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## Density-dependent reproductive success in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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## ABSTRACT

Two strains of *Tribolium castaneum*, one being susceptible to malathion (Asm) and the other malathion-specific resistant (PRm), were used to assess the effect of population density and its interaction with genetic background in reproductive success. A highly significant allometric relationship between female body weight and fecundity ( $R^2 = 0.413$ ) and another between female body weight and larval survivorship ( $R^2 = 0.561$ ) were found. Data showed that population density exerted an indirect effect on the reproductive success. The body weight of the Asm female was not affected by variations in population density but the body weight of the PRm female increased with reduced population density.

The genetic background (strain) and/or malathion-specific resistance greatly influenced reproductive success. Concerning the percentage survivorship of offspring of the two strains, there was an opposite trend with increased population density: in PRm, survivorship was lower at high density ( $76.64 \pm 13.75$ ; mean  $\pm$  SD) and higher at low density ( $88.39 \pm 7.61$ ), whereas in Asm, survivorship was higher at high density ( $53.39 \pm 15.57$ ) and lower at low density ( $43.99 \pm 20.08$ ). The PRm female laid more eggs than the Asm. Reproductive success was significantly higher in PRm than in Asm. In addition, the genetic background (the strain) and/or the pleiotropic effect of malathion-specific resistance had a significant effect on the fecundity of PRm because the differences in fecundity and reproductive success between Asm and PRm went beyond the body weight of the female and might be explained by their genetic background and/or the pleiotropic effect of malathion-specific resistance.

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### 1. Introduction

Most of the mutations conferring resistance to pesticides entail pleiotropic cost, which decreases the fitness of the resistant insects in the absence of any application of pesticide (Abedi and Brown, 1960; McKenzie et al., 1982; Carrière et al., 1994; Groeters et al., 1994). Such a cost originates from the disruptive effects on the normal metabolic balance by the overproduction of enzymes of detoxification which diverts energy normally reserved for reproduction (Uyenoyama, 1986; Lenski, 1988; Roush and Daly, 1990; Carrière et al., 1994). However, in the case of *Tribolium castaneum* (Herbst), it has been shown that a resistant strain had higher fitness than the susceptible strain (Arnaud et al., 1999, 2002). Although many studies have been devoted to *T. castaneum* (Sokoloff, 1977; White, 1987), none has considered the joint influence of population density and genetic background on reproductive success.

In this paper, the main intent was to determine whether the reproductive success (fecundity and larval survivorship) of the females of two strains of *T. castaneum* differing both in their specific resistance to malathion and their geographical origins were affected by a difference in rearing population density. For that purpose, the relation between the female body weight and the reproductive success was studied first. The influence of the joint effect of population density and specific resistance to malathion on their off-spring production was then examined. The use of two strains was intended to improve understanding of the pleiotropic effect of malathion-specific resistance and the importance of the genetic background in strain survival potential.

### 2. Materials and methods

#### 2.1. Insect strains

Two strains of *T. castaneum* were used: one specifically resistant to malathion, and the other susceptible to malathion (Haubruge, 1995). The resistant strain, PRm, was obtained from the Natural Resources Institute, Chatham, UK, and the susceptible

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one, Asm, was obtained from the Institut National de Recherche Agronomiques, Bordeaux, France. The PRm strain possessed a malathion-specific-resistance mechanism based on enhanced carboxylesterase activity (Haubruge, 1995; Haubruge et al., 2002). Both insect strains were bred (insecticide free) on whole-meal wheat flour with added brewer's yeast at a ratio of 10:1 (wt/wt) (Sokoloff et al., 1966; Imura, 1991), in continuous darkness at  $30 \pm 1$  °C and  $70 \pm 5\%$  relative humidity in plastic boxes (20 cm  $\times$  20.5 cm  $\times$  8 cm).

## 2.2. Selection of lines

Subsets of each laboratory strain were selected separately for low- and high-density rearing for eight generations according to the following procedure. Thirty couples of each strain were bred, respectively, on 100 and 800 g of food and yielded, respectively, 12 and 4 individuals/g as output density. To avoid confusion, the insect strains will be referred to throughout the paper as malathion-(or insecticide-) resistant or susceptible, and the population density as high-density-resistant line (HDR) and low-density-resistant line (LDR) or high-density-susceptible line (HDS) and low-density-susceptible line (LDS).

