eggs

Given that the males influence the egg laying rate of females mated to them by increasing it in a great amount, we were interested to know if some males influence more or less or on the contrary if the presence of the male (fecundation) produces only some kind of threshold stimulus but no more. In other words, will the laying rate of a fecundated female be different depending on the male to which she was mated?

Four experiments were run mating every male to two (exp. A, B and C) or four (exp. D) females and later evaluating the laying rate of each female individually. In this way it is possible to compare the part of the variance due to males with the individual variance and to conclude whether there exists some individual effect from the males.

A strain of *Tribolium castaneum*, so-called "Consejo" was used. The environmental conditions were: Temperature 32°C; humidity 70% R.H.; medium standard (95% wheat flour, 5% brewer's yeast). The mating was just immediately after emergence from the pupa stage. Seven days later the females were placed in individual containers with 3 gms. of fresh medium, discarding the males. Four days later the eggs laid per individual female were separated by suction and counted.

Table 1 gives some values from the analysis of the four trials run and Table 2 includes the analysis of the four as a unit. Experiment A gives

* Partial and preliminary study included in Grant No. FG-Sp-137 of P.L. 480, contract with the USDA.

no significant effects for males, but the other three give significance to different levels (0.05; 0.005 and 0.10 for B, C and D respectively). The pooled analysis gives significant effects for males to 0.005 level, with a quite large figure for df.

From these results we can conclude that under our conditions, the egg-laying rate of a fecundated female appears to be affected by the individual male to which she was mated.

Table 1. df, MS and F values for four experiments studying the influence of the male in the egg laying rate of fecundated females.

Experiment	Sources	df	MS	F
A	Males	39	223.31	0.71
	error	38	314.75	
B	Males	59	477.05	1.79 [†]
	error	50	266.76	
C	Males	48	663.35	2.48 [‡]
	error	44	266.95	
D	Males	48	429.18	1.41 [*]
	error	140	304.82	

Table 2. Analysis of variance for the pool of the four experiments.

Sources	df	SS	MS	F
Experiments	3	20,086.50	6,695.50	1.57 [‡]
Males	194	89,296.64	460.29	
error	272	79,718.83	293.08	
Total	469	189,101.97		

* significant to 0.10 level
[†] " " 0.05 "
[‡] " " 0.005 "

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*Addition to established linkage groups

The following two genes should be added to the maps previously revised by Sokoloff, 1965 (TIB 8:141-144).

platinum eye (pte) is a mutant resembling pearl (p) but behaving in a sex linked fashion. It is possible that this gene is allelic with ring (rg) (Yamada, 1961, reported by Bell, 1962). The mutant pte has been located about 18 units from py which is near a value reported for rose (rs) (Reynolds, reported by Bell, 1964). When rg and rs are released for further studies, tests of allelism will have to be carried out between pte and these genes to rule out the possibility that these mutants modifying eye color are allelic.

Spatulate (Spa) is a dominant with recessive lethal effects affecting the antennae (see illustration in Sokoloff, 1966, "The genetics of Tribolium and related species"). The available data place this gene about four units either to the right or to the left of sooty, more likely to the left of this gene, with the result that the arrangement in the fourth linkage group appears to be Be-(s)-Spa-(s)eju. Spa may be a recurrence of Eddleman's Df (Deformed) (Eddleman, 1961), but again, this mutant has not been released for comparative purposes.

The above data have been drawn from Sokoloff, Hoy and Johnson, 1966 (see Bibliography for full reference).

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*A behavioral difference of two mutant strains in Tribolium castaneum

Dawson (1964, TIB 7:50), McDonald and Fitting (1965, TIB 8:125) and Pratt (1965, TIB 8:126) have reported behavioral differences in various T. confusum strains which appear to be inherited. Dawson used inbred strains of this flour beetle without body markers; McDonald and Fitting contrasted the behavior of wild type and McGill black (the latter being more active), while Pratt compared wild type and ebony. Dawson, and

McDonald and Fitting noted that some strains tend to remain on the surface of the flour more so than other strains.

A similar observation has now been made in different strains of Tribolium castaneum. Surviving male beetles used by G. R. Johnson (Genetics 53: 111-115, 1966) were Be +/+ s and Fta +/+ c in genotype. The former were mated in a shell vial containing 8 grams of flour with + s/+ s virgins. The latter were mated in another vial with + c/+ c virgins. The two vials were kept in a room where neither light nor temperature were controlled. The old flour was not removed, but at intervals of approximately three months fresh flour was added on top of the old flour.

Observations were made regularly at daily intervals in the evening, and in addition, several observations were made on weekends, during the day and evening. The difference in behavior of the two strains in the evening was clear-cut. The Fta +/+ c × + c/+ c descendants remained under the surface in the flour, while the vial containing the backcross progeny of Be +/+ s × + s/+ s showed from one to two dozen beetles on the surface of the flour. During the day, at various times, these differences were cancelled out (equal numbers of beetles of the two strains being present on the surface in the two vials) or on the contrary, the Be +/+ s × + s/+ s backcross progeny disappeared from the surface while the Fta +/+ c × + c/+ c backcross progeny appeared on the surface of the flour.

Since the medium was variously conditioned, it would appear that, at least for T. castaneum, one of the factors suggested by Dawson (accumulation of quinones to account for the similar behavior of T. confusum strains) can be ruled out.

