

## Biological differences between strains of *Tribolium castaneum* selected for resistance to hypoxia and hypercarbia, and the unselected strain

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**Abstract.** A strain of the red flour beetle *Tribolium castaneum* (Herbst) was selected separately for resistance to hypoxia and hypercarbia. At the 21st generation, adults and pupae of the line selected for resistance to hypoxia were significantly heavier than those selected for resistance to hypercarbia and the unselected line. It also exhibited a more prolonged developmental range than the unselected line. The line selected for resistance to hypercarbia was also significantly heavier than the unselected line. When the selected and unselected lines were subjected to starvation at the 26th generation, metabolism in both selected lines proceeded at a lower rate than in the unselected line and consequently the selected lines survived for much longer periods.

**Key words.** *Tribolium*, flour beetle, resistance, hypoxia, hypercarbia.

### Introduction

Studies of resistance by insects to fumigants may provide insight into the mechanisms of resistance to modified atmospheres (MAs) since both affect the respiratory system and metabolism. These have shown that resistant strains may exhibit different phenotypic, physiological and biological characteristics from the original strain from which they developed. In studies of selection for resistance of *Sitophilus granarius* (L.) to methyl bromide (Monro, 1964; Uptis *et al.*, 1973) it was found that after forty-four selections the selected strain was heavier, had an extended life cycle and lower respiratory rate. Saxena & Bhatia (1980) developed a strain of *Tribolium castaneum* resistant to phosphine by selecting adults through sixteen generations. They obtained a resistance factor of  $\times 5.9$ . Female fecundity of the resistant strain was 24% lower, but differences in duration of development were not detected. Nakakita & Winks (1981) showed that a phosphine-resistant strain of *T. castaneum* was significantly lighter than the susceptible one. Experiments showed that respiration rates per mg did not differ between the strains, although on the basis of oxygen ( $O_2$ ) consumption per individual, the resistant strain had a significantly lower respiration rate.

Matheson & Parsons (1973), discussing mechanisms of resistance in *Drosophila melanogaster* Meig. to 100%

carbon dioxide ( $CO_2$ ), theorized that the response to anoxia was important. During anoxia, changes in metabolism result in building up an oxygen debt (Wigglesworth, 1972). This occurs by switching to metabolic pathways that are not  $O_2$ -dependent, but which are usually less efficient in energy production, thereby enabling survival for a limited period only. The survival time depends on the energy requirements of the particular organs of the individual concerned and these may be measured in a general way as the overall metabolic rate before anoxia. The higher the metabolic rate, the greater the energy requirement during anoxia and the shorter the length of time the organism would be expected to survive. A positive correlation was shown for *D. melanogaster* between metabolic rates and mortality due to 100%  $CO_2$ , and negative correlations between body weights and both mortalities and metabolic rates.

It is feasible that development of increased tolerance by stored product insects to hypoxia or to hypercarbia through selection, may also be accompanied by biological and physiological changes. These changes may improve their capability to survive environmental stress; they may also affect the intrinsic rate of increase of the resistant populations and thereby influence their intraspecific competition with susceptible strains under normal atmospheric conditions. The following study was made to examine the possible effects on the biology of *T. castaneum* caused by laboratory selection of two strains, one selected for resistance to a low  $O_2$  concentration atmosphere (LOC), and one selected for resistance to an atmosphere of high

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CO<sub>2</sub> concentration (HCC). These were compared with the original unselected laboratory strain (Donahaye, 1990a, b).

## Materials and Methods

### *Duration of developmental cycle*

Durations of developmental cycle of HCC-selected, LOC-selected and the unselected lines were examined at the 21st generation. A standard culture technique was employed for the selection of the resistant lines. This included the use of 1 litre jars containing 3000–5000 adults in approximately 250 g wheat flour for oviposition. Jars from each line were sieved, and eggs removed on two consecutive days, the eggs of the second day oviposition being 0–1 day old. These eggs were separated from free grains of flour and weighed. A batch weighing 210 mg (c. 5000 eggs) was taken from each culture and transferred to a culture jar containing 145 g standard culture medium (Donahaye, 1990a), and all three jars were held together in the same incubator at  $30 \pm 1^\circ\text{C}$  and 60% relative humidity (r.h.) until the first pupae were observed. Pupae were then removed every 3 days and held together in 50 ml jars until adult emergence. Duration of development from egg to adult of each individual was recorded.

