Scar: A Temperature Sensitive Mutation in Tribolium castaneaum Herbst

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In 1959 the author and a group of students at Salem-Washington Township High School undertook the isolation and characterization of mutants of *Tribolium castaneum* Herbst which would be useful as markers in irradiation, genetic, and ecological competition studies. One of the mutants isolated has been named scar *(Sc)*. This paper describes the scar mutant, presents data on its mode of inheritance, and reports the effect of temperature on penetrance of the mutant phenotype. Professor A. E. Bell, Purdue University, who first noted that temperature affected penetrance of the mutation, generously provided supplies and space for the studies reported here.

Materials and Methods

Purdue Foundation A served as the wild type in all crosses and was the strain from which the mutant was isolated.

The scar *(Sc)* mutant was isolated from the third inbred generation of x-irradiated (3,000 r.) wild type imagoes. The mutant is characterized by a depression in the metasturnum just anterior to the coxa (Fig. 1B). The surface of the affected area is rough and is delimited anteriorly by a fold. The mutant phenotype is visible in pupae several hours before eclosion.

Culture medium consisted of 95 parts sifted whole wheat flour and 5 parts brewers yeast. Relative humidity was 65 $\pm$ 5%.

Temperature shift experiments involved collecting heterozygous eggs, at one day intervals, at 27° C and allowing development to continue at this temperature until a specified age at which time they were transferred to 37° C for completion of the life cycle. Similar down-shift experiments were also performed (from 37° to 27°).

Results and Discussion

There being no evidence of sex-linkage, the results from reciprocal matings have been pooled and are presented in Table 1. Each of the percentages reported in Table I is the median from 1 to 5 separate experiments totaling 500 to 2000 beetles, but there were significant variations between certain replicates. One-degree fluctuations in temperature could account for the observed variations. In view of the probability of temperature variations within this range, the data of Table I are qualitative rather than quantitative.

Dominance and expressivity of the scar mutant is complicated by the effect of temperature on its penetrance (Table 1). Among homozygotes, one finds phenotypes ranging from typical scars on both sides (Fig. 1B), through single scars (Fig. 1C), to reduced scars (Fig. 1D) and even wild types. Thus, expressivity is variable and penetrance is incomplete. Since heterozygotes *(Sc/+)* have the same range of phenotypes as the homozygotes *(Sc/Sc)* without a new intermediate phenotype being present, the scar mutant is dominant over its wild type allele. Since penetrance of the mutant phenotype varies from zero at

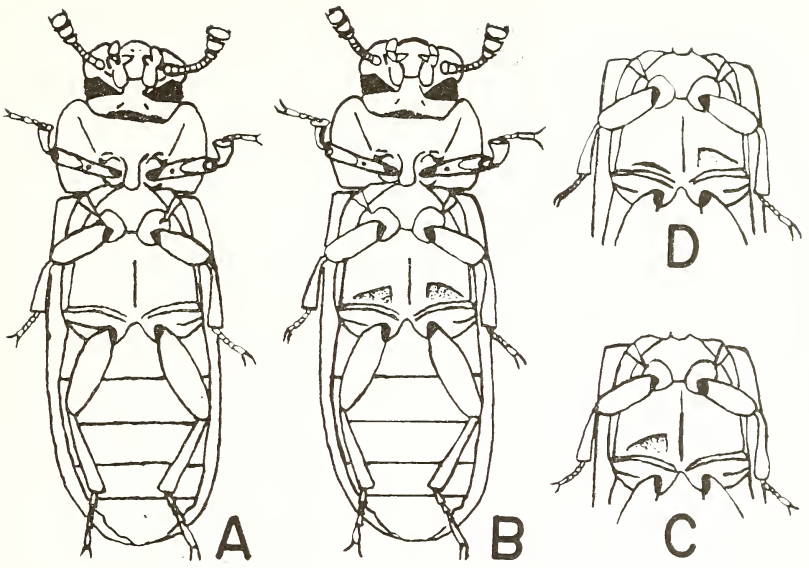

Figure 1. A is the wild type. B is the common scar phenotype. C and D are variations in expressitivity of scar and constitute about one-fifth of the scar phenotypes.