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*"Competition" between Tribolium anaphe, T. castaneum, T. confusum, T. destructor and T. madens by pairs. I.

We have available in the laboratory six species of Tribolium. T. anaphe, T. confusum and T. destructor belong to the confusum group. T. castaneum and T. madens to the castaneum group, and T. brevicornis to the brevicornis group. Although weight data are not immediately available, it can be easily seen that T. castaneum and T. confusum are far the smallest, and the other species are considerably larger. (For body measurements see H. E. Hinton, 1948. Bull. of Entomol. Res.)

We have introduced T. castaneum and T. confusum and other species two at a time, in whole wheat flour plus yeast to test the outcome. While we refer to this association as "competition," some published results (see for example Sokoloff, Lerner and Ho, 1965. Am. Nat.; Sokoloff, Franklin,

Overton and Ho, 1966. J. Stored Prod. Res.; and Sokoloff, Franklin and Lakhampal, 1966. J. Stored Prod. Res.) suggest that what is being observed is really the outcome of a double predator-prey relationship. Nevertheless the term "competition" is so descriptive and useful that it will continue to be used, despite the fact that this meaning is not included in the definitions given by Birch (1957, Am. Nat.).

The experimental conditions were as follows: Ten pairs of beetles of each species in each of 10 replicates containing standard medium (19 parts whole wheat flour, one part brewer's yeast). The shell vials were introduced into an incubator maintained either at 29° and 70 per cent or at 29° and 40 per cent. Adults were scored and discarded at monthly intervals (referred to below as a "transfer") when the medium was renewed following the method devised by Lerner and Ho (1961). The results, as available up to this date, are given in Tables 1-7, and the following summary:

<u>Species</u>	<u>Medium</u>	<u>R.H.</u>	<u>Winner</u>	<u>Transfers before disappearance of losing species</u>
<u>AN</u> vs <u>CS</u>	WY	70	<u>CS</u>	2
		40	<u>CS</u>	4
<u>AN</u> vs <u>CF</u>	WY	70	<u>CF</u>	3
		40	<u>CF</u>	3
<u>AN</u> vs <u>DS</u>	WY	70	<u>AN</u>	2
		40	--	--
<u>AN</u> vs <u>MD</u>	WY	70	<u>MD</u>	3
		40	<u>MD</u>	3
<u>CS</u> vs <u>MD</u>	WY	70	<u>CS</u>	3
		40	<u>CS</u>	3*
<u>CF</u> vs <u>MD</u>	WY	70	<u>CF</u>	7†
		40	<u>CF</u>	3‡
<u>DS</u> vs <u>MD</u>	WY	70	<u>MD</u>	2
		40	--	--

* MD survived for five transfers in five vials.

† MD was the surviving species in one vial at the end of eight transfers; and in four vials it is still surviving at the end of eight transfers.

‡ MD is still co-existing in low numbers at the end of five transfers.

It is clear that CS and CF are usually the winners against AN, DS, and MD. Sometimes, however, DS can hang on for a number of transfers (six in the case of vials containing CS, seven in the case of CF vials). MD appears the stronger competitor when introduced with AN and DS, but the experiments with DS need to be repeated: the vials containing this species and MD had to be discontinued because of an infection with the bacterium Streptococcus foecalis var. liquefaciens. One further remark: The method devised by Lerner and Ho (1961, Am. Nat.) works well for species which develop in about 30 days. By choosing an interval of 30 days it is possible that an extreme penalty is placed on slow developing species either because of characteristics of the species or because of the inadequacy of the nutrients in the medium where the species are reared. Thus, for example, CS and CF have been reared in the Naylor (1964, Canad. J. Zool.) medium without yeast. If one introduces 20 pairs of each species into this medium in separate vials, and discards the adults at every 30-day interval, at the end of the first 30 days one finds the original parents, at the end of 60 days fewer F_1 progeny than the numbers of P_1 introduced, and this number gradually declines CS declining more rapidly (becoming extinct at the end of four months), CF also becoming extinct, but only at the end of eight months in one vial and 11 months in the other vial. Thus, the disappearance of these two species may be an artifact imposed by the technique chosen. Similarly, in the interspecies experiments reported here, it is possible that one species goes out of existence rapidly because it is eliminated from those vials before the adults are able to reproduce. This problem needs further attention.

T. brevicornis has only recently become available, and a parasite-free culture of T. destructor is not yet available. These two species will be "competed" with the other available species in the near future. However, if any conclusion is to be drawn from the data so far available, it is that large size does not necessarily give a particular advantage to a species when in "competition." The experience of the senior author so far indicates that it is the smaller species of Tribolium and Drosophila that win over the larger species under the prevailing experimental conditions. In the case of Drosophila, a slight advantage in the developmental period and its smaller size enables D. persimilis to win over D. pseudoobscura under extremely crowded conditions. Quite likely in the above reported experiments with Tribolium, developmental rates are also playing a part, especially in setting up a cyclic preponderance of species at one transfer and of the other species at another transfer as those observed in the case of vials 5 and 7 in the upper block of Table 6, involving T. madens and T. confusum.

Table 1. Adult T. anaphe (numerator) and total T. anaphe plus T. castaneum (denominator) at a given transfer. Upper block, 29°C, 70% R.H.; lower block, 29°C, 40% R.H.

