NUTRITIONAL STUDIES ON THE CONFUSED FLOUR BEETLE, TRIBOLIUM CONFUSUM DUVAL.*

By ROYAL N. CHAPMAN.
(From the Division of Entomology and Economic Zoology, Department of Agriculture, University of Minnesota, St. Paul.)

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INTRODUCTION.

During the past 15 years a considerable amount of work has been done on the nutrition of insects. Guyénot (1913, 1917), Delcourt and Guyénot (1910, 1911), Loeb (1915), Northrop (1917), Baumberger (1917, 1919), Bacot and Harden (1922), and others have worked chiefly on the fruit-fly, Drosophila, which lives in decaying fruit. Bogdanow (1906, 1908), Wollman (1911, 1919), and others have studied the nutritional requirements of the blow-fly, Calliphora vomitoria L., but aside from the work of Portier (1919) on the meal worm, Tenebrio molitor L., the investigations have been confined to insects which normally live in media containing large numbers of microorganisms.

The work on the fruit-flies and the blow-fly has shown that the microorganisms are essential for normal growth in these insects. A further analysis of their diet is thus rendered very difficult. Bacot and Harden (1922) have attempted to analyze the diet of Drosophila in order to determine its vitamin requirements. They concluded that vitamin B is required and that vitamin C is not required. Vitamin A may be required although they were not sure that something other than the vitamin of butter fat may have been present making it possible for the flies to develop in the yeast extract when the butter fat was present but not in its absence. However, these authors make no mention of the fact that Baumberger (1919) was able to rear Drosophila...
phila on yeast alone. Neither do they suggest that something other than the vitamin B in the yeast extract may furnish the necessary nutrients.

Portier (1919) got complete development of Tenebrio molitor in flour which had been sterilized at 130°C. This author is sure that certain granules found in the epithelium of the digestive tract are not symbionts, and that his experiments prove that Tenebrio can live in a sterile medium and that microorganisms are in no way essential to its normal growth. For this reason it has seemed that an insect, such as the confused flour beetle, Tribolium confusum Duval, which normally lives in flour or meal is a desirable form for a study in nutrition. The contents of the digestive tracts of several larvae were stained and examined bacteriologically but no living organisms were found. In addition its normal life cycle requires but a few weeks, while that of Tenebrio molitor requires about 1 year.

In recording the rates of growth of insects, Baumberger (1919), Herms (1907), and others have used the weights of the larvae. Hirshler (1914) reviewed various methods of measurement and adopted length and thickness. Neither length nor weight seem entirely satisfactory for two reasons: in the first place they do not lend themselves to accuracy and in the second place they take no account of the attainment of maturity. The length of nearly all insect larvae varies greatly as the body elongates and contracts during locomotion. Consequently it is very difficult to assign a definite length to a larva at any time. The weighing of small larvae is subject to the errors incident to the weighing of any small object and it also takes no account of variations in moisture content.

The attainment of maturity cannot be recorded by either the measurement or the weight for, as Herms (1907), Baumberger (1919), and many others have found, larvae may reach maturity and pupate at various sizes. Herms found that he could control the size of the pupae and adults within certain limits by varying the amount of food eaten by the larvae of Lucilia. Those which fed longest grew largest and became the largest adults while those which fed the shortest length of time transformed while they were small. Overfeeding was found to prolong larval life due to retarding pupation and in some cases they did not pupate at all. Popovici-Boznosanu (1910) obtained the
same results with *Osmia rufa* and concluded that maturity is reached at smaller size when food is reduced and at large size when food is plentiful.

Wodsedalek (1917) found that the museum beetle *Trogoderma tarsale* could endure starvation for a long time, molting its skin and decreasing in size until it again obtained food when it resumed growth.

From these results and the general knowledge that the adult insects of nearly every species vary in size it may be concluded that growth, in the sense of increasing size, may be distinguished from the attainment of maturity. Kunkel (1918) believes that the size of flesh-fly pupae may be controlled by feeding ductless glands. It is later stated that mammalian thyroid retards growth and reduces size while thymus hastens pupation. But his data show such small differences of size and time that they would seem to be within the limits of experimental error. Dewitz (1902, 1913) believes that an oxidase controls the process of metamorphosis and that pyrogallic acid and CO₂ may retard it. Baumberger (1919) believes that certain changes in the nervous system and other organs must go on at a certain (minimum) rate if the minimum food substances are present. This might account for the variation in size when maturity is reached. However this may be, there would seem to be a good possibility of studying growth and the attainment of maturity in insects as affected by various foods.