### *Examination of oviposition rate*

Differentiation between the sexes of *Tribolium* spp. can be made easily at the pupal stage by examination of the urogomphs (Hinton, 1942). This was used to separate male and female pupae of each line at the 21st generation. The separated pupae were kept apart in glass jars until adult emergence. On emergence, for each line, twenty-five males and twenty-five females were placed together with 50 g wheat flour in 90 ml jars and held in an incubator at  $30 \pm 1^\circ\text{C}$ , and 60% r.h. The flour in each jar was sieved daily with a 60 mesh sieve and the number of eggs was counted. Adults were also examined for mortality. Over the first 11 days no mortality was recorded. Therefore this period was chosen to assess the oviposition rate per female per day for each line.

### *Comparison of adult and pupal weights between selected and unselected lines*

Sets of 100 pupae were taken at random from culture jars of the three lines at the 21st generation and weighed. Ten culture jars from each line formed ten replicates and differences in weight between the lines were analysed by one-way analysis of variance and Duncan's multiple range test. An identical procedure was used for comparing adult weights at the 22nd generation using 10–15-day-old adults taken from culture jars used to hold adults from the time of emergence until they were exposed to the respective MA.

### *Adult survival of the three lines under starvation conditions*

At the 26th generation groups of 100 adults (10–15 days old), from the three lines were exposed to air at 95% r.h. in flasks without food. This was done by pumping air through an exposure apparatus designed to supply humidity controlled gases at a uniform flow rate to sets of 100 ml Erlenmeyer flask in which the insects were exposed (Donahaye, 1990a). Each group was weighed before exposure.

For each line one flask was removed from the exposure apparatus at intervals to provide regular coverage of the exposure period. Immediately after removal from the apparatus, mortality of the exposed insects was recorded, the insects were reweighed, and water content was calculated after drying the insects in an oven at  $104^\circ\text{C}$  for 2 h (Donahaye, 1992). Mortality over the experimental period was calculated by probit-mortality log-time analysis. Weight loss was calculated and changes in water content and dry-weight loss were extrapolated from results of water content and dry weight of insect samples measured before exposure to starvation in air.

## Results and Discussion

### *Duration of developmental cycle*

The duration of development from egg to adult for the three lines is shown in Fig. 1. Total emergence for the HCC-selected, LOC-selected and unselected lines was 2547, 2682 and 2523 adults, respectively. The figure shows that for all three lines, peak emergence occurred 22 days after oviposition. For the HCC and unselected lines emergence had dropped to below 0.5% on the 29th day, whereas emergence of the LOC-selected line showed a pronounced trailing-off effect which continued until the 34th day.

Although emergence of all lines peaked on the same day, there is a clear indication of a greater range of development of the LOC-selected line.

### *Oviposition rate*

Average oviposition (eggs/female/day) over the first 11 days of adult life in the three lines was not significantly different ( $\chi^2$ -square analysis).

### *Adult and pupal weights*

Pupal weights (21st generation) and adult weights (22nd generation) are given in Table 1.

Of the three indices examined for the three lines, the heavier weights of the selected lines were the only ones giving a significant difference. It is interesting that Bray *et al.* (1962), selecting for weight of *T. castaneum* pupae, indicated the presence of large numbers (perhaps hundreds) of genes controlling body size of this insect.