Replicate	Transfer						
	#	0	1	2	3	4	5
1	20/40	20/45	1/90	1/117			
2	20/40	19/42	0/139	0/108			
3	20/40	20/48	0/158	0/137			
4	20/40	20/48	0/126	0/99			
5	20/40	20/40	0/136	0/101			
6	20/40	19/40	0/99	1/129			
7	20/40	20/40	0/132	0/98			
8	20/40	19/41	0/186	1/78			
9	20/40	20/40	0/190	0/140			
10	20/40	20/44	0/142	1/111			
1	20/40	19/42	11/153	1/94	0/143	0/46	
2	20/40	20/48	2/206	0/48	0/131	0/280	
3	20/40	20/44	10/263	0/137	0/113	0/168	
4	20/40	20/45	11/198	0/146	0/87	0/129	
5	20/40	20/42	7/234	0/143	0/123	0/197	
6	20/40	19/46	2/191	0/62	0/140	0/169	
7	20/40	20/52	2/221	0/100	*		
8	20/40	18/46	0/246	0/85	0/35		
9	20/40	20/47	1/207	0/114	0/116	0/317	
10	20/40	20/49	9/189	0/105	0/125	0/199	

* Discarded - infected with Nasema whitei.

Table 2. Adult T. anaphe (numerator) and total T. anaphe plus T. confusum (denominator) at a given transfer. Upper block: 29°C, 70% R.H.; lower block: 29°C, 40% R.H.

Replicate	Transfer					
	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
1	20/40	20/40	15/313	0/51	0/249	0/32
2	20/40	19/40	8/252	0/47	0/231	0/31
3	20/40	19/39	2/285	0/50	0/290	0/21
4	20/40	20/41	4/259	0/71	0/230	0/29
5	20/40	20/40	1/366	0/32	0/219	0/32
6	20/40	20/40	0/224	0/38	0/230	0/56
7	20/40	20/40	26/345	0/35	0/271	0/16
8	20/40	20/41	2/300	0/24	0/244	0/44
9	20/40	20/42	0/310	0/16	0/340	0/22
10	20/40	20/40	0/289	0/20	0/250	0/20
1	20/40	20/40	8/334	0/54		
2	20/40	19/40	0/240	0/23		
3	20/40	19/40	8/243	0/53		
4	20/40	20/40	10/276	0/35		
5	20/40	20/40	3/257	0/70		
6	20/40	20/39	2/252	4/23		
7	20/40	20/41	24/268	0/65		
8	20/40	18/38	13/250	0/31		
9	20/40	21/41	10/269	0/53		
10	20/40	18/38	10/256	0/48		

Table 3. Adult T. destructor (numerator) and total T. anaphe plus T. destructor (denominator) at a given transfer. Upper block: 29°C, 70% R.H.; lower block: 29°C, 40% R.H.

Replicate	Transfer				
	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
1	20/40	16/36	0/63	0/42	0/27
2	20/40	14/34	0/61	0/0	0/45
3	20/40	19/39	0/29	0/42	0/72
4	20/40	16/35	0/79	0/0	0/99
5	20/40	15/33	0/60	0/0	0/83
6	20/40	20/40	0/34	0/47	0/8
7	20/40	18/37	0/45	0/82	0/10
8	20/40	17/37	0/82	0/60	0/7
9	20/40	19/38	0/66	0/0	0/45
10	20/40	18/37	0/53	0/56	0/6

Table 4. Adult T. anaphe (numerator) and T. anaphe plus T. madens (denominator) at a given transfer. Upper block: 29°C, 70% R.H.; lower block: 29°C, 40% R.H.

Replicate	Transfer						
	<u>#</u>	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
1		20/40	19/39	7/105	0/45	0/23*	
2		20/40	18/38	9/129	0/6	0/27*	
3		20/40	20/40	4/121	0/26	0/59*	
4		20/40	20/39	16/144	0/9	0/73*	
5		20/40	20/39	14/116	0/53	0/38	
6		20/40	20/40	20/150	0/52	0/57	
7		20/40	20/40	17/132	0/79	0/63*	
8		20/40	20/40	5/102	0/5	0/16*	
9		20/40	20/40	13/119	0/49	0/85	
10		20/40	20/40	9/129	0/64	0/33*	
1		20/40	20/39	33/161	0/30	0/34	0/47
2		20/40	20/39	35/161	0/35	0/78	0/51
3		20/40	19/37	15/161	0/90	0/13	0/56
4		20/40	20/39	14/174	0/16	0/22	0/49
5		20/40	20/40	27/171	0/30	0/30	0/76
6		20/40	20/40	9/111	0/59	0/10	0/79
7		20/40	20/40	35/213	0/3	0/80	0/17
8		20/40	20/39	22/118	0/46	0/10	0/171
9		20/40	20/38	62/97	0/5	0/64	0/86
10		20/40	20/40	31/120	0/47	0/10	0/75

*Dead adults infected with Streptococcus foecalis var. liquefasciens - whole set discarded.

Table 5. Adult T. madens (numerator) and total T. madens plus T. castaneum (denominator) at a given transfer. Upper block: 29°C, 70% R.H.; lower block: 29°C, 40% R.H.