The object of the present work was to determine whether growth and maturity could be controlled experimentally and whether the flour beetles could be used in nutritional work of broad biological application. The experiments which furnished the material for this paper consisted of two series. The first series is a study of individual insects while the second series is a study of the populations of certain cultures. The proteins used in the second series of experiments were furnished by Dr. R. A. Gortner who also contributed many valuable suggestions during the course of the work.

**EXPERIMENTAL.**

*Experiments with Individual Insects.*

In the first series of experiments the measurements of the head have been used as the criterion of growth (Mercer (1900) and Chapman
STUDIES ON TRIBOLIUM CONFUSUM DUVAL (1914–15)). Since the head capsule is chitinous and can expand only at the time of molting, the number of times which the larva has molted can readily be determined by measuring the width of the head. One has only to know the number of molts required for maturity in order to determine just what portion of the larval life has been completed at any particular time. The head measurement also serves to distinguish the undersized larvae or “starvation forms” which may have more than the usual number of molts. When length or weight are relied upon it is impossible to know whether a larva of a certain size is about to pupate, is an undersized but mature larva, or whether it is still immature. When the head measurements are followed, the undersized mature larvae can be distinguished from the immature larva by the greater number of molts which they have experienced. The head measurement is thus a measurement of maturity or development as well as of growth, when time is taken into consideration.

The larvae of Tribolium confusum have been cultured in various foods including different grades of wheat flours and several of the so-called wheat flour substitutes. It was found that the records in the wheat flours were very similar and therefore they were adopted as controls in all of the experiments. In plotting the results these controls were so plotted that the curve of development would bisect the angle of the ordinate and the abscissa. This was done by letting $x$, the growth between two molts, equal $y$, the number of days between these two molts. Maturity is thus measured by the number of molts and growth by time. The curve of development of the controls is a straight line at an angle of 45° representing the development of the larva from the time that it hatched from the egg until it molted and became a pupa.

In the period just before the last larval molt the larva not only grows as it has in the previous periods, but it also undergoes metamorphosis so that when it has molted once more it is no longer a larva but has the structure of a pupa. The plotting of the curve of development in such a way that it will be a straight line up to the time of the emergence of the pupa, assumes that the process of metamorphosis, which is undergone during the prepupal period, has a normal rate in proportion to the rate of growth which has preceded it.
A study of the curves for the five grades of wheat products shows that the lower grades retard growth very slightly. These products range from the middlings flour, the highest grade, through the sizings flour, low grade flour, and tailings flour, to the bran, which is the coarse outer portion of the wheat kernel. Each individual curve on these graphs represents the record for an individual beetle and it will be noticed that there are some slight differences in the rates of development among individuals fed on the same food. In the middlings,
however, three individuals had exactly the same curve. The lower grades of the wheat flour not only retard growth to a slight degree, but they also retard metamorphosis, as is indicated by the break in the curve during the last larval period.

The curve for growth in barley flour is essentially like that for the best grades of wheat flour, while that for steel cut oats shows a greater retarding effect. The curves of growth in rye flour are most instructive for they show a growth which is essentially normal up to the last larval period at which time there is a marked decline. This last period is divided between a certain amount of growth which takes place during the earlier part, and the process of metamorphosis during which the larva is seemingly inactive as a so called prepupa. By observation of the larvae it is practically impossible to determine what portion of the period is devoted to each of these functions, for they seem to overlap and intergrade.

Further study of the curves of development in the rye flour shows that the growth has proceeded at the normal rate from one molt to another until the last period is reached. And, even after this point is reached there is no reason to believe that the rate of growth has been changed but rather that it is the new process of metamorphosis which is introduced at this time which is responsible for the change in the curve during this period alone. This seems to be substantiated by the fact that the size of the adults is very constant regardless of whether the larval period was short or long. Upon the basis of this assumption, which seems to be well founded, the deviation of the curve during the
last larval period is a measure of the effect of the food upon metamorphosis, and it also serves to distinguish between the effect of food upon development which has to do with increase in size and that which has to do with the transformation from the larval to the pupal stage.

The curves of development of the beetles in corn flour show that both growth and transformation were retarded to about the same degree. It will be noticed that there was one subnormal individual, which evidently had a slow rate of metabolism, and this individual was evidently slow both in growth and metamorphosis. Since no examination was made for parasites it is possible that it was pathological.

![Graph](image)

**Fig. 10.** Curve of larval development in rice flour.

The curves of development in the rice flour, which was made from polished rice, show only a slight retarding effect upon growth but a very marked effect upon metamorphosis. Ordinary polished rice contains a small amount of embryo, which was removed in one lot of rice which was then ground in a mortar and used as a nutrient medium. The curves in this experiment show a very marked effect upon growth and only one of a large number of larvae survived to pupate. The majority of the beetles died during the early part of larval life.

