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The Impact of Sperm Precedence in Malathion Resistance Transmission in Populations of the Red Flour Beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract—Malathion resistance in the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is actually a worldwide problem, and studies on resistance transmission are needed to improve insecticide resistance management. Females of *Tribolium castaneum* commonly mate with several males, and the last batch of male sperm preferentially fertilizes subsequent eggs. This phenomenon, a particular form of sexual selection, helps to increase resistance transmission in populations of stored product insects. We confirmed the last male sperm precedence and, in the absence of further matings, examined the evolution of mixed susceptible and malathion-resistant progeny during a 3-month period. The proportion of resistant phenotypes in female progeny was 99.6 and 3%, respectively, after the first mating with a resistant male and the second mating with a susceptible one. When females thus mated twice were isolated from males, the proportion of the resistant phenotype increased to 34.1% after 30 days. From 72 days onwards, this proportion ranged from 14.2 to 29.7%. © 1997, Published by Elsevier Science Ltd

Key words—malathion, insecticide resistance, *Tribolium castaneum*, sperm precedence, sperm competition

INTRODUCTION

Recently there has been considerable interest in the study of insecticide resistance as an evolutionary phenomenon. Malathion resistance in the red flour beetle, *Tribolium castaneum*, has become a worldwide problem (Champ and Dyte, 1976). Of particular interest with respect to insecticide resistance management are differences in the biological parameters which affect the net reproductive rate, but other characteristics like behavioural differences also can be important (Ferrari and Georghiou, 1981; Dyte, 1990). Rowlands (1988) has provided evidence, using strains of two *Anopheles* species, that resistant males were less competitive in mating.

Sperm competition has been demonstrated across a wide variety of animal taxa (Parker, 1970). In many insects, females remate before sperm from previous matings are exhausted. When this occurs, sperm from the latest male preferentially fertilize subsequent eggs (Gwynne, 1984). Many male adaptations which help to increase paternity result from sperm competition, a form of sexual selection that acts during, as well as after copulation.

The precedence of second male sperm in the progeny led to hypotheses that: (a) first male sperm is displaced and removed when females remate; (b) sperm is stratified in the female storage organ

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(Birkhead and Hunter, 1990). Schlager (1960) caged female *Tribolium castaneum* with alternating types of males and found that the proportion of offspring arising from the last male present gradually fell when females were maintained on their own, indicating that sperm from previous matings was being used to an increasing extent as the last male's sperm became depleted.

The purpose of this study was to determine whether sperm transferred from a susceptible male in later copulations takes precedence over sperm from a resistant male already in the spermatheca of female *Tribolium castaneum*. The impact of male absence on sperm utilization was also studied.

MATERIALS AND METHODS

Sperm precedence was studied using an autosomal, dominant and monofactorial genetic marker, malathion-specific resistance, in *Tribolium castaneum*. Two strains of the flour beetle were used in this study. One strain specifically resistant to malathion called 'PRm' was obtained from the Natural Resources Institute in Chatham, England. It was originally collected from storage systems in The Philippines and reared under laboratory conditions at this Institute for many generations (Champ and Dyte, 1976). The second strain 'Asm', susceptible to malathion, was collected from storage facilities at Abidjan in the Ivory Coast (Haubruge *et al.*, 1992). The insects were reared with whole wheat flour enriched with brewer's yeast (12:1) and kept in the darkness at 27 ± 1°C and 65 ± 5% relative humidity (r.h.).

Firstly 15 virgin malathion-resistant males were placed separately with 15 virgin susceptible females in a 2.5 cm diameter glass tube containing 1 g of wheat flour. After 6 days, the resistant males were removed and replaced by susceptible males in Petri dishes containing wheat flour for