Replicate	Transfer									
	#	0	1	2	3	4	5	6	7	8
1	20/40	20/40	0/88	0/193	0/113	--				
2	20/40	20/43	1/91	0/234	0/125	0/143				
3	20/40	19/39	6/93	0/171	0/114	0/111				
4	20/40	20/40	10/66	0/139	0/98	0/155				
5	20/40	19/39	2/57	0/296	0/118	0/159				
6	20/40	20/40	0/69	0/360	0/94	--				
7	20/40	20/40	0/67	0/194	0/101	--				
8	20/40	20/40	6/55	0/167	0/145	0/133				
9	20/40	20/40	4/92	0/173	0/83	0/103				
10	20/40	20/40	9/111	0/190	0/70	0/106				
1	20/40	20/41	3/92	0/110	1/75	0/236	0/40	--		
2	20/40	20/40	27/103	1/186	8/73	0/156	4/74	0/160		
3	20/40	20/40	49/109	0/160	15/58	0/140	6/41	0/149		
4	20/40	18/38	79/127	0/76	16/54	0/161	3/76	0/146		
5	20/40	20/38	14/78	0/138	0/83	0/216	1/71	--		
6	20/40	20/38	10/108	1/101	4/164	0/195	0/40	0/300		
7	20/40	19/39	3/113	0/138	0/127	0/190	0/30	--		
8	20/40	20/40	12/98	0/197	0/88	0/264	0/45	--		
9	20/40	20/39	37/110	1/118	3/71	0/292	6/38	0/273		
10	20/40	20/40	7/113	0/148	0/70	0/170	0/40	--		

Table 6. Adult T. madens (numerator) and adult T. madens plus T. confusum at a given transfer. Upper

block: 29°C, 70% R.H.; lower block: 29°C, 40% R.H.

Replicate #	Transfer								
	0	1	2*	3	4	5	6	7	8
1	20/40	20/40	51/91	3/55	12/131	0/78	0/86	0/110	--
2	20/40	20/40	45/90	2/93	19/66	0/66	0/54	1/75	0/136
3	20/40	20/40	44/100	3/72	28/174	3/58	0/98	0/109	0/240
4	20/40	20/40	36/93	1/89	1/129	0/240	0/35	0/218	--
5	20/40	20/40	49/93	0/98	13/158	0/29	41/43	0/32	23/23
6	20/40	20/40	30/85	1/122	11/144	0/63	3/81	0/159	10/175
7	20/40	20/40	46/73	7/77	8/186	13/26	0/47	64/69	2/22
8	20/40	20/40	37/58	6/78	12/114	4/48	2/121	0/86	0/121
9	20/40	20/40	11/71	1/144	3/219	7/115	1/80	4/86	1/134
10	20/40	20/40	45/78	8/41	27/74	20/37	0/60	3/104	5/65
1	20/40	20/40	64/117	1/39	63/65	27/27			
2	20/40	20/40	73/147	5/78	30/48	8/60			
3	20/40	20/41	14/110	2/72	8/93	0/35			
4	20/40	20/40	51/104	1/68	34/66	1/10			
5	20/40	19/38	58/113	1/2	155/705	4/31			
6	20/40	18/38	38/113	0/63	70/81	3/80			
7	20/40	20/40	9/62	1/257	1/55	0/34			
8	20/40	20/40	57/116	0/124	23/91	0/24			
9	20/40	18/38	13/105	2/232	14/144	0/26			
10	20/40	20/40	33/101	1/91	53/54	6/13			

* Heavy non-microbial mortality of MD at eclosion in both sets of vials.

Table 7. Adult T. destructor (numerator) and adult T. destructor plus T. madens (denominator) at a given transfer. Upper block: 29°C, 70% R.H.; lower block: 29°C, 40% R.H.

Replicate	Transfer				
	<u>#</u>	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>
1		20/40	17/37	0/88	0/74
2		20/40	16/36	0/69	0/41
3		20/40	16/36	0/88	0/39
4		20/40	14/34*	--	--
5		20/40	12/31*	--	--
6		20/40	18/38	0/80	0/20
7		20/40	18/38	0/87	0/1
8		20/40	13/33	0/102	0/0
9		20/40	14/33	0/81	0/54

*DS infected with Streptococcus foecalis var. liquefaciens. Discarded.

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Effect of removing the male on egg laying rate of fecundated females in *Tribolium castaneum**

In line with our studies on the influence of the male on egg laying rate of fecundated females, we have tested whether this effect might influence the correct evaluation of the character considered.

When a female is mated to a male just at the emergence from the pupa stage and we evaluate the egg production during a certain period, the figure obtained if we maintain the male with her, could be different to that attained after removing the male. This operation is necessary when several females are mated to one male and afterwards we have to evaluate the egg laying rate individually.

Our interest was to test the effect of removing the male at day 7 after the emergence on the eggs laid during the following four days. Given that the egg production of females without males (really virgin females) is very much smaller than the laying figure with males, it is thought that the laying rate obtained after that operation could be smaller than that evaluated maintaining the male with the female.

Three trials were run mating individual pairs of male and female just after adult emergence. Seven days later the male is removed from one-half of the matings taken at random. At that time fresh medium is put in every container to obtain the lay to be measured. After four days the eggs are separated from the flour by suction and counted. An analysis of variance is run on every trial to compare both treatments and a pool of the three runs is also made to get a better estimation of the means and possible differences.

The strain, technical methods and environmental conditions were the same as in the report of Ruano and Orozco elsewhere in this Bulletin.