## 2.3. Assessment of the density effect on fecundity

Prior to the experiment, each female and each male was weighed in an attempt to assess the relationship between the weight of the female and her reproductive parameters (fecundity and reproductive success).

From each of the four lines, two 10-day-old virgins, one female and one male (marked with an elytral dot with an organic solvent-free pen) were separated by sex as pupae at random and placed together in a glass tube containing 0.5 g of food. For each line, at least 15 replicate pairs were prepared. After 4 days, the male was removed and discarded. The female was moved to a Petri dish (55 mm  $\varnothing$ ) containing 5 g of food medium (0.2 mm particle size). To eliminate the effect of egg cannibalism, the eggs were collected and counted every other day by sieving (250 mesh/in). Then the eggs were returned to the flour for hatching. After 40 days, the emerged adults were recorded.

## 2.4. Allometry relations between reproductive success and female body weight

The relationship which links the female body weight and their reproductive success was studied with a fitted least-squares linear regression model. While fitting the linear regression model, an aberrant result (out of range) was detected, which was discarded. Thereafter, to preserve a balanced model, this point was re-estimated by using the curve of the linear model of regression. The degrees of freedom of the later tests were consequently corrected (reduction in the degrees of freedom of the residual by 1). The averages of the various combinations of factors were established on the basis of corrected data.

## 2.5. Strain-by-density effect on female fecundity and female fertility

The joint effects of the strain and high or low population density, and their interaction, on the reproductive success of the females were tested disregarding the body weight of the female by a two-way analysis of variance (AV2). The model includes the factor "strain" with two alternatives crossed with the density of population with two levels, and their interaction effect. When the interaction (density  $\times$  strain) was significant, the split of the AV2 model into two AV1 models according to the "strain" enabled

assessment of whether the population density influenced the two lines of the same strain. The interaction was then plotted.

## 2.6. Strain-by-density effect on female body weight in Asm and PRm strains of *T. castaneum*

To assess whether the population density and the strain, and their interaction, had a significant effect on the weight of the females, data were analysed with two-way ANOVA (AV2). When the interaction (density  $\times$  strain) was significant the effect of the interaction was represented by graphs and the preliminary AV2 model was divided into two AV1 models, according to the strain, to assess how the body weight of each strain was influenced by the population density.

## 2.7. Joint effect of strain, density and body weight of Asm females and PRm females on their reproductive success

Two models of ANOVA were compared to assess whether the body weight of the female alone was sufficient to explain the difference in reproductive success. The two models were general linear models (GLM) as follows:

- *Model 1*: a linear regression fitted for the reproductive success of the females according to the body weight of the females;
- *Model 2*: an analysis of the covariance (ANCOVA) fitted for the reproductive success according to the body weight of the female, the strain and the density including the density-by-strain interaction.

Comparison of these models addressed the following two questions:

1. What was the level of fecundity or the reproductive success of the females due to the joint action of the population density and the strain?
2. How important was the effect of population density on the reproductive success, and considering population density and the strain, which had the major effect?

All statistical analysis was carried out with R Statistical programming tools (Ihaka and Gentleman, 1996), release 1.5.1 for Windows NT4 SP6, and MINITAB (Minitab, 2000) release 13.20. The graphic analysis and the corrections of the degrees of freedom were carried out with Microsoft Excel, release 2000.

## 3. Results

### 3.1. Allometric relation between female body weight, fecundity and reproductive success

The results obtained for the body weight and reproductive parameters of the females of *T. castaneum* are shown in Table 1.

The least-squares linear model of regression showed that the body weight of the female and fecundity were bound by a highly significant linear relation ( $R^2 = 0.413$ ;  $F_{(1,57)} = 40.27$ ;  $P < 0.001$ ) (Fig. 1). The equation of this relation is  $F = -71.29 + 97.16W_f$  where  $F$  represents the number of eggs laid and  $W_f$  the body weight of the female (in mg).

