

INTERNAL AND EXTERNAL FACTORS GOVERNING THE FLUCTUATION  
OF TRIBOLIUM CASTANEUM POPULATIONS

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**ABSTRACT:** Laboratory experiments were designed to determine the external and internal factors responsible for the cyclic fluctuations of *T. castaneum* populations at constant environmental conditions of 29.5°C and 70% RH. Experiments were initiated by introducing 200 fresh eggs into 8 dram vials with 8 grams of standard medium. Thereafter populations were censused once a week. All individuals were classified as egg, larva, pupa or adult, counted, and returned to their original vial. Also the numbers of newly emerged adults during the past week were estimated from these data. The medium was replaced every 1, 2, 4, or 8 weeks during the 35 week tenure of the experiment. In all populations, the numbers of adults were relatively constant after the 10th week. The numbers of eggs, larvae, and pupae fluctuated regularly with a 4 or 5 week cycle, except in 8-week medium renewal populations where major peaks appeared in 8-week cycles with minor peaks at 4-5 week intervals. Cycles within various life stages were most clearly seen when the auto-correlations were calculated for each stage. The time lags of peaks among various life stages were also clearly seen with cross-correlation analyses. Parallel experiments demonstrated that the size of the environment (amount of medium) determines only the absolute populations size, and not the mode of fluctuation. The reduced amplitude of intermediate peaks observed in 8-week medium renewal populations is largely due to reduction of the medium (> 25%) by the 4th week after renewal. From the adult population constancy after the 10th week, we may assume that the number of eggs produced per day is also rather constant. Consequently, the large cyclic fluctuations in egg populations and subsequent fluctuations in larval, pupal, and newly emerged adult populations are most likely a function of internal regulation by high larval cannibalism of eggs.

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**INTRODUCTION:** Due to the long adult life and relatively shorter duration of immature stages, experimental *Tribolium* populations inevitably develop into overlapping generation systems unless special procedures are employed to prevent such development (e.g. [1,2]).

Simple mathematical considerations suggest that populations with overlapping generations will tend towards a stable age distribution [3,4]. However, this is not the case for *Tribolium* populations.

As early as 1928, when Chapman [5] used *Tribolium* for the first time in population studies, he noticed that the stage structure in *Tribolium* populations was not stable even after 170 days. Subsequent studies with *Tribolium* spp. always showed rather large population fluctuations over time in each of the developmental stages of *Tribolium*.

In the present study, we investigated the behavior of populations of *T. castaneum* under specific environmental conditions and attempted to understand the mechanisms causing the fluctuations and cyclic changes in the population sizes of the various developmental stages.

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**EXPERIMENT I: Method** - The experimental populations were initiated by introducing eggs (less than one day old) collected from stock cultures of *T. castaneum* into 8 dram vials containing either 4 or 8 g of standard medium (whole wheat flour enriched with brewer's yeast in a ratio of 100:5). The number of eggs introduced, treatment employed, and number of replicates are summarized in Table I. All populations were established on the same day. The populations were kept at 29.7°C and 71% RH.

The populations were censused once a week; the flour was sifted and individuals were separated into egg, larval, pupal, and adult stages, counted, and returned to the vials. Dead larvae, pupae, and adults, if any, were removed from the vial. When census time did not coincide with the flour renewal time, the old flour was also returned to the vial. At the flour renewal time, flour was replaced with 4 or 8 g of standard medium. The experiment was continued for 36 weeks. The number of newly emerged adults for each week was determined by; number of living adults plus number of dead adults recovered minus number of living adults a week before. Later this calculation was found to be underestimating the number of new adults, as some dead adults were lost due to cannibalism. However, this estimate closely approximates the magnitude of adult emergence over the preceding week, and was used for later analysis.