Table 1 includes means, df, MS and F values for the three experiments run, and Table 2 gives the analysis for the pool. It is observed, from the results obtained, that the means from the females without males during the period are slightly smaller than those from the females with males, but in no case are the differences significant. This is also true in the analysis of pooled data.

* Partial and preliminary study included in Grant No. FG-Sp-137 of P.L. 480; contract with the USDA.

We conclude that under our experimental conditions and when the character is the laying rate in the above mentioned four days, we can evaluate that trait equally well either by removing the male or by leaving him with the female.

Table 1. Means, df, MS and F values from three trials studying the effect of removing the male during the testing period on the laying rate of fecundated females.

Experiment	Means and st.e.	Sources	df	MS	F
A	No male 62.78	Treatments	1	247.69	1.09
	Male 67.37 ± 3.11	Error	45	227.76	
B	No male 47.96	Treatments	1	5.78	0.02
	Male 48.64 ± 3.11	Error	48	241.93	
C	No male 45.65	Treatments	1	10.48	0.04
	Male 46.33 ± 2.51	Error	89	287.53	

Table 2. Analysis of variance and means for the pool of the three experiments.

Means and st.e.	Sources	df	SS	MS	F
No male 50.35	Experiments	2	12,023.55	6,011.77	0.80
Male 52.45 ± 1.66	Treatments	1	205.50	205.50	
	Error	184	47,511.14	258.21	
	Total	187	59,740.19		

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Irradiation treatments

Adults and larvae of several stored-product insect species were subjected to gamma irradiation in a mobile cobalt-60 source to determine the effects of different dosages on longevity and fecundity of survivors. Mortality assessments were made each week and the survivors were transferred to fresh food. Sixteen weeks after exposure to 6,500 rads *Tribolium castaneum* adults had only 5% mortality compared to 60% mortality in the controls;

Cryptolestes ferrugineus had 75% mortality compared to 100% mortality in the controls. Adult survivors of the irradiation treatments were able to damage wheat to the same extent as the controls but were unable to reproduce. Observations are being continued.

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Locomotor activity of spider beetles in wheat

The periodic locomotor activity of the hairy spider beetle, Ptinus villiger (Reit.) was assessed by the numbers of beetles found every three hours in water traps and dry traps at the surface of stored grain. The traps consisted of 6-fluid-oz. glass jars sunk into the grain with the tops level with the grain surface. During a 45-hour period, more insects were caught in water traps during the late morning than at other times, indicating a periodic humidity response. Locomotor activity as expressed by numbers of insects caught in dry traps appeared to be arrhythmic throughout the observation period.

NOTES - TEACHING

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*Linkage group identification by the "dominant synthetic lethal" effect

There are at present the following dominant mutations:

linkage group 4: Bar eye (Be); Spatulate (Spa)
 linkage group 6: Microphthalmic (Mo)
 linkage group 7: Short antenna (Sa, Sa-1, Sa-2, Sa-3 and Sa-4) and
 Fused tarsi and antennae (Fta)

Crosses within and between these various dominants produce the following:

1. Be/+ × Be/+ : +/+ : 2 Be/+
2. Fta/+ × Fta/+ : +/+ : 2 Fta/+
3. Mo/+ × Mo/+ : +/+ : 2 Mo/+
4. Sa/+ × Sa/+ : +/+ : 2 Sa/+
5. Spa/+ × Spa/+ : +/+ : 2 Spa/+
6. Be/+ × Fta/+ : +/+ : Be/+ : Fta/+ : Be Fta
7. Be/+ × Mo/+ : +/+ : 3 abnormal eyes (Mo or Be)
8. Be/+ × Sa/+ : +/+ : Be/+ : Sa/+ : Be/Sa
9. Mo/+ × Sa/+ : +/+ : Mo/+ : Sa/+ : Mo/Sa
10. Fta/+ × Spa/+ : +/+ : 3 abnormal antennae
11. Fta/+ × Sa/+ : +/+ : 2 abnormal antennae
12. Be/+ × Spa/+ : +/+ : Be/+ : Spa/+

The first five crosses clearly indicate these genes behave as dominants with recessive lethal effects. The next five crosses give the expected ratios of the dominant genes when they are not linked. Crosses 11 and especially 12, on the other hand, clearly indicate that a dominant synthetic lethal effect is produced. In Mating 11, Fta/+ × Sa/+, the Fta and Sa class appeared but the Fta Sa failed to do so. Since both of these genes affect the antennae and the legs the fact that the Fta Sa class failed to appear might be interpreted in various ways, but fortunately a semi-dominant allele of Sa was available. This gene, sa, does not produce the dominant lethal effect, and thanks to it the Fta and Sa genes have been located in the same linkage group. No such difficulties are encountered in the crosses involving Be/+ and Spa/+. These two genes affect different parts of the

body of the beetle (the eye and antenna), and the absence of the Be Spa class is readily determined.

It would appear from these results that for dominant genes having recessive lethal effects, the first crosses that should be attempted to establish linkage relationships is between the newly discovered mutants and those serving as dominant markers for these chromosomes (IV, VI, and VII). If the dominant synthetic lethal effect is absent, then these new dominant mutations having recessive lethal effects are not found in those linkage groups. In a separate series of crosses, set up at the same time, the new dominants can be mated systematically to stocks bearing the recessive mutants associated with the various linkage groups. Since the double heterozygotes (say $\underline{M}/+\underline{m}$) will have to be crossed back to the double recessive ($+\underline{m}/+\underline{m}$) to determine whether the new mutant is linked to these genes, the dominant synthetic lethal effect is an obvious labor saving device and a useful exercise in laboratory courses in genetics since, if it can be recognized by mating two dominants with recessive lethal effects, linkage relationships can be established in one generation instead of two (which would be required by mating these mutants to the recessive markers).