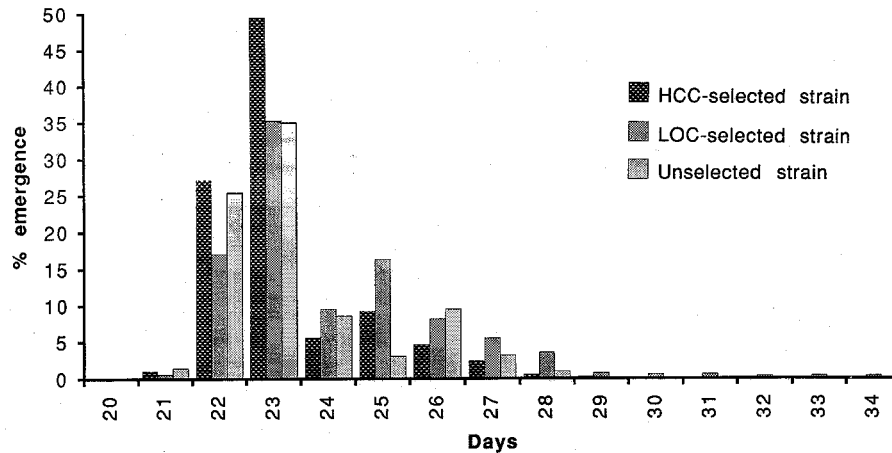


Fig. 1. Development from egg to adult of three lines of *Tribolium castaneum* at 26°C and 60% r.h. after twenty-one generations of selection.

Table 1. Comparison of pupal and adult weights of the lines of *Tribolium castaneum* at the 21st and 22nd generation.

	Mean wt/pupa* (mg)	Mean wt/adult* (mg)
Unselected line	2.163 (A)	1.848 (a)
HCC-selected line	2.211 (B)	2.022 (b)
LOC-selected line	2.263 (C)	2.085 (c)

\* Values in each column followed by different letters are significantly different at  $P < 0.05$ .

#### Adult survival of the three lines under starvation conditions

Times required to produce mortality and changes in water content and dry matter under starvation for the three lines are given in Table 2 and Fig. 2.

Table 2 gives results of probit analysis of the mortality data. From Fig. 2, it can be seen that the loss in dry weight of the unselected line was very rapid initially (about 10% of the initial dry weight per day during the first 3 days), followed by decreasing loss in dry weight as mortality increased. The 50% mortality level ( $LT_{50}$ ), occurred on the 9th day, when dry weight loss had reached approximately 40%. In contrast, loss in dry weight of the two selected lines was initially low and uniform, as indicated by the straight section of the weight-loss curve, and consisted of c. 1.45% and 1.35% loss per day over the first

Table 2. Mortality rates for exposure to starvation in air for adults of the three lines of *Tribolium castaneum* at the 26th generation.

