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OBSERVATIONS ON THE GENERAL BIOLOGY OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM*

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INTRODUCTORY REMARKS

TRIBOLIUM CONFUSUM, the flour beetle, possesses characteristics which commend it to the attention of experimentalists. It is cosmopolitan and easily obtainable; it has a moderately short life-cycle; it is hardy and requires no elaborate equipment for its maintenance; both adults and immature forms are readily recoverable from the medium for study by a simple technique, and it spends its entire life-history in various pulverized grains such as flour. As has been pointed out before, this latter feature is particularly advantageous in many kinds of experimentation since, with a total environment such as flour, considerable stability of both intra- and extramedium relationships can be obtained. By using similar flour in all experiments and by expressing this flour quantitatively in terms of weight and surface exposure, as well as surrounding the flour by as constant external conditions as obtainable, a total environment can be established which is relatively quite constant and reproducible. Despite these attractive requisites it is only recently that *Tribolium* has come to the attention of biologists interested in theoretical problems. The economic zoölogists have been vigorously aware of the existence of this form for many years and have done much in providing data on the general ecology of the species. However, past this point, these workers, possibly unfortunately, have seemed more interested in devising methods of exterminating the form than recommending it to the attention of experimentalists. Babcock (1912), an early worker with *Tribolium*, used this beetle in studying the problem of metabolic water, finding the insect maintained a tissue water level about 40 per cent greater than the moisture content of an environment containing 10 per cent water. Davey (1917, '19), another early investigator, reported results showing that the length of life of these beetles could be prolonged if mass cultures received a small daily dosage of X-rays. Probably the principal credit in introducing *Tribolium* as an experimental organism, however, belongs to R. N. Chapman who in 1918 reported on its life-history, in 1924 on its nutritional requirements, and in 1928 on its suitability for population study. These papers will be discussed at length later: it is only important at this point to remember that they emphasized the practicability of *Tribolium* for experimental purposes by presenting a technique of handling the beetles as well as actual data. Since *Tribolium* does seem destined to merit more experimental attention, and since experimentalists need a factual background about the animal of their endeavors, it seems permissible at this stage to review fairly completely certain details about the flour beetle in the hope that such a review will be helpful and possibly stimulating to future investigators in the field. With this in mind the following general topics about *Tribolium confusum* will be dealt with: first, taxonomic and historical considerations including a brief discussion of closely related species; second, a review of the food relationships of the flour beetle along with discussions of certain nutritional problems; third, a detailed discussion of the life-cycle and its relation to environmental factors; fourth, a discussion of productivity (fecundity and fertility); fifth, a treatment of some of the obvious behavior reactions of the beetle from both a statistical and an observational viewpoint, including some original observations upon cannibalism, copulation, and defecation; sixth, notes on the parasites of *Tribolium confusum*; seventh, a discussion of the technique of culturing and studying populations of the flour beetle, and last, a summary. It should be pointed out that no attempt will be made to cite or deal with every paper which has been written on *Tribolium*. This would be futile and not pertinent to the problem at hand due, in part, to the economic nature of many of the reports. The emphasis will be placed on a discussion of those investigations yielding information most likely to be required by future workers confined primarily to experimental research.

TAXONOMIC AND HISTORICAL

The genus *Tribolium* is a member of the Order Coleoptera, Family Tenebrionidae and sub-family Ulominae. The Tenebrionids are commonly referred to as the 'Darkling beetles' and include such forms as certain fungus-beetles, the pinacate-bugs, and the common meal worm *Tenebrio molitor* which has certain points of resemblance to *Tribolium*. There are several other species of *Tribolium* besides *confusum*. The other common American form is *Tribolium ferrugineum* Fab. Doctor Good, of the Department of Agriculture, informs me that the latter species is probably more properly referred to as *Tribolium castaneum* Hbst. These two American flour beetles seem to be remarkably similar in general structure and function. There are several criteria used by taxonomists in distinguishing the two from each other. *T. ferrugineum* = *castaneum* possesses a distinct three-jointed antennal club while *T. confusum* has a gradually enlarging club. Good (1933) points out the fact that the eyes of *T. confusum*, when viewed from the ventral surface, are smaller than those of the other species. The evidence at hand indicates that *T. confusum* has, in general, a more northern range of distribution than *T. ferrugineum* = *castaneum*. This paper will deal entirely with *T. confusum* largely because more is known of its general ecology. It should be emphasized, however, that the other species would probably lend itself as well to experimentation.

At the present time the genus *Tribolium* is very widely distributed over the world. It is largely disseminated in grains transported by commerce. The exact origin of the grain dwelling habit is not known but a probable interpretation is advanced by Good (1933: p. 328) who says,

> Almost without exception, the beetles of the subfamily Ulominae, of which Tribolium is a member, occur either as pests of stored products or else under the bark of trees and in rotting logs. It seems evident that all members of this group originally lived in the latter habitat and have recently adopted the flour-feeding habit. Two species of Tribolium, *T. madens* Charp. and *T. indicum* Blair, are found almost exclusively in such situations, and the two flour pests *T. confusum* and *T. ferrugineum* are themselves occasionally found there.

Tribolium confusum was really first described unknowingly by Etienne Mulsant who, in attempting to improve upon the account of the already known *Tribolium ferrugineum*, published a description of *confusum*. However, since Mulsant thought he was simply redescribing *T. ferrugineum* his reference to *confusum* has been neglected. The credit of the description has been ascribed to P. N. Camille Jacquelin du Val who in 1868 recognized *Tribolium confusum* as a distinct species and published his account. Due to the fact that du Val's (now commonly written Duval) description typifies much of the taxonomic work of his era it is considered permissible to append it below as it existed originally.

(1) *Tribolium confusum*, Jacq. du V.

T. ferrugineo simillimum, et descripto ulterior supervacua. Ab illo differt capite latiusculo, genis ad oculos destinctius angulatis, pronoto postice leviter sed destincte sensim angustato, cum angulis posticis acutiusculis, interstitiis elytrorum paulo magis elevatis et praesertim antennis sensim elavatis, —Long. 0,0034–42.–

Je dois ajouter pour compléter la distinction des deux espèces en question que, chez le *T. ferrugineum* la tête n'est pas plus large que longue et offre des joues très obtusement angulées auprès des yeux, le pronotum est légèrement et régulièrement arqu suré les côtés, n'est pas plus large ou même est un peu plus étroit en avant qu'en arrière et offre des angles postérieurs droits, mais sans former de pointe saillante, enfin les antennes sont terminées par une massue tres distincte et assez brusque de trois articles, tandis que chez le *T. confusum* lamassue est insensiblement formée par les 4 ou 5 derniers articles. L'espèce que je viens dec signaler se trouve confondue dans diverses collections françaises avec le *T. ferrugineum*, et a été decrite pour cette dernière par M. M. Kuster et Mulsant. La phrase suivante, "*Antennae articulis tribus perfoliatis*," insérée par Fabricus dans son *Supplementum Entomol. Syst.* p. 179, nous fixe sur l'espèce de cet auteur. Le *Colydium castaneum* de Herbst appartient aussi à n'en pas douter à cette dernière. Quant à l'*Ips cinnamomea* de Herbst, la figure qu'en donne cet auteur démontre que ce ne peut être un *Tribolium* ni même à mon avis un Ténébrionide." (p. 181)