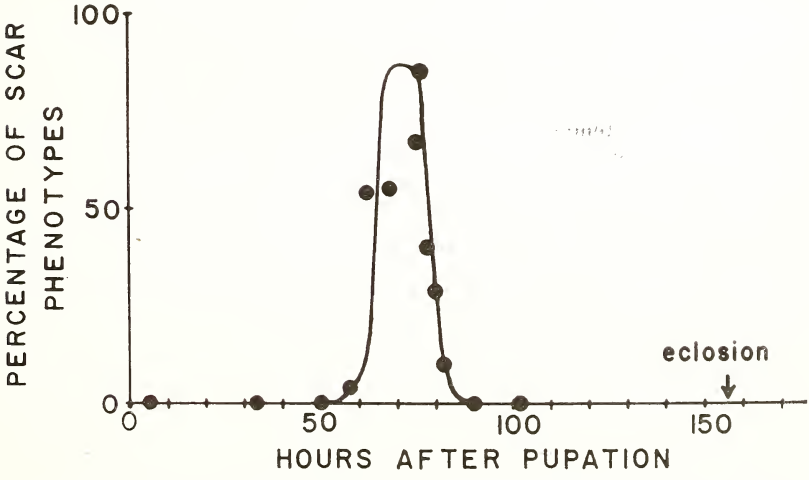

Figure 2. Pupae, collected at 2-hour intervals, were held at 27° except during a 10-hour exposure to 37° C. Penetrance of the scar phenotype is plotted against the median pupal age at the midpoint of the exposure to 37° C.

27° C. to 100% in some experiments at 37° C. (Mating B, Table 1), scar will be a useful marker only at temperatures approaching the lethal limit for the species.

The results of Mating C (Table 1) show that a second outcross to wild type does not appreciably reduce penetrance of the scar mutation

—this indicates that the scar phenotype is controlled by a single gene. On the other hand, the results of single pair inbreeding experiments suggest that other genes affect penetrance of the scar phenotype; however, quantitative data in support of this hypothesis are not presently available.

Temperature shift experiments which tested each day of the life cycle showed that penetrance of the scar phenotype in heterozygotes depended solely upon the temperature during the middle portion of the pupation period. Thus, pupae held at 37° during the middle portion of the pupation period develop scar phenotypes, but pupae of similar age held at 27° developed no scar phenotypes regardless of the temperature during the larval and early pupal or late pupal stadia.

The duration of the temperature sensitive period was determined by means of temperature pulse experiments. Pupae, collected at 27° at 2-hour intervals, where held at 27° until they had reached various post-pupation ages and were then transferred to 37° for ten hours and then returned to 27° until eclosion. The results are presented in Fig. 2. The percentage of scar phenotypes observed in each sample, consisting of 20 to 50 individuals, is plotted against median pupal age at the midpoint of the exposure to 37°. The plot shows that the midpoint of the temperature sensitive period is 71 hours after pupation at 27° and that the temperature sensitive period spans approximately one-fifth of the pupal stadium.

To investigate whether shorter exposures at 37° would result in scar phenotypes, 135 pupae, collected at 27° over a 6-hour period, were divided into two lots. One lot was exposed to 37° for 6 hours

Table 1
Effect of temperature on matings of the scar mutant

Mating	Percentage of scar phenotypes among progeny at various temperatures (°C)			
	37°	35°	33°	27°
A. *Sc*/*Sc* x *Sc*/*Sc*	N.T.	95	73	N.T.
B. *Sc*/*Sc* x +/+	96	69	9	0
C. *Sc*/+ x +/+	N.T.	33	2	0
D. +/+ x +/+	N.T.	1	0	0

N.T. = not tested

at the median post-pupation age of 71 hours and 46% developed the scar phenotype. The other lot was similarly exposed for 3 hours and 8% of these developed the scar phenotype. Thus, longer exposures to 37° resulted in greater penetrance but this observation may involve two factors. Firstly, the effect of temperature during the sensitive period may be accumulative. Secondly, there are variations in the developmental age of individuals within a sample, and the greater penetrance at longer exposures to 37° may be the result of higher percentages of individuals maturing into the temperature sensitive period during the exposure.

It is not likely that the scar mutation was induced by x-rays because scar phenotypes are occasionally observed when the wild type stock is incubated at 35°. However the frequency of the scar gene in the wild type stock is not great enough to invalidate the qualitative data presented in Table 1.

The temperature sensitivity of the scar mutant may be due to temperature sensitivity of the synthesis of a mutant protein or other substance or due to instability of a previously formed protein at high temperature. It would be interesting to determine whether the defect in morphological development of the mutant is coincident with or preceded by the period of temperature sensitivity.

Summary

The scar *(Sc)* mutation of *Tribolium castaneum* Herbst involves a a depression in the metasternum just anterior to the coxa. One or both sides may be affected. Penetrance of the mutation in heterozygotes varies from zero at 27° C to 100% in some matings at 37° C and depends solely upon the temperature during the central one-fifth of the pupal period. The mutant phenotype is visible in pupae several hours before eclosion.