**Results** - Both replicates for each treatment showed similar trends. Fig. 1 shows the population changes of replicates chosen from treatments 1, 2, 4, and 5. The scales for treatment 1 (1-4-1) are doubled for better comparison with other replicates. Several statements can immediately be made from this figure: First, the fluctuations exhibited by treatments 1 (1-4-1) and 2 (1-8-1) are the same except that the absolute numbers in treatment 1 are about half of those in treatment 2, which shows that the absolute population sizes of any stages are almost completely determined by the amount of flour available (or, perhaps by the space generated by flour, see [6]). Second, the adult populations stay rather constant after the 14th week (i.e. 3rd wave of adult emergence) with

TABLE 1. Summary of experimental conditions in experiment 1.

	Interval of flour renewal (weeks)	Amount of flour renewed (g)	Number of replicates	Number of eggs initially introduced	Code name
(1)	1	4	2	100	1-4-1, 1-4-2
(2)	1	8	2	200	1-8-1, 1-8-2
(3)	2	8	2	200	2-8-1, 2-8-2
(4)	4	8	2	200	4-8-1, 4-8-2
(5)	8	8	2	200	8-8-1, 8-8-2

about 35 individuals per 1 g of flour. Thirdly, in treatments 1, 2, 3, and 4, there is a cycle of 4 weeks in egg populations, which is followed by cycles of 4 week periodicity for larval, pupal, and newly emerged adult populations, but with 1 to 2 weeks time lag each. In treatment 5, the major egg peak appeared every 8 weeks (corresponding to one week after flour renewal) with a minor peak at 4 weeks. The cyclic change of numbers of individuals in various developmental stages and time lags among different developmental stages can be best seen by time series analysis [7]. Fig. 2 shows some results of the analysis. The two upper figures in Fig. 2 are typical correlograms of autocorrelation of various life stages. In both cases, the adult population shows a monotonous decrease in auto correlation value as the distance (time) increases. This is easily understood from the relative constancy of the adult population over time. All the other stages (egg, larva, pupa, and newly emerged adult) show significantly high positive correlations at distances of 4, 8 and 12 weeks, clearly showing that there is a definite 4 week cyclicity within each of these stages. With replicates 8-8-1 where the flour was renewed every 8 weeks, the auto correlation of egg stages shows the highest value at 8 weeks difference, although in other stages 8 week cyclicity was less conspicuous than a 4 week cycle.

To determine if significant cross correlation exists between populations of two life cycle stages, a cross correlation analysis was conducted. For larval and pupal populations (Fig. 2, bottom left), this analysis shows the very high correlation coefficient at distances of 2, 6 and 10 weeks, suggesting that the pupal population size at any time is most highly positively related to the larval population size two weeks before. Similarly, the right hand side figure shows that the larval population size at any moment is most positively correlated with egg population size one or two weeks before.

Similar cross correlation analysis for other combinations of stages showed that pupal fluctuation was preceded by egg fluctuation by 4 weeks, and changes in newly emerged adult population were preceded by egg fluctuation by 4 to 5 weeks.