FOOD AND NUTRITION

Tribolium confusum lives in almost any kind of flour, cracked grain, breakfast food or meal. A list of specific foods in which these beetles are found has been compiled by Chittenden (1896, 1897) and includes whole-wheat flour, bleached and unbleached white flour, bran, rice flour, rye flour, corn meal, barley flour and oat meal. Good (1933) also reports the beetles living in chocolate, spices (red pepper), various kinds of nuts, and sometimes feeding on specimens in insect collections. Chittenden has found *Tribolium* in snuff, orris root, baking powder, ginger, slippery elm, peas and beans. The beetles are unable to feed on whole grains, as pointed out by Chapman (1931), because their mouthparts are not adapted for attacking large, hard pieces of food. Typically, the entire life-history of a *Tribolium* is passed within its original environment.

Chapman (1918) has studied the relative susceptibility of wheat flour to the invasion of *Tribolium* in order to determine, as he states it, if there is any 'preference' exhibited by adult beetles for specific flours. This investigator divided an experimental jar into five equal portions each containing a different kind of wheat flour which varied from finely ground flour (first middlings) to bran. These flours were in continuity with each other so that a beetle could pass from one type to another. Then adult *Tribolium* were introduced into the center of the jar, and, being surrounded by equal parts of the five types of cereals, were allowed to migrate. After a varying length of time the flours were examined and a census made of their beetle population. Chapman found that the bran contained essentially twice as many beetles as did the other media and concluded that these data indicated a preference selection on the part of *Tribolium.* In repeating the experiment with larvae no evidence of such a 'preference' was found. Observations were also made with rice flour, rye flour, barley flour and corn flour and the results of these experiments, in harmony with the first ones, indicated that the beetles were reacting, not necessarily to the richest food, but to the most coarsely ground medium. Chapman ingeniously tested this further by running a varying series of sawdusts differentially ground and again corroborated the fact that the beetles react positively to the coarse material which they can enter readily and move through with facility. However, after the experiments had run a longer time it was observed that all of the flours, whether coarse or fine, were equally populated. This was due to the fact that the beetles eventually honeycombed the finely ground flour with tunnels and were then able to move freely through it.

Chapman (1924) reared *Tribolium* on various media and concluded, first, that microörganisms did not play an important part in their nutrition; second, that the nutritional requirements for growth, or increase in size, were less exacting than those for maturity or metamorphosis, and third, that wheat germ most closely satisfied these physiological requirements. Sweetman and Palmer (1928) investigated the vitamine requirements of *Tribolium*, and found that Vitamine B was necessary for their development. Their investigations showed that the wheat embryo was a rich source of Vitamine B. The conclusion to be drawn on the basis of these experiments seems to be that flours represent a well balanced diet and that *Tribolium* is typically able to live in them with its complete nutritional requirements well satisfied.

Under the caption of nutritional effects it might be interesting to mention briefly certain experiments of Holdaway (1933) dealing with the effect of starvation on sex ratio. This investigator subjected first instar larvae to different degrees of starvation as follows: the first group served as controls and were not starved; the second group received one day of starvation; the third group two days starvation, and the fourth group three days starvation. These experimental groups were incubated until the pupal stage was reached at which time sex was determined. The larvae starved one day exhibited a higher proportion of males than did the controls. This proportion did not exceed the limits of random sampling and can not be taken too seriously. However, the groups starved two and three days showed a statistically greater number of females than the controls. This was a significant difference. Holdaway shows that this alteration of sex ratio can not be explained on the basis of a differential mortality existing between the sexes and concludes that the starvation is the critical factor.

Certain other observations have been made on the physiological effects of various kinds of foods upon *Tribolium* and these will be discussed in later sections of this paper.

METAMORPHOSIS AND LIFE-CYCLE

Many references to the life-cycle of *Tribolium confusum* may be found in the literature. Most of these reports are incomplete, abbreviated, and frequently incorrect. In most ways, the best statements on the subject are those of Chapman (1918), Brindley (1930), Holdaway (1932), and Good (1933). These papers all present the results of original observation and do not resort to the recopying of often erroneous textbook statements. In the present discussion on *Tribolium* metamorphosis and life-history the information will be drawn largely from these four papers. For a succinct account in German the papers of Zacher (1927, '33) and Kunike (1931) can be recommended. Other pertinent references are those of Chittenden (1896) and Dean (1913).

As is typical with holometabolous insects, *i.e.*, forms displaying complete metamorphosis, *Tribolium confusum* possesses in its life-cycle, egg, larval, pupal, and adult stages. In order to clarify this discussion it is deemed wise to describe briefly these various types.

The eggs are ovoidal in shape when naked but usually appear somewhat irregular due to the fact that they are surfaced with flour particles which adhere to the sticky egg membrane. Brindley (1930) reports the following egg measurements: mean width, .40 mm., mean length, .64 mm., with a standard deviation of .02 and .04 mm., respectively. Chapman (1918) draws attention to the fact that the contour and general appearance of the eggs varies somewhat according to the type of medium in which they are found. Thus eggs in finely milled wheat flour would appear smaller than those from coarser cereals. Stanley (1932) determined the mean moisture content of 30,000 eggs and found it to be 44.9 per cent.

The eggs hatch into small, white larvae which measure, according to Brindley, on the mean 1.18 mm. in length, and 0.18 mm. across the head capsule. The first instar larvae weigh 0.028 mg. The moulting behavior of the larvae may be described in the words of Chapman (1918, p. 75) who says,

> For a short time before each moulting, the larva is inactive and the body is large in proportion to the head. The skin splits dorsally over the head and thorax, and the larva emerges. It is at first white, like the larva of the first instar, but after twenty-four hours it takes on a yellowish color. Immediately after moulting, when the larva has expanded as a result of being freed from the old skin, it has often been observed to remain quiet for a time.

An interesting question with regard to *Tribolium* metamorphosis is that dealing with the number of larval instars. Chapman and Brindley, both reporting original observations, have noted that there are six larval instars in the development. These writers measured the larval head width during the different instars and used this as a criterion of development: i.e., a certain head width indicated a specific larval stage. Good (1933) studied the number of instars by counting the number of exuviae (larval skins) deposited in the flour after each larval moult. This author concludes (p. 331): ". . . . the writer has determined that there is no fixed number of larval moults, but that the number ranges from 6 to 11 or more and is normally 7 or 8 instead of 6. This variation is due both to external conditions, such as food, temperature, and humidity, and to individual characteristics entirely apart from external influences."