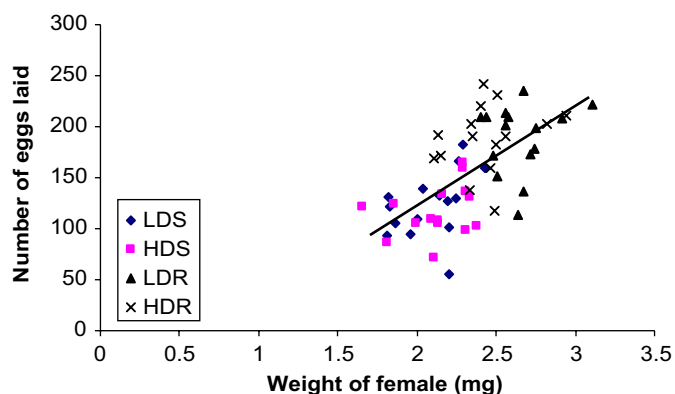
A similar relationship also exists between the body weight and the reproductive success of the female ( $R^2 = 0.561$ ;  $F_{(1,57)} = 72.76$ ;  $P < 0.001$ ); the equation of this relation is  $SR = 205.29 + 133.66W_f$  where  $SR$  is the number of emerged adult progeny and  $W_f$  the body weight (mg) of the female.

**Table 1**

Body weight and reproductive parameters (fecundity, reproductive success and fitness) of both susceptible (Asm) and malathion-specific-resistant (PRm) females of *Tribolium castaneum* at high and low population density

Density (means ±SD)	♀ body weight (means ±SD)	Fecundity (means ±SD)	Fertility (means ±SD)	Survival (% ±SD)
HDR	2.43 ± 0.23	188.07 ± 33.61	143.00 ± 31.71	76.64 ± 13.75
LDR	2.65 ± 0.18	188.93 ± 33.87	158.27 ± 35.91	88.39 ± 7.61
HDS	2.12 ± 0.21	117.33 ± 25.36	63.13 ± 23.84	53.39 ± 15.57
LDS	2.09 ± 0.19	123.40 ± 32.26	56.60 ± 27.54	43.99 ± 20.08

HDR and HDS = high-density lines resistant and susceptible, respectively; LDR and LDS = low-density lines resistant and susceptible, respectively. Data are means ± standard deviation of 15 replicates.



**Fig. 1.** Allometric relationship between the female body weight and the number of egg laid per female per 10 days in *Tribolium castaneum*.

Thus, the fecundity and the fertility of females of both strains were strongly dependent on the body weight. This linear relation showed that on average, the higher the female body weight, the more eggs the female laid.

### 3.2. Joint effect of *T. castaneum* strain and density on female body weight

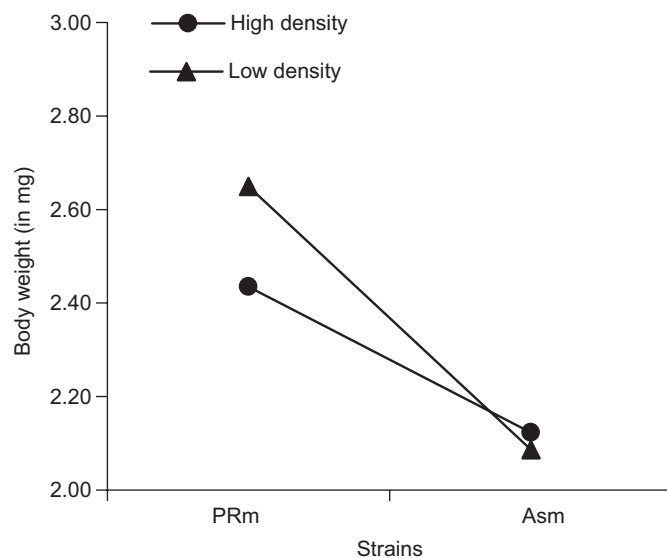
The joint influence of the population density and the strain on the body weight of the females was estimated by an AV2 model, which highlighted a significant “density-by-strain” interaction ( $F_{(1,55)} = 5.48$ ;  $P < 0.01$ ). Thus, the population density alone only partly explained the difference in body mass between females, which was influenced by the combined effect of density and strain. The effect of population density was more important for the PRm strain than for the Asm strain which had a lower mean body weight (Fig. 2).