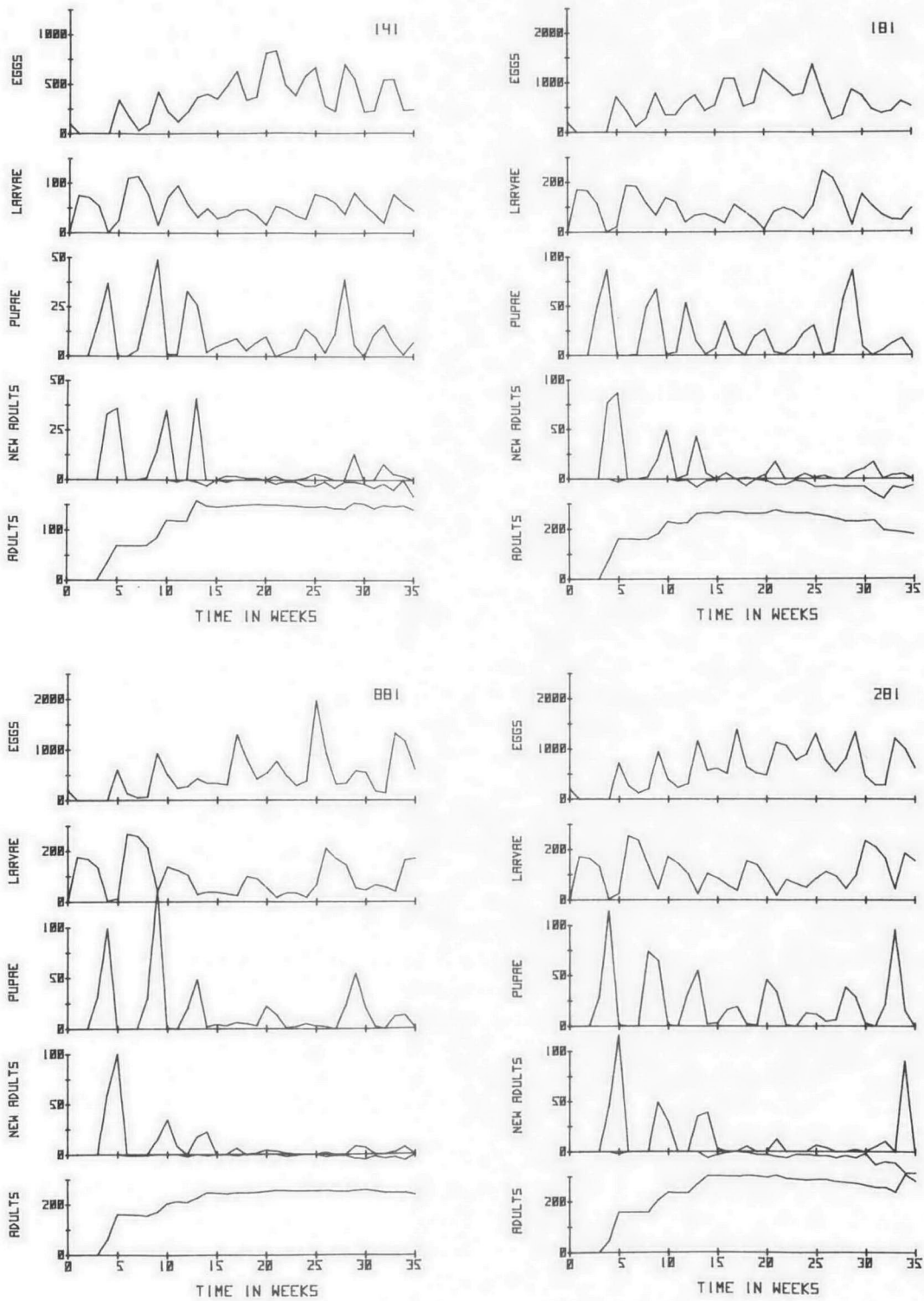


FIGURE 1. Population dynamics of various developmental stages in Experiment 1. For code name, refer to Table 1. The line below the abscissa for new adults shows the number of dead adults recovered at each census time, with the same scaling as new adult but inverted.



**EXPERIMENT II:** In order to see the effect of conditioned flour on the development of immature stages and consequently on the population dynamics, the following experiment was conducted.

Method - At 16th weekly census of Experiment I, when all the replicates received new medium, the old flour removed from the vials was retained. The weight of the recovered flour was similar in replicate vials. Percent reduction in original weight increased with time since flour was changed (Table II). Flour from treatments 2 through 5 only was used for this experiment. Flour from each treatment was well mixed, and divided into four 8 dram vials, each receiving 2 g of flour. One hundred stock culture eggs less than one day old were introduced into each of two of four vials, and 10 eggs each into the other two vials. All 16 vials were set up at one time and maintained at 29.7°C and 71% R.H. After 28 days all the vials were censused. Individuals were classified into larva, pupa, or adult, counted, and individuals in the same stage were weighed in mass. Flour remaining in the vial was also weighed.