The larvae gradually increase in size with every moult: Brindley shows the fourth instar larvae, for example, to have

TABLE 1

Larval measurements of Tribolium confusum. (Taken from Brindley by permission of the Ann. Ent. Soc. Am.)

INSTAR	AGE IN DAYS	LENGTH		HEAD CAPSULE WIDTH		WEIGHT
		Average	Standard deviation	Average	Standard deviation	
		mm.	*mm.*	*mm.*	*mm.*	*mg.*
1	0	1.18	0.05	0.18	0.01	0.028
2	3	1.64	0.11	0.22	0.03	0.035
3	6	2.38	0.08	0.29	0.01	0.119
4	9	3.23	0.20	0.40	0.01	0.332
5	12	4.00	0.44	0.53	0.04	1.09
6	15	6.00	0.70	0.69	0.03	2.40

a mean length of 3.23 mm., a head breadth of .40 mm., and a weight of .332 mg. The last instar larvae are 6.0 mm. long, .69 mm. broad across the head, and weigh 2.4 mg. As the time for pupation advances these last instar larvae become more and more quiescent and contracted and finally pupate. An original drawing of a last instar larval form is appended as Figure 1. Table 1 gives Brindley's measurements of *Tribolium* larvae.

The pupae of *Tribolium* are naked and, with the occasional exception of a slight abdominal movement, they are inactive. They are whitish-yellow when first formed but turn yellowish with age, being brown at the time of emergence. Brindley finds the pupae have a mean length of 3.46 mm., and a width of 1.12 mm. There is a tendency for the female pupae to be longer than the male. A question of great practical importance for experimentalists working with *Tribolium* is the method of determin-

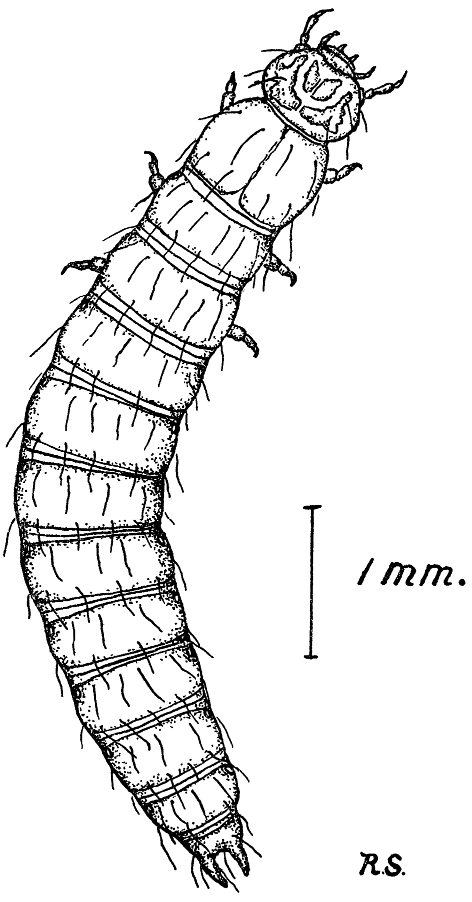

Fig. 1. Drawing of a Last Instar Larval Form

ing the sex of the beetles. As far as is known, the only reliable external sexual characteristic for any stage is found in the pupal stage. When the ventral posterior ends of the male and female pupae are examined under low magnification this sexual distinction is obvious. On the terminal segment the female has a pair of small appendages whch are reduced to indistinct elevations in the male. Figure 2 brings out this distinction between the two sexes.

Marshall (1927) has described the development of the compound eye of *Tribolium confusum*, and, since this is a contribution to the metamorphosis of these beetles, it is considered wise to outline the essentials of this development here. The compound eye makes its first appearance in a late larval instar as a grouping together, in the eye regions, of a few hypodermal cells to form a spindle-like body. These cells are the visual cells and by increasing in size they form the retinula. At the distal end of this retinula four nuclei enlarge and, with their surrounding cytoplasm, become the crystalline cone cells. With development there is an increase in the size of these four cells and they eventually become the largest part of the ommatidium. In their growth they push the distal portion of the retinula away from the eye surface. At this stage there have been formed two distinct parts to each developing ommatidium: a proximal portion consisting of the retinula, and a distal portion composed of the crystalline cone cells. Pigment is laid down first in the visual cells, then in the corneal cells, and finally in the cells between the ommatidia. Each crystalline cone cell possesses a large clear space in its proximal part which is an anlage of the crystalline cone. In *Tribolium*, however, this cone disappears during the pupal period and is absent from the eyes of adult beetles. Early in its development each ommatidium becomes convex over its exterior surface and pushes out the cuticula covering it. This is the start of the cuticular lens. The cells of the cornea lying between the ommatidia secrete cuticula which moves inwards to the crystalline cone cells and forces them from their original position. With this secretion of cuticula the lens development is completed.

A good idea of the proportions of *Tribolium confusum* adults may be obtained from Figure 4. Brindley records their mean length as 3.4 mm., width across the thorax 1.02 mm., mean male weight 1.48 mg., and mean female weight 1.78 mg. There seems to be a quite consistent tendency for the females to be larger than the males but there are enough exceptions to this fact to prevent the size of the adults from being used as a reliable criterion of sex. Immediately after emergence the chitinous exoskeleton of the adult beetle is soft; the forms are inactive, and are a light brown in color. In one or two days, however, the beetles have assumed the typical reddish-brown color with the exoskeleton quite hard. In very old *Tribolium* the adults are nearly black. Good (1933) reports observations on the longevity of adult *Tribolium*. This investigator observed 50 individuals and found 13 of them alive after 24 months of life. Dr. Good has informed the writer that of these 13, one male lived approximately three years nine months, another male three years eight months, and a third male three years four months. The present author has observed that the majority of individuals which successfully emerge and meet with no accidents live at least six months. This long life is, of course, frequently valuable from the viewpoint of the experimentalist.