Other experiments were tried using a synthetic medium which contained all of the ingredients of a Pasteur's solution except the water. In this medium there was no protein. None of the larvae in these experiments survived to pupate and the mortality was very high.
Fig. 11. Curve of larval development in embryo-free rice flour.

Fig. 12. Curve of larval development in synthetic food.
during the early periods. No method was employed to prevent cannibalism and the survivors undoubtedly fed on those that died. Nevertheless it seems rather surprising that growth was as rapid as the curves show it to have been.

The results as shown in these curves indicate that the head measurement is a very satisfactory criterion of the development of these insects. Furthermore it is possible by the use of the head measurement to distinguish between the effect of food upon growth and upon metamorphosis.

The effect of any given food upon the rate of growth may be determined by dividing the rate of growth on this food by the rate of growth in the normal food. In this case the time for growth in the first middlings flour is taken as the normal and, since it required 16 days up to the last instar, it may be expressed as 16. In the steel cut oats the time varied from 17 to 20 days and may be expressed as 20. Growth has then been retarded 1.25 in the steel cut oats. A comparison of the time required for the abnormal individual in the corn flour shows that its growth was 2.25 times that in the normal diet.

The effect of any food upon metamorphosis may be determined in much the same way by comparing the time required in the last instar when the larva were fed upon different foods. The time for the last instar was 7 days in the first middlings flour and 11 days for the average in the steel cut oats. This gives us a prolongation, for the last instar, of 1.51 times that in the first middlings flour.

We have already found that the first instars were prolonged 1.25 times that in the first middlings flour. In the case of these instars growth may be assumed to be the principal activity, but in the last instar metamorphosis also occurs. We notice that the ratio in this last instar is 1.51 instead of 1.25. The comparison of these two ratios may therefore represent the comparison of the effects of this food on growth alone and upon metamorphosis.

*Experiments with Mass Culture.*

The study of mass culture instead of individuals was resorted to because the task of making the micrometer measurements of the individuals was so exacting as to make it impossible for more than a few insects to be under observation at one time. The mass culture
method was chosen with the hope that it might develop into an easy routine which would facilitate nutritional investigations. Since these beetles are small, large numbers of them can be kept in a small space and the shortness of their life cycle makes many generations possible in a short period of time. Consequently they would be valuable animals to use in nutritional work if they proved to be sufficiently like higher animals in their requirements. (See Osborne and Mendel (1913, 1914); also Kianizin (1915–16).)

The death rate and times of emergence of adults in the cultures were used as the measure of the nutritional value of the various foods. The death rates were plotted to give a summation curve of the death rates of each population much as Davey (1919) did for cultures of the adult beetles. The per cent of the total population which was dead each day was recorded on the larval curve and the per cent which had transformed was recorded on the adult curve. When a larva pupated it was counted as dead on the curve for the larval population but it was also recorded on the curve for transformation. At the end of the experiment the per cent dead plus the per cent which had transformed always equaled 100.

Each culture was started from eggs which were carefully guarded to prevent any infestation with parasites. The containers were sterilized and larvae were examined from time to time but no infestation was found. About 1,000 newly hatched larvae were placed in each culture at the start.

The control cultures were in wheat flour and all cultures were kept side by side in an incubator maintained at 28°C. Lacking adequate humidity control it was not possible to get the same results in winter when the humidity in the laboratory was low as in the summer when it was high. In order to continue the work throughout the year it was necessary to run a control culture in wheat flour along with each series of experimental cultures. A comparison of the control curves for summer and winter shows that in the latter metamorphosis was retarded. On the other hand, control cultures which were run in duplicate at the same time of the year gave curves which were practically identical.

The populations were counted each day to record the number of living larvae. There can be little doubt that the daily handling of the
FIG. 13. Curve showing larval death rate expressed as the per cent of larvae dead each day and the per cent of adult beetles emerged from a culture in wheat flour at high humidity.

Larval death rate in corn-starch 77 gm., lactalbumen 20 gm., and Osborne and Mendel salt mixture 3 gm. (Emergence of adults in lower right.)

Larval death rate in corn-starch 75 gm., casein 20 gm., vitamin B 2 gm., and salts 3 gm.