The effect of population density on the body mass of the females was also assessed by two combined AV1, one for each strain. From these two AV1, it appeared that the body weight of the malathion-susceptible females was not influenced by the population density ( $AV1$ ,  $F_{(1,55)} = 0.23$ ;  $P = 0.63$ ) while the body weight of the heavier malathion-specific-resistant females was significantly reduced by increased population density ( $AV1$ ,  $F_{(1,55)} = 7.99$ ;  $P < 0.01$ ) (Fig. 2).

The density thus exerted an indirect influence on the fecundity of the malathion-specific-resistant strain, since fecundity was related to the female body weight.

### 3.3. Joint effect of population density and *T. castaneum* strain on female fecundity

The joint influence of the strain, and of the density, and their interaction on fecundity was tested using AV2 and revealed that only the strain had a highly significant effect on fecundity of the



**Fig. 2.** Density and strain interaction effect on the body weight of both susceptible (Asm) and malathion-specific-resistant (PRm) females of *Tribolium castaneum*.

female ( $F_{(1,55)} = 69.07$ ;  $P < 0.001$ ). The fecundity of the females of *T. castaneum* was not directly dependent on the population density ( $AV2$ ,  $F_{(1,55)} = 0.1$ ;  $P = 0.75$ ; Fig. 3), nor did the combined action of population density and strain have any effect on the fecundity.

### 3.4. Joint effect of population density and *T. castaneum* strain on reproductive success of females

From the previous AV2 analysis, it appeared that the reproductive success of the females was significantly influenced by the strain ( $AV2$ ,  $F_{(1,55)} = 64.77$ ;  $P < 0.001$ ), but not by the population density ( $AV2$ ,  $F_{(1,55)} = 0.12$ ;  $P = 0.73$ ). The interaction of density and the strain on the reproductive success of the females was also significant ( $AV2$ ,  $F_{(1,55)} = 4.30$ ;  $P = 0.043$ ) (Fig. 4).

Fig. 4 shows the opposite effect of population density on the reproductive success of Asm and PRm females. Indeed, the reproductive success of the PRm females was higher at low density than at high density, but splitting of the AV2 analysis into two AV1 models did not reveal any significant effect of the density on the reproductive success of the females for either the malathion-susceptible strain ( $AV1$ ,  $F_{(1,55)} = 2.91$ ;  $P = 0.09$ ) or the malathion-specific-resistant strain ( $AV1$ ,  $F_{(1,55)} = 1.50$ ;  $P = 0.22$ ).

### 3.5. Triple interaction “strain–density–female body weight” on reproductive success

The analysis of covariance showed a highly significant effect of the strain on fecundity ( $AV2$ ,  $F_{(1,54)} = 21.51$ ;  $P < 0.001$ ). Thus, the strain had a direct effect, which could partly explain the difference

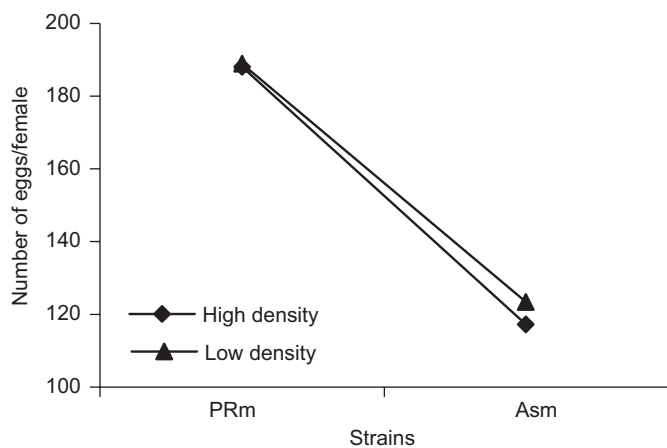


Fig. 3. Strain–density interaction effect on fecundity of both susceptible (Asm) and malathion-specific-resistant (PRm) females of *Tribolium castaneum*.