Brindley (1930) has reported on the life-cycle of *Tribolium confusum* under carefully controlled conditions of humidity and temperature. This author subjected the beetles to a temperature of 29.7° C., and a relative humidity of 73 per cent. His findings, as to the duration of the various stages, are recorded in Table 2. These data bring out the fact that all of the larval stages are passed through rather rapidly except the last instar which is comparable in extent to the egg and pupal periods. As indicated by the standard deviation this last larval instar also displays the greatest time variation in its duration. It is interesting to note that this is related to the observation of Chapman (1918) who points out that the last larval instar is more influenced by ecological changes, with respect to its duration, than any of the other

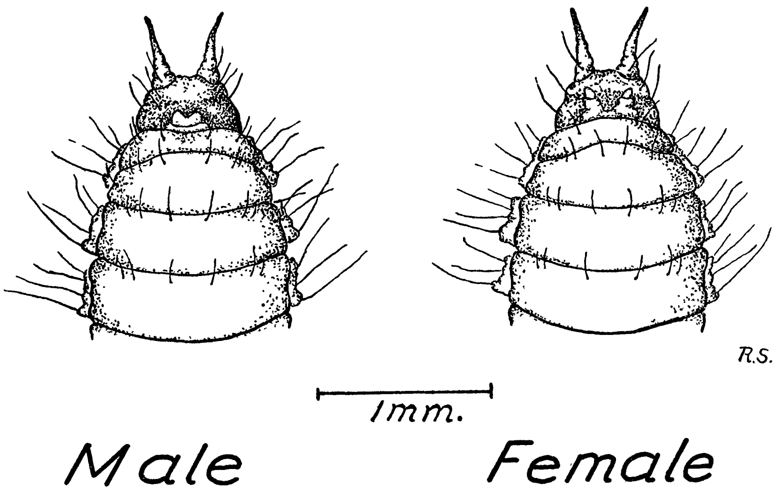

FIG. 2. TERMINAL VIEW OF MALE AND FEMALE TRIBOLIUM PUPAE SHOWING THE SEXUAL CHARACTERS

developmental stages. The average length of time required for the completion of a *Tribolium* life-cycle under Brindley's conditions of temperature and humidity, and food is 30 days. These data of Brindley's demonstrate that for a particular set of conditions, and a presumably in-bred stock, the variation in the length of the cycle is surprisingly small for observations based on 100 different individuals. It would be erroneous to generalize, however, that 30 days represents the usual length of the cycle. Good (1933), to illustrate, shows that the length of the cycle varies, for one thing, according to the food the larvae feed on. At 27°C., this writer finds that the larval period lasts 31 days when the forms had been reared on middlings as compared to 89 days when the larvae had been raised in white flour. The latter observation differs from the experience of the present author, however, since, in white patent flour at a temperature of 28°C., and a relative humidity of approximately 50 per cent, an entire life-cycle from egg to adult is completed in about 50 days. The cycle becomes longer as the temperature lowers. Good reports that *Tribolium* adults spend the winter in unheated Maryland flour mills in a semi-dormant condition, resuming breeding as the spring approaches. The larvae and pupae do not seem so able to withstand low temperatures as do the adults. Chapman (1931) reports that *Tribolium* adults die in a few weeks if subjected to a temperature as low as 7°C., indicating their inability to assume a true dormancy. On the basis of experimentation (Chapman, 1931; Chapman and Baird, 1933; Stanley, 1932) the relation of temperature to the length of the life-cycle can be summarized as follows: at 32°C., the development from egg to adult is passed through in about 27 days; at 27°C., in about 37 days, and at 22°C., in about 93 days. These figures were all obtained under constant conditions of humidity (75 per cent) with whole wheat flour used as food.

TABLE 2

Length of the stadia of Tribolium. (Taken from Brindley by permission of the Ann. Ent. Soc. Amer.)

STAGE	LENGTH OF STAGES (IN DAYS)			
	Minimum	Maximum	Average	Standard deviation
Egg	5.5	5.5	5.5	0.00
1st. instar	2.0	2.0	2.0	0.00
2nd. instar	2.0	3.0	3.0	0.05
3rd. instar	2.0	3.0	2.5	0.68
4th. instar	2.0	3.0	2.7	0.55
5th. instar	2.0	3.0	2.8	0.36
6th. instar	5.0	7.0	5.5	0.71
Pupa	6.0	7.0	6.2	0.44
Totals	26.5	33.5	30.2	

An essential point to be emphasized about the life-cycle of *Tribolium confusum* is that it must be expressed relative to the specific environmental conditions obtaining at the time of observation. Although no actual data are available as yet it is most probable that the duration of metamorphosis also varies according to the genetic pattern of the beetles as well as with the surrounding ecological conditions.

Holdaway (1932) has reported at length upon the effects of atmospheric moisture on *Tribolium*. This author deals with the following aspects of the subject: first, the effect of humidity on the size of adult populations; second, the rate of population growth as related to humidity; third, the effect of change of humidity on population equilibrium; fourth, the relation of humidity to the rate of development and the viability of the various metamorphic stages, i.e., physiological effects of humidity, and last, the importance of humidity in affecting entire populations. Holdaway finds adult *Tribolium* increase in numbers as the humidity increases from 25 per cent to

75 per cent. In his experiments there were 9.5 beetles per gram of flour at 25 per cent R. H., 11.4 beetles per gram of flour at 50 per cent R. H., and 15 per gram of flour at 75 per cent R. H. Above 75 per cent the number of adults declines due to the development of fungus in the flour. The author also noted that the adult populations kept at 75 per cent R. H. had a faster growth rate than those at lower humidities. By taking, for example, an asymptotic population of adult beetles characteristic of a 25 per cent humidity environment (9.5 beetles per gram of flour) and subjecting this same group to a humidity of 75 per cent the population assumed the concentration typical for a 75 per cent humidity (15 beetles per gram of flour). This indicates that the effects of humidity on the life-cycle are probably not irreversible in nature. In studying the relation of humidity to viability of immature stages Holdaway found that, in general, more eggs hatched in low humidities than in high. On the other hand, the larvae showed a greater survival in the high humidity environments with the optimum at 75 per cent. The pupae had coefficients of survival similar to those of the eggs since more pupae failed to develop as the surrounding atmospheric moisture increased. A consideration of the effect of humidity on rate of metamorphosis showed that the eggs and pupal stages were little influenced by humidity conditions while the larvae developed more rapidly as the humidity increased from 25 per cent to 75 per cent. Holdaway investigated the influence of 25 per cent, 50 per cent, and 75 per cent relative humidity on oviposition and concluded that no apparent effects were discernible. A further statistical analysis of his data (Table XV, p. 296), however, suggests that the fecundity rate is significantly higher for beetles in a 50 per cent humidity environment when compared with those in 25 per cent. The females in 75 per cent humidity also produced significantly more eggs than those in 25 per cent. When the females of the 50 per cent environment were compared with those in the 75 per cent environment there was a suggestion (1.9 times the standard error) that the former were ovipositing more rapidly. The effect of humidity on oviposition can not be considered definitely closed at the present time.