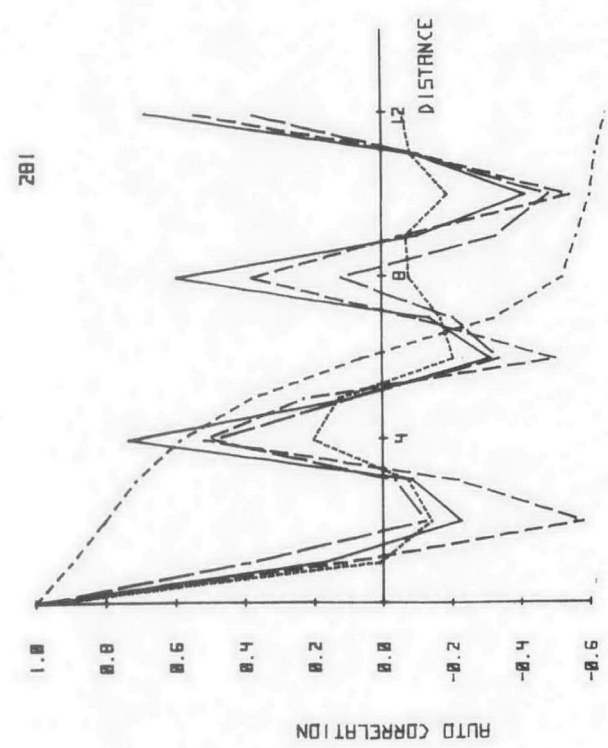
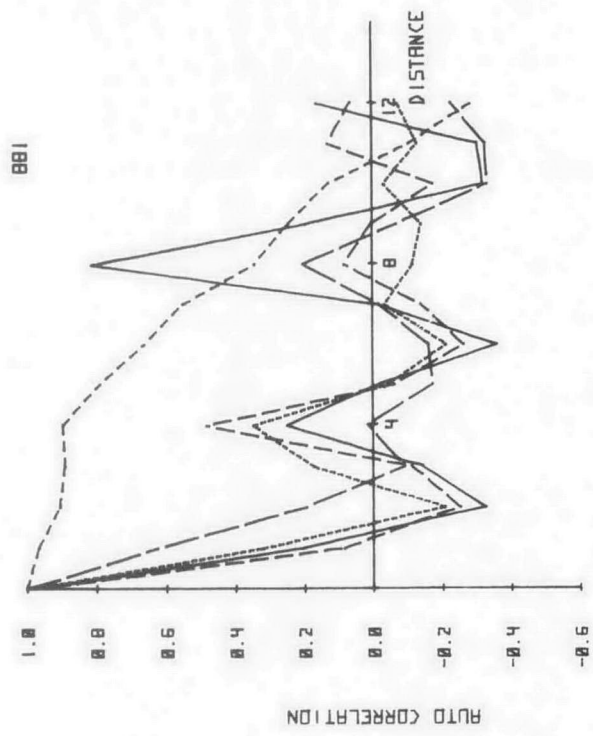
**TABLE II.** Amount of flour recovered at 16th week in experiment I.

Treatment	Flour Supply Time	Amount of flour supplied (g)	Mean amount of flour recovered (g)	% reduction in weight from the original supply
(1)	1 week before	4	3.73	6.8
(2)	1 week before	8	7.23	9.6
(3)	2 weeks before	8	6.63	17.1
(4)	4 weeks before	8	5.95	25.6
(5)	8 weeks before	8	4.90	38.7

Results - Fig. 3 shows the proportions of various stages on the 28th day. The two replicates of each experiment gave similar results. So only mean values are shown in each bar.

The total number of individuals recovered on the 28th day are shown on the top of each bar. Analysis of variance showed that there is no significant effect on the overall survival rate (= total number recovered/initial number of eggs) due to initial egg density, age of flour, or their interaction. Similar non-significant results were obtained for mean individual weights of adults, pupae, or larvae. The mean amount of flour recovered after 28 days was 1.92 g (8 replicates) at low density and 1.52 g at high density. Within high or low density there was no significant effect on the amount of flour recovered due to the age of the flour.