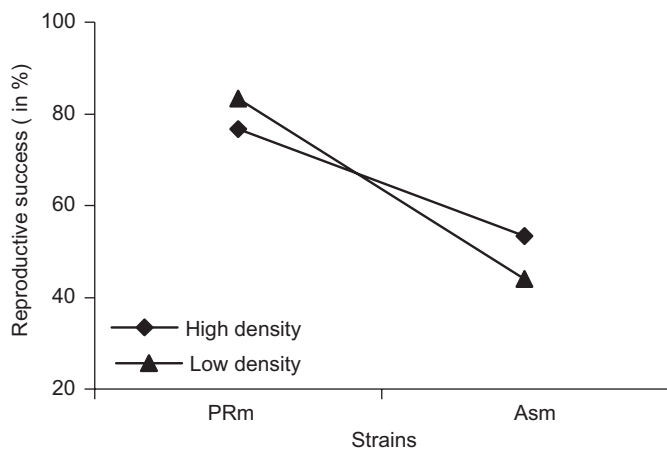


Fig. 4. Strain–density interaction effect on the reproductive success (percentage survivorship of eggs to emerging adults) of both susceptible (Asm) and malathion-specific-resistant (PRm) females of *Tribolium castaneum*.

in fecundity of the females, even after correcting for female weight.

The analysis of covariance showed that the strain had a highly significant effect on female reproductive success too ( $AV2$ ,  $F_{(1,54)} = 16.22$ ;  $P < 0.001$ ). Thus, the effect of the strain on the reproductive success of the females went beyond a simple indirect effect related to female body weight.

#### 4. Discussion

The results were in agreement with the principle of resource allocation: the large females were more fertile and produced offspring of greater size (Longstaff, 1981; Gilbert, 1986; Arnaud et al., 2002). Our results are in agreement with those predicted by the theories related to the reproductive strategies of iteroparous stored products insects. According to this theory, the allometric relationship between insect body weight and number of eggs laid is 0.70 for iteroparous and 0.40 for semelparous insects (Holloway et al., 1987).

The results herein examined the relationship linking the body weight of the females and their reproductive success, using linear regressions. The correlation coefficients expressing the relationship between the body weight of the female and the reproductive success were, respectively, 0.643 for the relationship linking the

weight of the female with its fecundity and 0.749 for the relationship linking the weight of the female with its reproductive success. Holloway et al. (1987) showed, by way of a regression, that the number of reproductive units produced per day increased significantly with the body weight in iteroparous insects ( $R^2 = 0.30$ ), but not in semelparous insects ( $R^2 < 0.01$ ).

Our earlier results showed the superiority of the reproductive success of the malathion-specific-resistant strain, even in the absence of the malathion, compared with the malathion-susceptible strain (Arnaud et al., 1999, 2002). Life history theories support the view that body weight plays a central role in the optimization of fitness, even if the relationship between the body weight of the female and the characters which optimize fitness is not always convincing (Corkum et al., 1997; Klingenberg and Spence, 1997). In the present experiments, there was a significant allometric relationship between the female weight and reproductive success, over a 10-day period of observation. The strain had an important effect on the body weight of the females, those of the malathion-specific-resistant strain being on average 0.81 mg heavier than the susceptible females (which represented 20% of the total average body weight).

The period of egg laying of a female *Tribolium* is spread out over several months. However, according to Wade (1978), the production of eggs during the first week of egg laying was of major significance in the study of the factors which could influence the rate of growth of populations of *T. castaneum*. In the same way, Arnaud et al. (2002) showed that the estimate of the fecundity of various strains of *T. castaneum* over a relatively short period led to reliable conclusions.

The population density had a significant effect only on the PRm females. Indeed, for this malathion-specific-resistant strain, the females from low density were 0.22 mg heavier than the females from high density. Other factors related to the strain (i.e. genetic background) also showed a highly significant effect, even after pooling the direct influence of female body weight on fecundity.

Thus, the females of the malathion-specific-resistant strain were more fertile than expected, considering the body weight. Conversely, the susceptible strain was less fertile than its body weight would have predicted. Females of the resistant strain had a reproductive success, which was 31% higher on average than the reproductive success of the females of the susceptible strain. From the information generated by this study, it would be imprudent to claim that the differences in fecundity and reproductive success are only due to the pleiotropic effect of malathion-specific resistance, since the two strains used have different geographical origins and consequently are likely to differ in their "genetic background".

In conclusion, this paper indicates the need to incorporate the study of life history traits in genetical and molecular biological studies for the development of strategies for the management of insecticide resistance. Indeed, the integration of life history data into theoretical models predicting the spread of insecticide resistance through pest populations should strengthen their accuracy and widen their applicability.

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