As an elaboration of the life-cycle of *Tribolium confusum* it might be well to describe briefly some of the facts known as to the growth of entire populations of these forms. Chapman (1928) has experimentally approached this problem with interesting results. This author set up six environments of whole-wheat flour which increased in size geometrically. The environments consisted of 4, 8, 16, 32, 64, and 128 grams of flour. Adult *Tribolium* were introduced into these environments to provide initially one beetle to each two grams of flour. Thus the four gram culture contained one pair of *Tribolium*, the eight gram culture two pairs, the 16 gram culture four pairs, the 32 gram culture eight pairs, the 64 gram culture 16 pairs, and the 128 gram culture 32 pairs. Counts of the number of eggs, larvae, pupae, and adults were made at various intervals in order to see how the total population was increasing relative to environmental size. The essential point of the data, as emphasized by Chapman, was that after a period of approximately 100 days the populations all reached an equilibrium which was similar for all the environments when measured in terms of beetles per gram of flour. In other words, the culture of 128 grams of flour with its initial population of 32 pairs of beetles, although much larger after 100 days in terms of total number of individuals, contained essentially the same number per gram of flour as did, for example, the 4 gram-1 pair unit. Chapman calculated this equilibrium point attained by *Tribolium*, under these specific conditions of temperature, humidity and kind of flour, to be 43.97 ± 2.88 individuals per gram of flour. Chapman considers this equilibrium to be the result of the interplay between the capacity of the species to reproduce (Biotic Potential) and the resistance of the environment. The author concludes that cannibalism is a most important factor in this resistance since, although thousands of eggs may be present in the environment at any one time, only some of them escape being eaten. It is this eating of eggs, which is directly proportional to the population concentration, that maintains the equilibrium of the total number of individuals. Nicholson (1933: p. 140) criticizes Chapman's method of calculating 'environmental resistance' as follows:

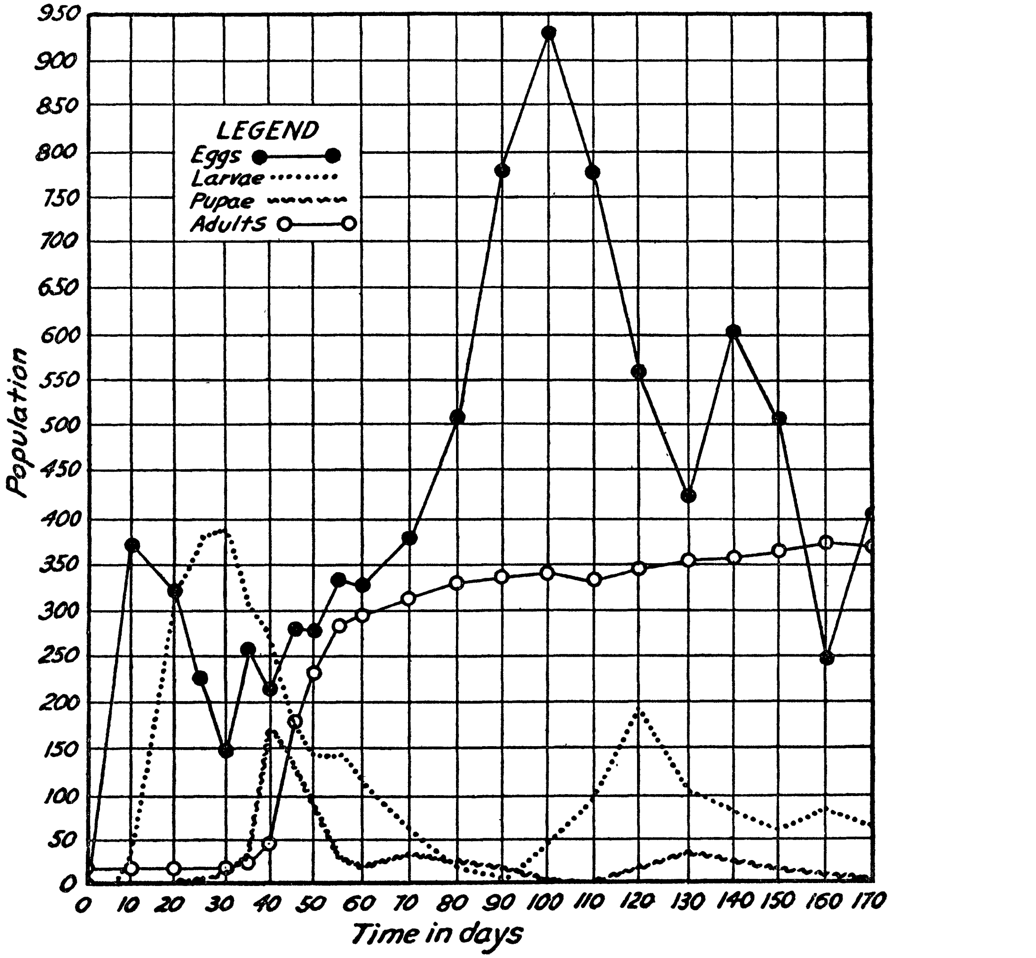

FIG. 3. POPULATION GROWTH OF TRIBOLIUM CONFUSUM AT 27°C., AND 75% RELATIVE HUMIDITY (Taken from Stanley, 1932, Canadian Journal of Research, 6, p. 668, by permission)

> . . . . Having experimentally determined the density of *Tribolium confusum* under certain conditions, he pro-

ceeds to calculate environmental resistance by means of his formula. A glance at this example (p. 120) shows that the only use made of the experimentally determined density is to multiply *both* sides of the equation by it. Clearly the result is independent of density, and Chapman's hypothesis completely fails to deal quantitatively with animal populations.

Gause (1931) analyzed the data of Chapman and showed that the *Tribolium* populations grew according to the well-known logistic curve of Verhulst (1838) and Pearl (1925). Figure 3, taken from Stanley (1932), shows the varying numbers of eggs, larvae, pupae and adults present in active *Tribolium* populations incubated at 27°C., for a period of 170 days. Chapman (1933), in a pre-publication abstract, calls attention to the fluctuation of numbers in *Tribolium* populations. This investigator has carried on four separate populations for nearly two years and has demonstrated a synchronous fluctuation of their numbers.

Allee (1931, 1934), Maclagan (1932), and Park (1932, 1933) have shown that in early stages of population growth there may be an optimal population size for growth which is larger than the initial minimum beetle concentration and smaller than the initial maximum beetle concentration. Allee has related these observations to the beneficial effects frequently resulting from crowding. Maclagan and Park have presented analyses as to the casual factors involved with a discussion of these two theories by Park (1934).