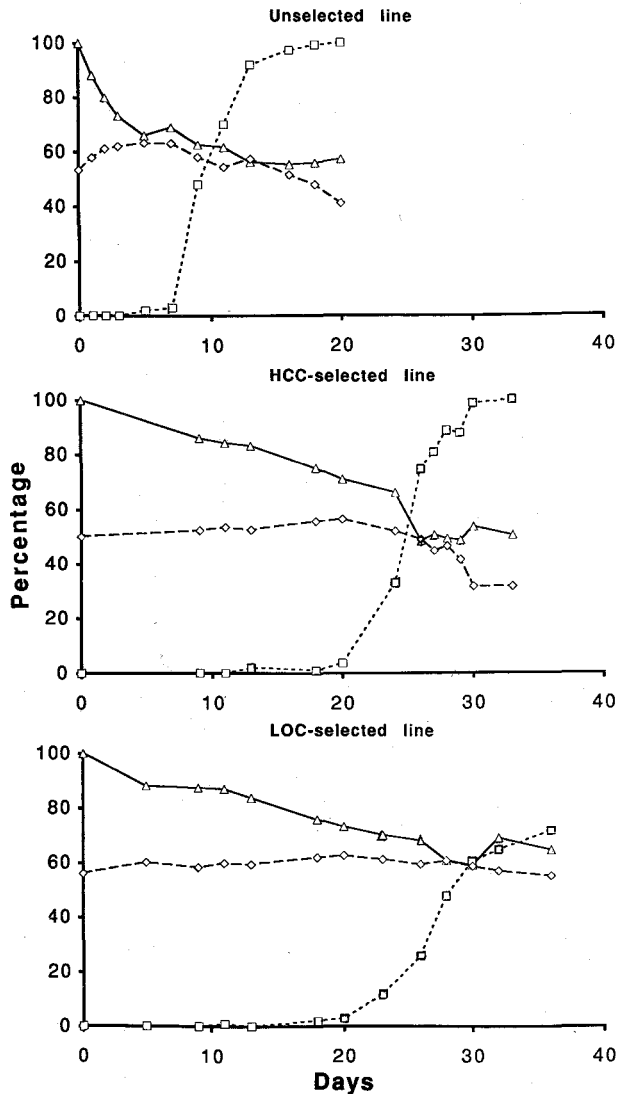
Line	$LT_{50}$ (days)*	$LT_{99}$ (days)*
Unselected	8.8 (7.3–10.3)	24.5 (18.2–49.3)
HCC-selected	24.6 (22.6–26)	32.5 (30.2–37.9)
LOC-selected	30.2 (28.4–32.3)	47.2 (42.37–56.3)

\* Numbers in parentheses give the 95% confidence range.

20 days' exposure of the HCC- and LOC-selected lines, respectively. At the  $LT_{50}$  stage after 24 days for the HCC-selected line and 30 days for the LOC-selected line, dry-weight losses were similar to the unselected line at c. 43% and 38%, respectively. At the  $LT_{99}$  stage, survival of the HCC-selected and LOC-selected lines were 1.71 and 2.48 times longer than that of the unselected line. However, the more heterogenic response of the unselected line gave rise to wider confidence bands at the higher mortalities and at  $LT_{99}$  mortality, differences between the three lines were not significant. It should be noted that losses in dry weight under starvation conditions were greater than those under exposure to MAs (Donahaye, 1992). This indicates the possibility of metabolism of energy reserves that were not available during exposure to MAs. Another possibility is that although observed activity was extremely limited within the flasks, some surviving adults did appear to be eating other dead insects.

In all three lines there was an initial increase in water content, due, apparently, to metabolic water produced from energy reserves. Towards the end of exposure, losses in water content were recorded as the water content of the dead insects came into equilibrium with the 95% r.h. of the atmosphere (Donahaye, 1992).

The above results show that survival of both the MA selected lines was far greater than those of the unselected line. The increased survival falls outside the range ( $LT_{50}$  of 10–13.4 days) shown by sixteen strains of *T. castaneum* adults examined by Sverdlov & Wool (1975) that were reared and exposed under similar conditions. This was in spite of the fact that average strain weights ranged from 1.95 to 2.09 mg/adult. From this it may be deduced that factors other than the correlation between survival and energy reserves as expressed by body weight contributed to prolonging the survival of the lines resistant to MAs. This is corroborated by the finding (Donahaye, 1992) that in air the metabolic rates of both resistant lines, as expressed by oxygen consumption and carbon dioxide production, were significantly lower than that of the unselected line.



**Fig. 2.** Mortality and weight loss of adults of the unselected, HCC-selected and LOC-selected lines of *Tribolium castaneum* during starvation in air at the 26th generation ( $\Delta$ , % dry wt;  $\square$ , % mortality;  $\diamond$ , % water content).

In conclusion, the advantage of the MA-selected lines in surviving conditions of starvation is evident. Although field resistance to MAs has not yet been recorded, the potential of stored-product insects to develop resistance to MAs has been shown (Bond & Buckland, 1979; Donahaye, 1990a, b, 1991) and this may be accompanied by a survival advantage in storage situations where grain is periodically removed, or storage structures are left empty for prolonged periods.

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