THE INFLUENCE OF THE HOMEOTIC MUTATION, "ANTENNAPEDIA", ON EGG CANNIBALISM IN *TRIBOLIUM CASTANEUM*

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ABSTRACT. — A comparison of egg cannibalism by adults of differing genotypes (normal wild type versus the homeotic mutation, antennapedia) in the flour beetle, *Tribolium castaneum*, was performed. Egg cannibalism over a 24-hour period was examined for adult density levels of one, two, four, eight, sixteen and thirty-two pairs. A significant reduction in egg cannibalism was observed for beetles homozygous for antennapedia from that of homozygous wild-type at all levels. The effects of antennal ablation were also examined for both genotypes and found not to exhibit a significant influence upon the total results observed.

Much work has been conducted on egg cannibalism in the flour beetles of the genus, *Tribolium*, however, the approach has been primarily from the standpoint of its influence upon population growth and regulation. An extensive review of this ecological approach and detailed references are found in Park *et al.* (1965).

However, the possible effects of different genetic influences upon such an important regulatory mechanism were not examined in previous studies. One approach to this question is an assessment of the influence of a single gene upon egg cannibalism. It is the purpose of this study to report such an influence upon egg cannibalism as affected by the homeotic mutation, antennapedia, *ap* (Englert and Bell, 1963a) in the red rust flour beetle, *Tribolium castaneum*.

MATERIALS AND METHODS

The genetic material tested was derived from a mass mated non-inbred population known as the Purdue "+" Foundation stock and a stock homozygous for the mutation "antennapedia," *ap*.

To insure that intrinsic factors such as age, viability, and fecundity rate were controlled as much as possible, the beetles used were collected as pupae and kept as mated pairs until 21 days after emerging as adults.

At this time they were introduced at their selected density levels to 20 ml glass creamers containing 5 gm standard culturing medium (95% whole wheat flour and 5% dried brewer's yeast). Homogeneously distributed throughout the

medium were 100 eggs which had been marked by using a neutral red dye in a manner similar to that used by Rich (1956). The culture creamers had been acclimated for ten days prior to introduction of adult beetles and marked eggs. The investigation was conducted in a Labline Incubator, Model R-1130, which provided constant environmental conditions for temperature and humidity at 33 ± 3°C and 65 ± 5%, respectively.

Density levels which were chosen for this study were one, two, four, eight, sixteen, and thirty-two adult pairs per 5 gm culturing medium.

At the end of 24 hours, both adults and eggs were removed, and all marked and unmarked eggs recovered were counted to determine the rate of cannibalism occurring. Cannibalism rate was calculated using the basic generalized formula of Rich (1956), later modified by Sonlietner (1961):

$$c = (1/bt) \log_e (M_o/M_t)$$

where c = the cannibalism rate; b = number of beetles; t = time in days; M_o = number of marked eggs introduced; and M_t = number of marked eggs recovered.

Results

The cannibalism rates presented in Table 1 clearly indicate that for all density levels the rate exhibited by the *ap* genotype is significantly reduced from that of the normal wild-type genotype. It is also evident, that as the number of adults increased, the rate of cannibalism decreased for both genotypes. However, the decline in cannibalism rate does not behave in a similar manner over densities for the two genotypes.

Table 1. Cannibalism rates (mean ± standard error)† of antennapedia and normal wild-type adult beetles at varying densities.

Number of Pairs	Genotype		Number of Replicates
	+/+	*ap/ap*	
1.....	6.62 ± 0.62	5.24 ± 0.64	10
2.....	5.27 ± 0.39	3.50 ± 0.44	10
4.....	4.57 ± 0.23	2.15 ± 0.02	5
8.....	3.19 ± 0.02	2.78 ± 0.04	5
16.....	4.55 ± 0.02	2.76 ± 0.16	5
32.....	3.16 ± 0.02	2.20 ± 0.01	5

† c X 10^2

An analysis of variance (Table 2) was performed on the data to determine whether the differences observed were of statistical significance. Since ten replicates were employed at the one and two pair density levels, thus presenting unequal subclass numbers, the analysis was performed using unweighted means and correcting the error term with the harmonic mean (Snedecor and Cochran, 1968). The main effects, genotype and density, were shown to be statistically significant, as suggested by the magnitude of the standard errors presented in Table 1.

Of equal interest is the GxD interaction term, which also reveals statistical significance, verifying our earlier suggestion that the two genotypes reacted in a different manner with regards to egg cannibalism at differing density levels. While the general pattern of decline for the two genotypes differs specifically at the eight and sixteen pair density levels, both do exhibit an interruption in the decline of cannibalism rate at these levels, suggesting possibly that this may be a critical level of adult density versus egg number for population control regardless of genotype.