PRODUCTIVITY

One aspect of the general biology of *Tribolium confusum* of obvious importance to experimentalists is the question of productivity. This can be interpreted as dealing with both oviposition, or fecundity, and fertility, or percentage of egg hatch. It is only recently that data have been reported on these considerations and much still remains to be done. Good (1933) reports a series of interesting observations on oviposition which are valuable since they record the number of eggs produced by females during the entire period of their egg-laying life. This period, according to Good, may last as long as 14 months, the average being approximately nine months. During this time a female *Tribolium* normally lays 400 to 500 eggs, although records of nearly a thousand eggs are not unknown. With regard to egg fertility Good reports that about 90 per cent of these eggs hatch. Brindley (1930) obtained oviposition data for ten newly emerged *Tribolium* pairs and found, as might be expected, that these young females oviposited at a much higher rate during this limited period than did the forms used by Good who obtained long-time records. Using Pearl's (1922) 'rate per female per day' method of measuring fecundity, Brindley reports, for this ten day period, an average of about 11 eggs per female per day with occasionally as high as 18 eggs being produced in a single day. These rates are higher than those of Chapman (1918) or Park (1932, '33) who also worked with young females over a short period of observation. One reason for this discrepancy is undoubtedly, as Brindley points out, the fact that he removed his adult beetles daily and introduced them into fresh flour thus essentially eliminating the eating of eggs by adults. In addition, other factors, both genetic and ecological, are quite conceivably operating in this case. As a general example, temperature has been demonstrated to affect oviposition. Stanley (1932) summarizes certain data of Chapman on the effect of temperature on egg-laying. At 22°C., the mean oviposition rate per female per day was 1.9 eggs; at 27°C., 6.24 eggs, and at 32°C., 10.73 eggs. These are all significant differences and clearly indicate the relation of moderate temperature variations to fecundity. Park (1932, '33), in studying population problems, had occasion to repeat frequently a standard experiment in which either one pair of young adult *Tribolium* or else a single fecundated female spent eleven days in 32 grams of patent flour at 28°C., with egg counts made at the end of that time. As a further contribution to the question of *Tribolium* productivity all cases falling within the above experimental type have been collected from the data of both papers and are biometrically presented in Table 3. These rates are, on the mean, slightly higher than those presented by Good but much lower than those of Brindley and Chapman as

TABLE 3

Egg productivity rates of Tribolium confusum (237 cases calculated from the data of Park, 1932, '33)

Maximum number of eggs after 11 days incubation	63. eggs
Minimum number of eggs after 11 days incubation	0.
Mean number of eggs after 11 days incubation	38.9 ± .64
Median number	31.2 ± .80
Standard deviation	14.8 ± .45
Coefficient of variation	38% ±1.17
Mean rate per female per day	3.5

reported by Stanley (1932). It should be emphasized that in these experiments no attempt was made to eliminate cannibalism: the rates presented here are not therefore *sensu stricto* absolute fecundity rates but simply measure the eggs remaining in the population after some have been consumed. The emphasis here was placed upon the study of natural populations in which egg-eating is an important function. Park used patent flour in his experiments while Chapman and Brindley used whole-wheat flour. Good (1933) has shown that the kind of flour influences egg laying. At 27°C., in whole-wheat flour he found the mean egg rate per female per day was 2.43 eggs; in bran, 1.26 eggs; in oatmeal, 1.04 eggs, and in white patent flour, 0.58 eggs. There is, in all probability, no real discrepancy between these data and those of other authors since the experimental conditions and procedure have been different with various ultimate aims in mind. On the other hand, there seems to be no patent biological reason why these rates and those obtained by Chapman and Brindley, who worked with different stocks and in a different location, should necessarily closely approach the same constant figure. This argument is further substantiated by the investigations of Good (1933) who obtained oviposition rates much lower than those of Chapman. It should be indicated that Good purposely ran his experiments over a long period of time and the rates he obtained may have been influenced by aging on the part of the beetles. On the other hand Good removed the adults from their flour daily so that egg-eating effects were minimized. About oviposition Good says, "The number of eggs laid per day is not large. In no case were more than 13 viable eggs laid in one day by a single female, and the average was only 2 or 3 per day. Under optimum conditions Brindley records 18 eggs in one day and a much higher daily average than is indicated here." (p. 330)

As can be seen from the standard deviation of Table 3, these particular egg-rates of *Tribolium* vary considerably between individuals even when experimental conditions are quite closely controlled. These data do not, of course, indicate whether the egg-laying and egg survival variation is due to non-controlled ecological factors, or to genetic differences between the beetles. Presumably both types of factors are operating. It is a common observation that one female may have a high oviposition rate which persists throughout a considerable portion of that individual's life, while another female, under as nearly similar conditions as can be obtained, has a consistently low oviposition rate. Facts such as these seem to indicate that the genetic constitution of the beetle in question is an important factor in fecundity as well as are ecological influences. With respect to egg fertility Park (1933) found that about 90 per cent of the eggs produced larvae providing they were laid by a female who had experienced more than a single initial copulation. Park also reported the oviposition rate of young virgin *Tribolium* females showing it to be much lower (about 20 times) than that of fecundated individuals.

An interesting, but anticipated, feature of *Tribolium* oviposition is that it is affected by certain environmental influences such as kind of flour, possibly humidity (Holdaway, 1932), temperature, conditioning of the flour by beetles living in it (Park, 1934), as well as population density relationships. Facts such as these where such a major function as reproduction can be shown to be sensitive to experimental changes enhance the value of the form for study. More work on this general subject seems forthcoming judging from advance abstracts presented by Chapman (1933) and Chapman and Baird (1933).

CERTAIN ASPECTS OF TRIBOLIUM BEHAVIOR

Certain observations on the behavior of *Tribolium* may be of interest. From common laboratory experience it is obvious that the adults are photo-negative: i.e., they loosely cluster in shaded portions of a container when subjected to direct light. In connection with their behavior to light it is instructive to know that *Tribolium* cultures can be maintained indefinitely in complete darkness as Chapman (1931) has noted. These beetles also react negatively to gravity and when located on a nearly vertical surface crawl to the top of the container. This gravity reaction seems to be markedly displayed, however, only when the beetles are not located in some kind of solid culture medium. In the latter medium the tendency to vertical stratificaton, at least in small environments, is slight.

In moving through the flour the beetles seem to employ only trial and error methods since their movement appears quite at random. This fact is possibly implied in the common name of the form, the 'confused' flour beetle, and has been observed a number of times. Stanley (1932, 1932a), working with *Tribolium* populations mathematically, makes the random movement one of his fundamental premises. Park (1933) has shown how the adults of a single colony of *Tribolium* were distributed entirely at random. This type of movement seems to be the logically expected one since such an environment as flour would probably limit the efficient operation of such senses as visual and olfactory. This is substantiated by Chapman's (1931) observation that *Tribolium* exhibits relatively little olfactory selection of food. Although all the evidence points to complete random movement by *Tribolium* in flour, at this stage, however, it can not be too dogmatically asserted that the beetles never exhibit any directed motion.

The fact that adult and larval *Tribolium* practice cannibalism has been reported by several authors. It is known that eggs, early instar larvae, and pupae are consumed. Chapman (1928) emphasized the importance of the egg-eating behavior in the maintenance of population equilibrium. Park (1933) experimentally obtained egg-eating rates by introducing a single adult beetle into an environment of 32 grams of flour which contained initially 30 eggs and making counts of these original 30 eggs after 11 days of incubation. It was found that virgin females, fecundated females, and males had a statistically similar egg-eating rate as tested by this experiment with the mean number of eggs eaten for the period approximately seven per beetle. It is to be expected, of course, that with other types of experiments these rates might change relatively.