There are only a few semi-dominant mutations in *Tribolium* (b, ca) and only one truly dominant mutation which does not have recessive lethal effects (see description of Nonpunctate prothorax, Npp, in New Mutant section). No matings have been performed between Npp and the various dominants with recessive lethal effects cited above. It would seem, however, that for the dominant synthetic lethal effect to be observed it is a prerequisite that a lethal effect be observed when two heterozygous dominants have, independently, a recessive lethal effect (i.e. $\underline{A}/+ \times \underline{A}/+$ would cause the death of $\underline{A}/\underline{A}$ and $\underline{B}/+ \times \underline{B}/+$ would eliminate $\underline{B}/\underline{B}$. If such is the case then in $\underline{A}/+ \times \underline{B}/+$ the dominant synthetic lethal effect would cause the death of the AB class).

It would be of interest to examine these dominant mutants cytologically to determine whether any cytological basis exists for the peculiar behavior of these single dominants and their interaction.

NOTES - TECHNICAL

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*Techniques used in a genotype X environment interaction study with *Tribolium castaneum*

Some degree of success with respect to the control of humidity has been attained at this laboratory with simple equipment. Still-air chicken-incubators (Oakes Model AO) such as those shown in Fig. 1 have been used.

Circumambient conditions

The room housing the equipment is heated by a thermostatically controlled electric heater. Relative humidity and temperature in the room are maintained at levels lower than those in the rest of the building during summer months when a thermostatically controlled air conditioner switches on. The room temperatures are recorded daily on the side nearest a window. The mean readings are approximately 20.6 and 15.9°C. for dry and wet bulbs respectively. Temperatures are generally lower from December through April and in this period the spread between the dry and wet bulb reading is greater, indicating a lower relative humidity.

High humidity environment in incubators

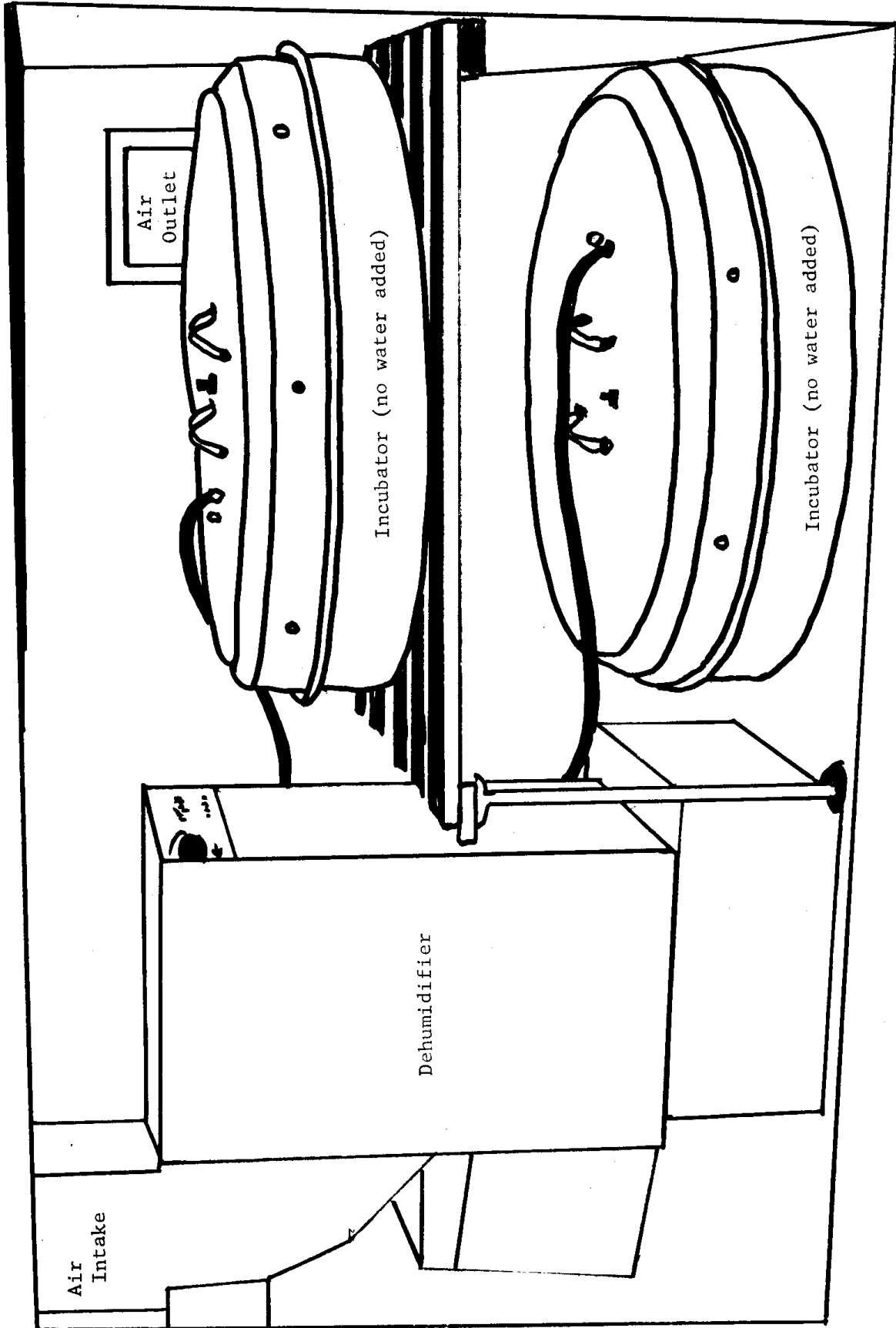
Water supplied in open dishes raises the humidity within the incubator and the spread between the dry and wet bulb readings ranges from 1.1 to 3.2°C. with dry bulb readings in the neighborhood of 27.8°C. Air holes are plugged with cotton to prevent moisture from escaping.