TABLE 2. Analysis of variance for egg cannibalism.

Source of Variation	d.f.	Mean Square
Genotype (G)	1	43.02**
Density (D)	5	17.70**
G x D	5	7.23**
Error (corrected)	68	0.87

** $P < .01$

In an attempt to learn whether the morphological effects of this homeotic mutation was that of reducing the searching ability of the beetle through a modification of its antenna, a supplementary experiment involving antennal ablation was conducted. Choosing a density level where the differences between the two genotypes were the greatest (four pairs), wild-type beetles were examined for differences in cannibalism rate for intact antennae, removal of left antenna only, and both antennae removed. Antennapedia beetles were compared only for differences between intact antennae and both antennae removed. The

results of this test are given in Table 3.

There appears to be a slight difference between the intact and the non-intact antennae for the wild-type genotype, but not for antennapedia. However, when an analysis of variance was performed, significance was observed only for genotypes and not for treatment or the treatment by genotype interaction (Table 4). Therefore, we can say that antennal ablation had no effect upon cannibalism rate, since the mutant maintained its basic difference under both treatments.

Discussion

While the results presented here were obtained under controlled and optimum conditions: unconditioned medium, 24-hour time restriction, optimum age of adults for high fecundity, and optimum temperature and humidity conditions, the difference noted between the two genotypes for cannibalism rate is considered real.

The entire question of egg cannibalism by adults regardless of genotype is based upon a number of assumptions being met. These in-

Table 3. Cannibalism rates (mean ± standard error)† following ablation of both normal wild-type and mutant antennae at a density of four pairs.

Antennal Ablation	Genotype		Number of Replicates
	+/+	*ap/ap*	
Intact	4.57 ± 0.28	2.15 ± 0.26	5
Both removed	3.50 ± 0.63	2.29 ± 0.50	5
Left antenna only	2.72 ± 1.10	.....	5

† c X 10^2

Table 4. Analysis of variance for egg cannibalism following antennal ablation.

Source of Variation	d.f.	Mean Square
Genotype (G)	1	11.39**
Treatment (T)	2	0.87
G x T	1	6.96
Error	20	1.37

** $P < .01$

clude: (1) no non-random searching for eggs exists, (2) adult beetles exhibit no appetite "satiation", and (3) marked and unmarked eggs are equally likely to be cannibalized.

To minimize any major deviations from the first assumption the time of adult exposure was limited to the 24-hour period, thus providing a common base for comparison between genotypes. Sonleitner (1961) has demonstrated for *T. castaneum* that the latter two assumptions do hold, therefore, no attempts have been made here to minimize these effects, other than keeping the number of eggs constant (100) for each density level.

The increase in adult density without a corresponding increase in egg density did, however, most likely contribute to the decline in cannibalism rate observed for both genotypes. Rich (1956) has demonstrated a positive correlation of egg cannibalism rate with egg density.

By restricting the number of eggs available to the adults, those adults at the lower densities actually have available more eggs per beetle than do the adults of the higher densities. It appears that the behavior of the two genotypes differ in the exact density level at which the decline is stabilized.

It might be argued that the difference in cannibalism observed for the two genotypes may actually then be a difference in fecundity rate, and thus the difference observed is purely a function of total number of eggs available, particularly to the wild-type beetles. However, Englert and Bell (1963b) have shown that the fecundity of the antennapedia mutation is as high as its normal wild-type counterpart, thus this factor cannot explain the differences observed.

An attempt to examine the role of the antenna *per se* in egg cannibalism has revealed no significant difference in the cannibalism rate at a density level of four pairs of adults for either genotype when the antennae are intact or removed, although the genotype difference still persisted. Such evidence is indicative of the fact, that although the antennapedia mutation changes the morphological structure of the antenna, its affect on whatever role the antenna may play in egg cannibalism is not similarly affected.

Thus, the cause for a reduction in egg cannibalism by antennapedia adults lies in an area other than antennal structure *per se*. One such cause which was not examined here is behavior of antennapedia in regards to the preference in movement upon the surface of the medium rather than randomly tunneling throughout the medium, thus reducing the number of eggs each adult will encounter.

Another possible cause, again one of behavior, is that the actual response of "egg encounter" is itself specific and non-random, and the egg cannibalism is the result of the encounter only if a "taste" or "satiation" preference exists. An alteration of such a preference for the antennapedia beetles may be an indirect result of the basic action of the gene itself. Such a behavioral change would be difficult indeed to examine.

Egg cannibalism is considered to be of importance in regulating the upper limits of population growth,

in that it plays a major role in reduction of the rate of larval hatching (Sonleitner, 1961). A reduction in such an important regulating mechanism brought about by a single gene mutation would place a population at a distinct disadvantage for self-regulation.

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