Outside of these statistical approaches to the study of cannibalism, however, there seems to have been little done on the observational side. It might be interesting at this point to resort to natural history and describe the behavior of an adult *Tribolium* in the process of eating an egg. It is impossible, naturally, to observe the beetle eating an egg when both are covered with flour. Therefore, an observation container was fitted up which consisted of a petri dish lined with filter paper containing a number of eggs and several adult *Tribolium.* To facilitate the observation of details the whole dish was placed on the stage of a low powered binocular microscope and the beetles examined through this magnification. In this dish the beetles move at random without any visible reference to the eggs. They occasionally walk over them or roll them to one side as they move about but seem to engage in no definite preliminaries previous to the actual consumption of the egg. It is not clear exactly which senses are employed in the location of a specific egg which is to be eaten. Presumably both visual and tactile senses aid the beetle in its reaction. In eating the egg the *Tribolium* follows a rather definite behavior pattern. The egg is grasped between opposing surfaces of the tarsal joints of the prothoracic legs with the tarsal claws usually puncturing the egg membrane. While the egg is being thus grasped the prothoracic legs are extended forward so that the femur and most of the tibia of both legs parallel the longitudinal axis of the body and may be partly covered by the head. As a result of this leg movement the part of the egg proximal to the beetle is brought into contact with the mouth parts. By means of mirrors it was possible to observe the mouthparts in action. The short, horny mandibles seem to function primarily in fragmenting particles from the egg while the maxillae and maxillary palpi aid in this fragmentation and also work the material into the mouth proper aided by the labium and labial palpi. The eating of the egg is facilitated by the fact that upon puncturing the egg membrane the fluid contents coagulate and, mixing with the flour which adheres to the external surface of all *Tribolium* eggs, produces a mixture of sufficient consistency to be handled by a form possessing biting mouthparts. As the beetle consumes the egg the prothoracic legs pull the remaining portion posteriorly so that it always approximately maintains its same position relative to the mouth. The mesa- and metathoracic legs usually brace the beetle by extension to the side and back. A general representation of the beetle eating an egg is depicted in Figure 4. It is not the purpose of this description to suggest that the behavior described is a fixed and non-variable one, for this would be fallacious. This report does describe, however, the typical chain of events as they were observed during the reaction. A beetle can completely consume an egg in fifteen minutes, although frequently it may take much longer. Quite often a *Tribolium* may leave an egg before it has been completely eaten. When the observation tray was covered with a thin layer of flour the egg-eating behavior remained unchanged.

Another behavior reaction of probable interest to experimentalists is the copulation one. The importance of copulation in certain *Tribolium* populations has already been reported by Park (1933) where it was demonstrated that, up to a certain point, the fecundity and fertility of *Tri-*

bolium females was increased by repeated copulations. Copulation seems to be a frequently practiced behavior among *Tribolium* as both statistical and observational data show. There do not seem to exist, however, any particularly elaborate or unvarying behavior reactions associated with the process. Males can occasionally be observed to attempt to mount other males. This suggests that sex recognition is not highly developed among these forms. The pre-copulation behavior varies greatly both as to its extent and nature. Often a male may follow a female and, attempting to mount her with no success, eventually cease his activities. Again the two sexes may meet and copulate without any visible preliminaries at all. In mounting the female the male beetle clasps her with all three pairs of legs extending them around the ventral surface of her body. The male lies somewhat posteriorly on the dorsal surface of the female so that his head coincides with the thorax of the female. This

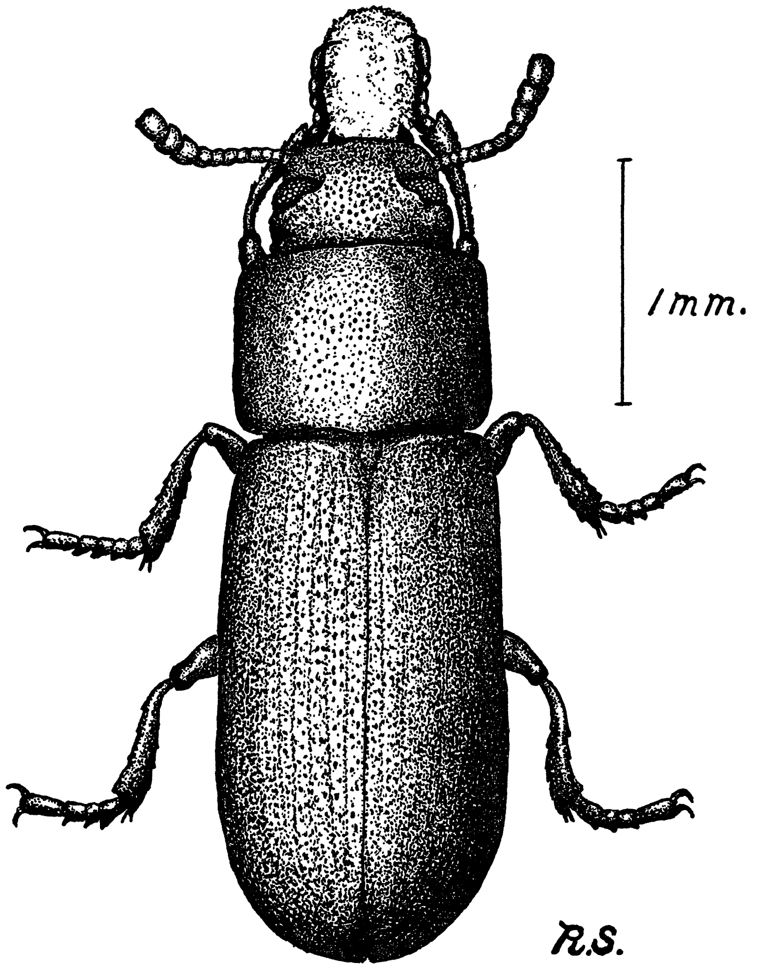

Fig. 4. Drawing Showing an Adult Tribolium Eating One of Its Own Eggs (from life)

allows the terminal end of the male's abdomen to project out behind that of the female. The chitinized penis is then projected downward and forward making contact with the vagina which is but slightly extruded. When this connection has been effected the seminal fluid, a mucous-like, slightly milky appearing secretion, enters the vagina. As far as the author's personal experience is concerned the mating pair may remain united for as long a period as fifteen minutes or, conversely, the copulation may be consummated within a space of two or three minutes time. During the copulation the females are sometimes observed to move about rapidly and at other times to remain motionless. Frequently the male completely withdraws the penis but remains clasped to the female and inserts it again after a varying length of time.