Although the age of the flour showed no significant effect on the proportion of adults or larvae at low density (both at 5% level), age of the flour had significant effects at high density on the proportion of pupae at both densities. Non-significant results at low density stems mostly from the small number of individuals employed (10 eggs/vial). This masked the effects of



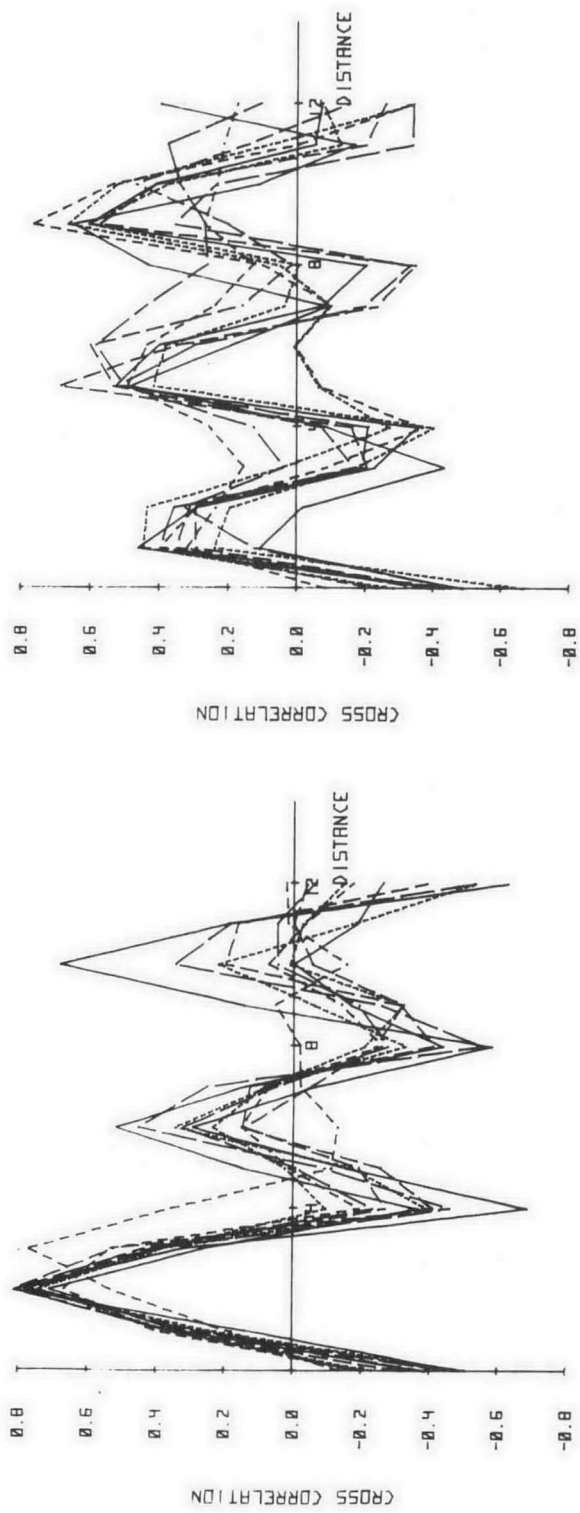


FIGURE 2. Upper panel: Correlograms of auto correlation of two replicates. Abscissa shows the distance by week. Lower panel: Correlograms of cross correlation. Left, larval stages leading pupal stages. Right, egg stages leading larval stages. Each line designates a replicate. Legend: \_\_\_\_\_ eggs; \_\_\_\_\_ larvae; \_\_\_\_\_ pupae; ----- newly emerged adults; - - - - - adults.

treatment since proportions changed significantly when differences in population sizes were as low as one individual. In spite of this non-significant result in analysis of variance at low density, the stage structure at two densities with the same kind of flour shows striking similarities, and although the high density (100 eggs/vial) seem to have caused, in general, a little delay in development, the effect of difference in density of 10-fold seems quite small (see [8]).