Low humidity environment in incubators

Air in the low humidity cabinet (Fig. 1) passes through a humidistatically controlled dehumidifier (Westinghouse Custom Supreme). The spread between the wet and dry bulb readings in this environment ranges between 5.0 and 6.6°C. with dry bulb readings similar to the high humidity environment. When this cabinet was first installed, attempts were made to dry humid air from a non-airconditioned room during summer months. The heat dispensed from the dehumidifier raised the temperature in the cabinet to about 32°C. When air was drawn from an air conditioned room such a problem was not encountered.

Results

At three weeks of age, *Tribolium castaneum* reared at approximately



27.8°C. are still in the larval stage in both of the environments employed here. Approximately 1400 progeny sampled into each environment from common parents yielded mean weights of 1922 and 604 micrograms in the high and 102 humidity environments respectively. The standard error of the difference was 23 grams.

A cursory analysis of similar data indicates that there is considerable genotype X environment interaction for larval weight measured under these conditions.

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*Cytological technique for the study of Tribolium chromosomes

Take four or five days old male pupae and place them in Carnoy's fixative (4 chloroform : 3 ethyl alcohol : 1 acetic acid) for a few minutes till they are dead. Take the pupae out, place them on a slide and cut the abdomen with a razor blade, just below the point of attachment of wings to abdomen and a few segments above the location of the testes. Then squeeze the abdomen with forceps, the testes along with fatty tissue come out. Take this tissue and fix it in a fresh Carnoy's fixative, in which acetic acid is replaced by acetic acid saturated with ferric-acetate. Leave in fixative for 6-8 hours and then squash the testes in 1% acetocarmine. A drop of acetic acid saturated with ferric-acetate (Mordant) can also be added to the stain on the slide, in order to get a dark staining of the chromosomes.

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*Population cages

In Tribolium Information Bulletin 3, Daniel J. McDonald described a population cage for Tribolium confusum. In some of our experiments it was necessary to have multi-niche and single-niche situations; therefore, the following population cage was constructed.

A population cage with a central body and eight side arms was first constructed entirely with acrylic plastic. This proved to be unsatisfactory because the cage lids could not be secured tightly, permitting Tribolium to escape. Another population cage (Fig. 1) was constructed which was escape-proof and efficient to handle during censuses. The following materials were used in its construction:

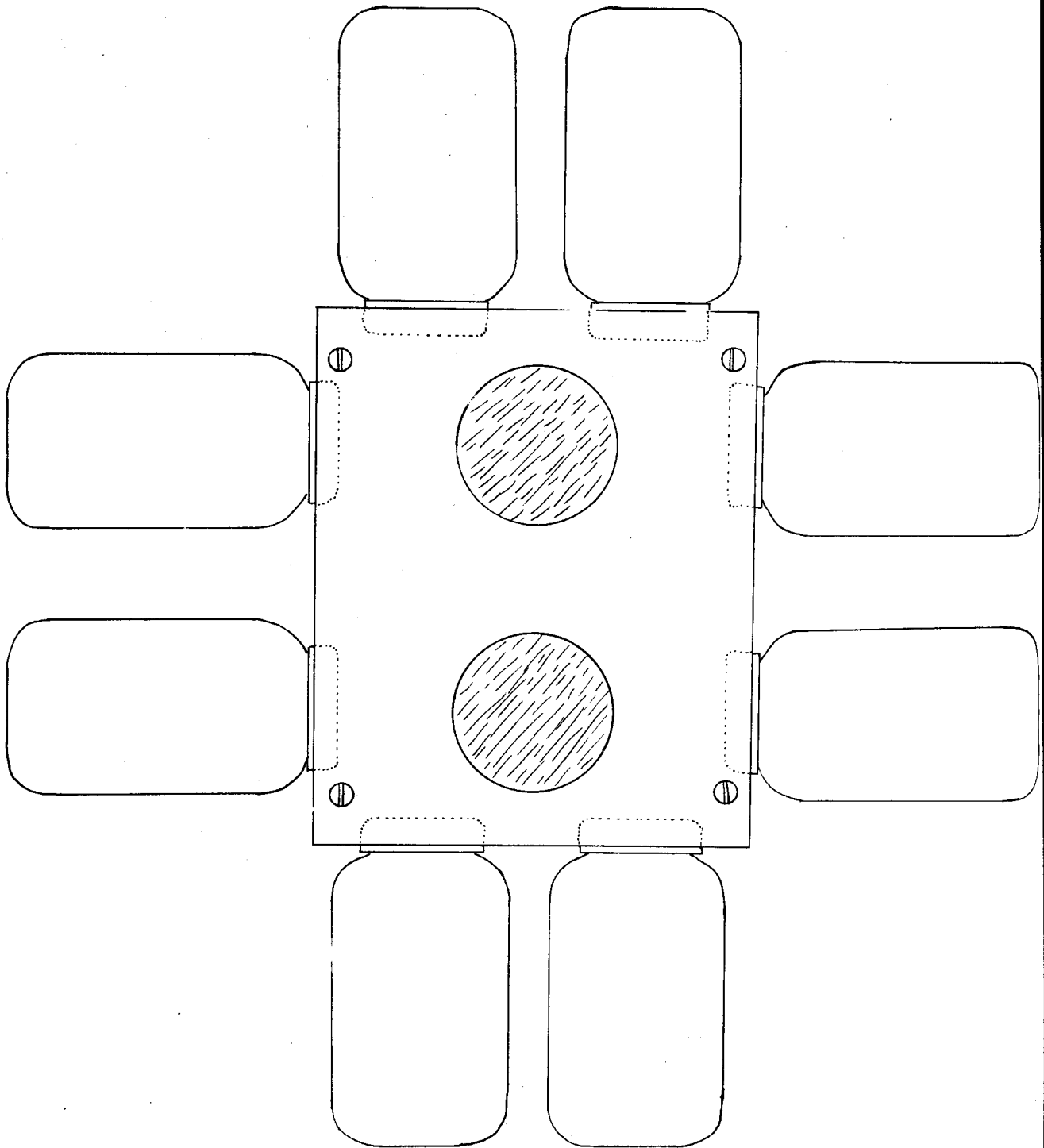


Fig. 1

1. Aluminum chassis box (4" x 5" x 3")
2. Polyethylene plastic bottles
3. Bakelite caps for the above bottles
4. 80 mesh-brass screen
5. Epoxy glue
6. Metal screws

Two openings on each of the four sides of the aluminum chassis box were cut by a 1-1/8" chassis punch. The caps, bored with a 1/4" hole, were ground slightly to fit snugly into the 1-1/8" openings and were permanently glued into these openings. Thus, the plastic bottles could be screwed securely into the caps and required no further support. Minute holes were drilled into the top surface of the plastic bottles for ventilation.

The bottom of the chassis box was permanently glued and secured with metal screws. Two openings were cut into the lid with a 1-1/2" chassis punch for ventilation. The screen with an aluminum metal back with comparable 1-1/2" openings was bolted and glued permanently to the lid. This lid could be removed by unscrewing four metal screws and made escape-proof by using masking tape around its edges. During censuses of the side cages, a small 1/4" rubber plug was inserted into the holes of the caps.

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*Food choice cages

This cage (Fig. 2) was entirely made of acrylic-plexiglas plastic with respective thicknesses of 1/16", 1/8" and 1/4" and the overall cage dimensions were 1-3/4" x 4" x 1-3/4". The 1/4" sides of the cage were grooved with a circular saw to hold the 1/8" partition which was drilled with 1/8" holes to allow *Tribolium* to move freely between the compartments. Another groove to hold the sliding 1/16" lid was cut in the top-inner surface of the sides. The lid was drilled with minute holes for ventilation. The ends and the bottom of the cage were constructed with 1/8" plastic.

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*Simplified techniques for obtaining Tribolium eggs

Two very simple devices have been designed for obtaining small numbers of eggs for embryological studies or for classroom exercises. Each may be constructed easily and cheaply from readily available materials.

The larger shaker is made from two 9" x 5" x 3" loaf pans. Aluminum pans may be used; the material is easy to work but becomes battered with extended use. The bottom is cut from one pan with a can-opener, tin snips, etc. and silk bolting cloth of about 56 threads/inch is used to form a new bottom. The bolting cloth is stretched over the bottom and glued to the outside of the pan with epoxy resin. In order to prevent eggs being lost in the crevices, it is necessary to saturate the area of the cloth extending onto the side of the pan. Two holes are drilled in each end of the pan, two inches apart, and about an inch from the bottom of the pan. One and a half inch bolts are placed in these holes with their heads in the interior of the pan. Matching nuts are screwed down tightly on the outside. Similarly spaced holes are drilled in each end of the other pan just under the rim. In one end the rim is snipped out over the holes transforming each into a groove. The extending ends of the bolts of the cloth-bottomed pan are slipped into the holes and grooves of the second pan. With this arrangement the bolting cloth-bottomed pan nestles within the other but is held above it forming a convenient hand sifter.

A much simpler sifter suitable only for very small quantities of medium may be made by holding a piece of bolting cloth taut in an ordinary embroidery hoop.

Eggs obtained with such sifting devices usually are contaminated with the feces of the adults and with certain particles of wheat. The eggs may be separated from the debris by placing the mixture on a piece of stiff paper and gently tapping and jiggling the paper.² Due to their shape, the somewhat yellowish eggs will roll to one region of the paper and may be guided off it into a receptacle. Two such treatments remove almost all foreign material not adhering directly to the eggs.

¹ These techniques were developed in the course of the tenure of National Science Foundation Science Faculty Fellowship No. 63087.

² This technique was described by Saunders and Krueger (1957, J. Econ. Ent. 50:693) and subsequently adapted for large populations (all instars, including eggs) by Sokoloff (1959, TIB 2:36).