A brief comment might be made about the defecation of *Tribolium* adults. This was observed by means of the same type of observation apparatus used in studying cannibalism. Defecation seems to be a rather frequent process judging from the accumulation of fecal matter on the filter paper. The feces are well formed, ovoidal in shape, and vary in color from almost colorless, through various degrees of green, to a dark black. The beetles observed had all been fed upon patent flour. In defecating a single pellet is usually extruded. In the cases observed this is rather quickly done. The beetles may defecate either while moving or quiescent.

Another behavior reaction may be mentioned since, although infrequent, it is of considerable biological interest. Chapman (1926) noted that when aggregated *Tribolium* adults were stimulated by rubbing they emitted a rather pungent gas, ". . . . which smells not unlike an aldehyde. It irritates the mucous membrane of the nose and turns flour and certain other materials pink, and in high concentration, affects the eyes." (p. 295) When the larval and pupal forms of *Tribolium* were subjected to the gas Chapman observed that in about 10 per cent of the cases abnormal forms were developed. The mature larvae emerged with a combination of larval and pupal structures and the pupae emerged as adults displaying some deformity. Chapman concludes that the development of these monsters is associated in some manner with the gas produced by the beetles when the latter are irritated by some unusual stimulus. The control larvae and pupae not subjected to the gas showed no incidence of this atypical metamorphosis. Doctor John Stanley (personal communication) has advised me that there is some evidence that the gas, along with certain fluids found in *Tribolium*, may be irritating to man resulting particularly in gastric disorders. He advocates discretion in the handling of the form for this reason.

Adult *Tribolium confusum* possess fairly well developed wings which leads to the question concerning their ability to fly. Good (1933) reports that he has never observed *Tribolium confusum* fly or even attempt flight. According to this author, however, the closely related species, *Tribolium ferrugineum=castaneum*, frequently attempts to fly and, when stimulated by a strong light, occasionally makes short flights of a few feet. Holdaway (1932) reports that on one occasion only has he observed *Tribolium confusum* fly and that was when the form had been stimulated by high temperature. The present author has never observed flight on the part of these beetles.

Adult *Tribolium* frequently practice death-feigning or letisimulation. In handling the beetles the author has often observed them draw in the legs and remain completely quiet despite the fact they were being moved about. This reaction is of short duration, however, a few seconds at the most, and the forms soon become active irrespective of outside stimulation.

TRIBOLIUM PARASITES

Good (1933) reports the occurrence of two mites *Acarophenax tribolii* Newstead and Duval and *Pediculoides ventricosus* Newport, and a bethylid (parasitic wasp), *Rhabdepyris zeae* Waterston as parasitic on *Tribolium*. This author states that these three forms are not very effective in controlling the beetles. Doctor Clay G. Huff, of the University of Chicago, and the present author studied a case of internal parasitism in *Tribolium confusum* which arose in the stock cultures. The parasites seemed to have their effect upon the pupal stages since the latter, when parasitized heavily, grew dark in color and failed to emerge as adults. Histological preparations were made of the pupae and the parasite studied. The conclusion was reached that this form was a coelomic coccidian probably belonging to the genus *Adelina* and previously referred to by Riley and Krogh (1922) and White (1923). The latter writer mentions that infected larvae and adults, as well as pupae, frequently die of the disease. Gregarines are also reported as infesting *Tribolium confusum*.

TECHNIQUE

In culturing stock *Tribolium* populations practically any type of receptacle may be used. Standard glass battery jars about 25 x 19 cm., are convenient and economical and may be stacked in incubators with a minimal loss of space. Many kinds of food can be used for breeding the beetles and most of the *Tribolium* workers seem to have favored whole wheat flour. Since the wheat husks so frequently resemble the larvae and since they (the husks) do not pass through fine meshed sieves it is advisable to use a flour which has these seed-coats removed. The present author uses 'Ceresota' a non-bleached, commercial, white patent flour. It is preferable to keep the stock jars in constant temperature incubators at a temperature between 27°C. and 30°C.. The humidity can either be accurately controlled or may be allowed to vary between 40 and 75 per cent. In order to maintain the stocks at asymptotic concentration it is necessary to change the flour frequently as Park (1934) has demonstrated.

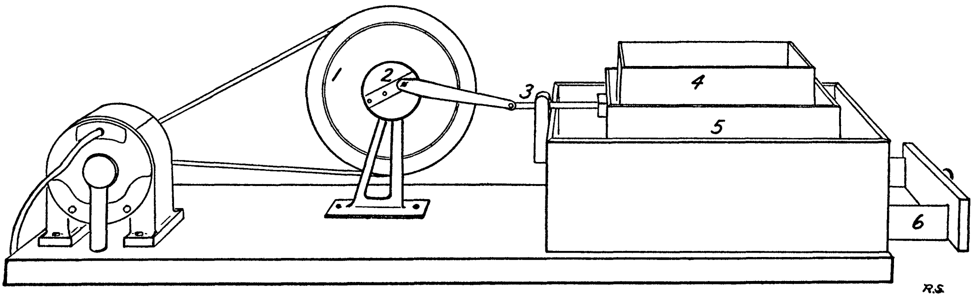

FIG. 5. DIAGRAM OF THE AUTOMATIC FLOUR SIFTER
1, belt wheel; 2, driving wheel; 3, driving rod; 4, flour sieve; 5, flour sieve holder; 6, removable collecting tray

A census can be taken of infected flour by passing it through fine meshed standard silk bolting cloth. Chapman (1918) used different meshed cloths and found that number 9, for example, would not pass any of the *Tribolium* stages but would permit finely milled flour to go through; number 3 separated eggs and larger larval stages from the flour; number 000 passed all eggs and larvae except the last instar forms, etc. Thus by using bolting cloth sieves of a specific mesh *Tribolium* stages may be retained for counting as desired. To facilitate the sieving of the flour the author uses an electrically driven sifting apparatus constructed for him by Mr. M. E. Carson, Whitman Laboratory, University of Chicago. This apparatus (Fig. 5) simply moves a framed piece of bolting cloth back and forth over a removable tray and separates the desired beetle stages from the flour which collects in the tray below. There is evidence to show that shaking affects adversely the oviposition of adult *Tribolium* (Stanley, 1932). For this reason, it is desirable to gently remove the adult beetles from the experimental flour before sifting is started. The beetles may be handled with brush or spatula.

SUMMARY

The twofold purpose of this paper has been to point out the desirable features exhibited by *Tribolium confusum* Duval for experimentation, and to present information about the general biology of the form which might be useful to present and future investigators. At the present time most of the *Tribolium* experimentalists are using the flour beetle in population studies. This is, of course, easily understood since the form is admirably suited for studies of this type due to the homogeneity and reproducibility of its environment. Many population problems remain to be solved here, however, and future work is to be encouraged. On the other hand, *Tribolium* does not seem limited to population studies alone; investigations on nutritional, radiation, behavior, metamorphosis, and possibly genetic problems can be approached by the use of this organism.

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