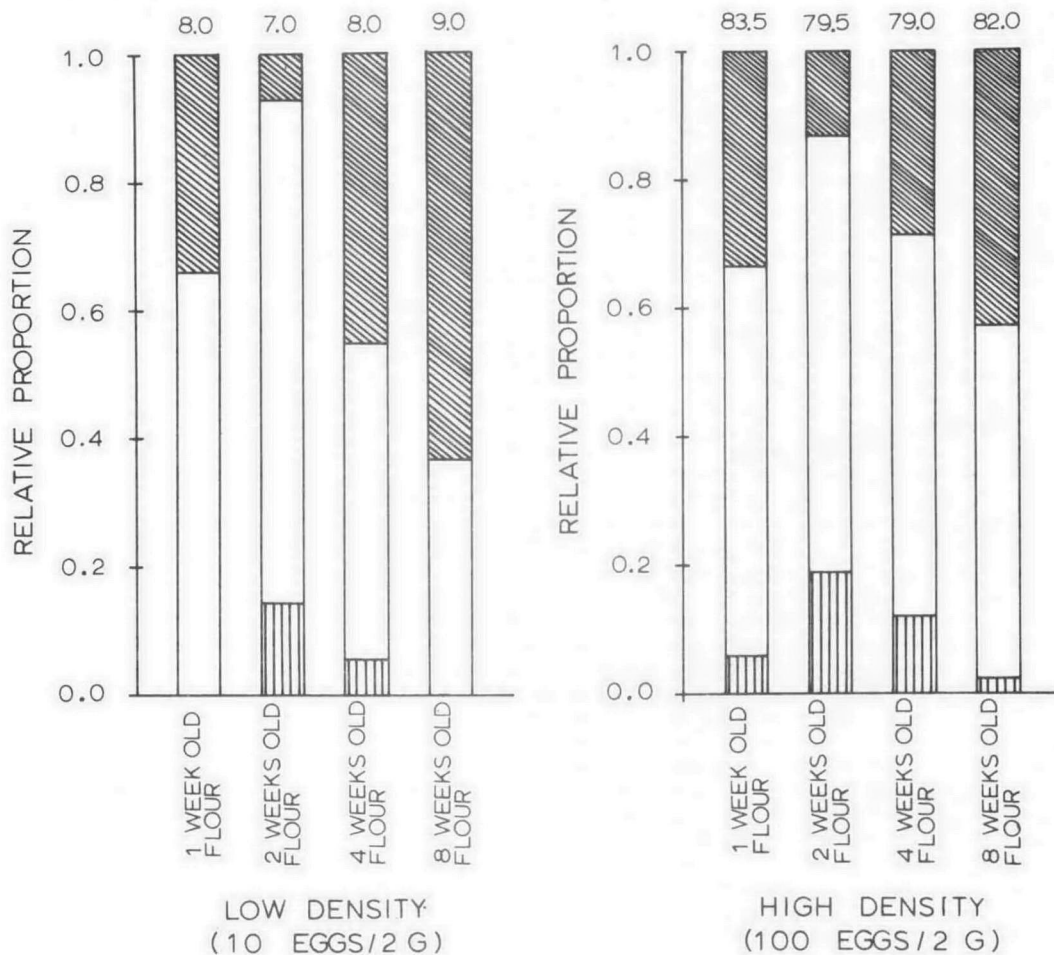


FIGURE 3. Stage composition at 28th day in experiment II. The figures on top of each bar shows the total number of individuals recovered. Key: larvae, pupae, adults.

The differences in stage structure at 28th day among the various kinds of flour were unexpected. Generally, older, more conditioned flour at both low and high densities resulted in faster development of *Tribolium* without affecting mortality in immature stages. The reasons for this are not clear and further experiments to clarify this pattern are planned.

**DISCUSSION:** It has long been known that *Tribolium* populations, under constant environmental conditions with regular flour renewal,



do not attain a stable distribution nor a constant equilibrium population size. However, the direct observations showing not only fluctuations but also the clear cyclicity in the change of population size with time were lacking.

Strawbridge [9] was the first to look into the dynamics of *T. castaneum* population in detail. He censused the population every three days, replacing the flour every 15 days. He discussed, among other subjects, the cyclic fluctuations of *T. castaneum* populations of several strains and under varying conditions. One of his population histories is seen in a published paper [10]. Although his data show large fluctuations in population sizes of various stages over time, the cyclic nature is not so conspicuous as seen in the present study. Many of the stages show rather erratic fluctuations, with possibly ca. 30 day cycle. However, it is significant that whenever he replaced old flour (every 15 days), large increases in egg populations were noted at the next census time. Similar results were obtained in treatments 4 and 5 in the present experiment.