Larval death rate in corn-starch 75 gm., wheat gluten 20 gm., wheat germ 2.5 gm., vitamin B 2 gm., and salts 3 gm. (Adults emerged in lower right.)
Fig. 14. Larval death rate in corn-starch 80 gm. and lactalbumen 20 gm.
Larval death rate in corn-starch 80 gm. and zein 20 gm.
" " " " " 78 " lactalbumen 20 gm., and vitamin B
from wheat germ or dextrin, 2 gm.
Larval death rate in corn-starch, 80 gm., zein 15 gm., and lactalbumen 5 gm.
Fig. 15. Larval death rate in corn-starch 75 gm., wheat gluten 20 gm., vitamin B 2 gm., and salts 3 gm.
Larval death rate in corn-starch 78 gm., zein 20 gm., and vitamin B 2 gm.
75 lactalbumen 20 gm., vitamin B 2 gm., and salts 3 gm.
larvae increased the death rate, as was shown by a comparison with the number of pupae from cultures which were not handled daily. However, since all of the cultures were handled alike the results from the different cultures are comparable. It was also found that the death rate was the same whether larvae were kept separately or in mass cultures.

All of the foods used had Kingsford’s corn-starch as the base to which various proteins, salts, and vitamin were added. The experiments follow the general plan of those of Osborne and Mendel (1913)

![Graph showing larval death rate in wheat flour and number of adults emerged at low humidity.](Fig. 16)
with mammals in order that the insects might be compared with other animals. The salt mixture of Osborne and Mendel (Loeb (1915)), lactalbumen, casein, zein, wheat gluten, protamine nucleate, vitamin from wheat germ, and the wheat germ itself were added to the starch as indicated on the graphs.

![Graph](image_url)

**Fig. 17.** Larval death rate in corn-starch 77 gm., zein 20 gm., and salts 3 gm. Larval death rate in protamine nucleate 100 gm.
- "" " corn-starch 80 gm. and zein 20 gm.

The curves resulting from these experiments are not smooth but are marked by more or less abrupt breaks. These occur in about the same portions of all of the curves and at about the right intervals to represent the time of molting. Since molting is known to be a crisis in the lives of larvae it seems reasonable to believe that death is more apt
to occur at this time than at any other. Observations on the individual insects support this view and a comparison of the control curves for summer and winter (high and low humidity) shows that these breaks are more abrupt in cultures exposed to dry conditions in which the molting of the dry skin is most difficult.

Diet Series I, No. 10 containing starch, wheat gluten, wheat germ, vitamin B on dextrin, and salts gave almost exactly the same curve as the control in wheat flour, except that the transformation was delayed about 2 weeks. Diet Series I, No. 9 was identical with No. 10.
except that it lacked the wheat germ. The death curve for this culture is like No. 10 up to about the time for transformation when the larvae all died.

The next approach to the control curve is that for Series II, No. 5 which is the same as Series I, No. 10 except that protamine nucleate has been substituted for wheat germ with a slight reduction in the number of adults emerged. Next in order of similarity to the control is Series I, No. 8 consisting of starch, casein, salts, and vitamin B on dextrin. When protamine nucleate is added the transformation curve shows reduction rather than an increase as is shown by Series II, No. 7. Series II, No. 2 shows that zein may be used as a source of protein without wheat germs although it gives a small number of adult beetles. The addition of protamine nucleate to this diet makes little difference, as may be seen in the curve for Series II, No. 3.

The other curves show that none of the other foods approach wheat flour and that wheat gluten and wheat germ are the best sources of protein tried. The vitamin extracted from the wheat germ does not give the results that wheat germ gives.

SUMMARY.

The confused flour beetle (*Tribolium confusum*) was chosen for this study because it lives in a food which ordinarily contains no living organisms. The death rates are greater in cultures which are handled daily than in those which are not handled but when all are handled alike the results are comparable.

The results from experiments with individual beetles in various kinds of flour were plotted with instars (larval stages) on the ordinate and time in days on the abscissa, using the results from control experiments in wheat flour to determine the length of the various instars from an “$x = y$” formula. The curves of development were found to be straight lines throughout all but the last instar. The curve for the last instar during which the larva transformed deviated from the straight line in certain foods, notably rice flour.

When mass cultures were used the death and transformation curves were plotted for each synthetic food. A comparison of the curves from wheat flour and the synthetic foods shows that the first parts
of the curves are very much alike in all cases and that a few resemble the control in every respect except that the transformation curve has been moved back for a considerable time.

The death curves for the mass cultures are not smooth but show sudden increase in death at approximately the times of molting. These curves may therefore be compared with the records from individual beetles.

CONCLUSIONS.

1. Insects which normally feed in flour or similar foods which do not normally contain living organisms are satisfactory animals for nutritional work.

2. The requirements for growth, in the sense of increasing size, seem to be much less exacting than for maturity or transformation.

3. Wheat germ more nearly satisfies the requirement for growth and transformation than anything else tried.

4. The vitamin from wheat germ does not seem to supplement deficient diets.

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