Mertz [11], during the study of experimental populations founded by 26 old adult *T. castaneum*, censused his population every 30 days at the same time when flour was renewed and found the cyclic population fluctuation in various life stages with about 120 days of period. He stated that a 120 days cycle is strictly an artifact of census schedule and there must have been a cycle of ca. 40 days period (roughly corresponding to the sum of egg and larval periods in his strain) which gave a superficial 120 days cycle. However, it should be realized that there is one reason to suspect the existence of real 120 days cycles in the pupal population. His data show that there are increases in adult population corresponding to the superficial pupal peaks. If every 120 days pupal peaks are purely an artifact, we expect that the adult population will increase stepwise every 40 days instead of every 120 days as seen in his figure.

Comparing his results with the present study, his conclusion that there was a major cycle of ca. 40 days period seems quite reasonable. However, I suspect there might have been another longer cycle corresponding to the adult increases in every 120 days. This may be related to the mean adult longevity, while the shorter cycles of 40 days in Mertz's study and 4 weeks in the present experiment correspond to the total length of egg and larval period as suggested by Mertz's model [11]. The suggestion that the period of cycle does not correspond to the sum of developmental periods of all the immature stages, but corresponds to the sum of egg and larval periods only is well confirmed by the time series analysis of present data: The cross correlation between egg and pupal stages showed the highest value with 4 weeks difference, and that of egg and newly emerged adults was 5 weeks. On the other hand, the auto correlation showed a 4 weeks cycle for any stages. This indicates that the egg population increases as soon as the larval population decreases.

The main interval mechanism producing this cycling in

population dynamics of various developmental stages is larval cannibalism on eggs. Although adult cannibalism on eggs is thought to be important for regulation in *Tribolium* populations [12], and obviously is the first constraint determining population size (although this is apparently closely related to the environment size), this is not a main source of cyclicity. Moreover, although conditioned flour is known to have deleterious effects on many aspects of population performance [13], Experiment II shows the minimal effect of conditioned flour on the survival and development of immature stages, at least for the strain employed in the present system.

Beside these internal factors, there exist external factors which modify the cycling in *Tribolium* populations. Among these, the size of the environment (i.e. amount of flour) and flour renewal schedule seem most important. The flour renewal schedule brings about the sudden quantitative increase in the size of environment and also the qualitative improvement of environment especially when the renewal interval is long.

The combination of these interval and external factors can explain *T. castaneum* population dynamics observed in the present study as follows: If the size and quality of the environment stays relatively constant when flour is renewed frequently (as in treatments 1, 2, and 3), the adult population size remains constant with balanced death and newly emerged adults, and egg input by adults also remains stable. The constant input into the egg population is reduced by adult cannibalism on eggs. However, this does not cause any fluctuation in egg population. It is the larval cannibalism on eggs which brings about fluctuations in egg populations. As soon as eggs hatch and larval populations build up, severe reduction in egg populations occur and continue until the majority of larvae advance to the pupal stage. As soon as the cannibalistic pressure by larvae on the eggs is removed, the egg population increases again and the cycle repeats with ca. a 4 week period.

This internal cycling is reinforced with 4 weeks flour renewal, which coincides with the build up of egg populations. Egg production is then enhanced by the quantitative increase in the environmental size and qualitative improvement of the environment.

With flour renewal at 8 weeks intervals, the egg input by adult populations decreases following the last supply of flour partly due to the conditioning of the flour which reduces the fecundity [13], and by sheer reduction in environmental size (i.e. amount of flour, Table II). Consequently when the increase in egg population by internal cycling is not matched with an external stimulus (flour renewal) the peak of egg populations is substantially lower than those seen in other treatments. When the internal cycling coincides with flour renewal, the large build up of egg populations is observed just as in 4 week flour renewal populations, and this large wave carries over to later developmental stages.

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