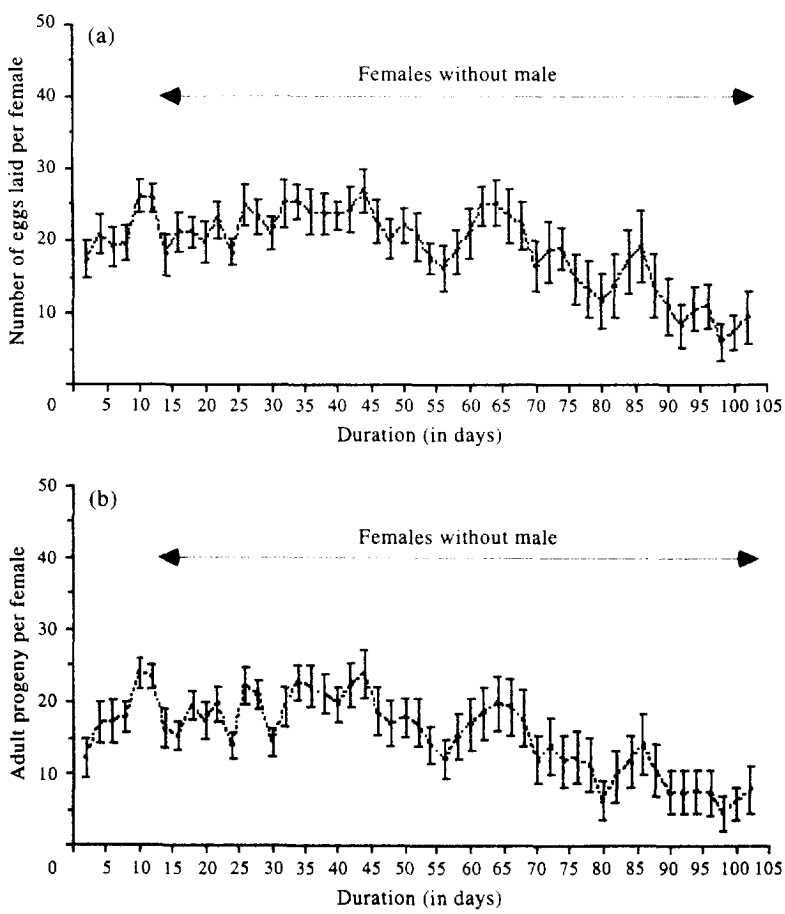

Fig. 1. Effects of remating and male deprivation on oviposition (number of eggs laid by female per 2 days) (a) and progeny (total number of insects produced per female in 2 days) (b) for a susceptible strain of *Tribolium castaneum* during 102 days.

a further 6 days. After the second mating period, the susceptible males were discarded and females were transferred to a new vial. Eggs were collected every 2 days during the period of mating and during an additional 90-day period. All eggs counted after each sieving were set up on 4 g of culture medium for their entire period of development. When the progeny of each susceptible female eclosed, the total number of adults was noted. After 7 days, the proportion of resistant insects was detected with a malathion contact bioassay. On a filter paper impregnated with a concentration of 1% malathion, susceptible insects died after 3 h.

RESULTS AND DISCUSSION

The number of eggs laid by *Tribolium castaneum* are summarized in Fig. 1a. The fecundity was estimated as 19.1 and 26.2 eggs per female per 2 days after 6 and 12 days, respectively. After the maximum rate of oviposition (27.1 eggs per 2 days) was attained at 44 days, it decreased progressively to 9.5 eggs per 2 days after 102 days. Percentage adult emergence ranged from 92% at 18 days to 54.7% at 80 days (Fig. 1b).

The proportion of resistant phenotypes in female progeny was 99.6 and 3%, respectively, after the first and second mating period. The latter proportion increased to 34.5 and 26.7%, respectively, after 30 and 102 days for females mated twice (Fig. 2).

Our results indicate that sperm from the last male is used for fertilization and so confirm the sperm competition theory (Parker, 1970). Malathion-susceptible females stored sperm of resistant males in the spermatheca for a long duration and used this sperm at fertilization when matings were suspended. There was some mixing of susceptible and resistant sperm in the spermatheca, since both phenotypes appeared together in the progeny of the malathion-susceptible *T. castaneum*.

Insects and mites are responsible for deterioration of stored food and they cause yearly losses estimated at about 30% of 1800 million tons of stored grain. Moreover, intensive control of these pests with pesticides such as phosphine and malathion has triggered the development of resistant strains. As trade of cereals in the world implies circulation of freight from one storage place to another, it allows the dispersal of pests from different geographic areas. This will result sometimes in the introduction of susceptible strains to silos already contaminated with insects that are resistant and vice versa. Such introductions will increase the spread of susceptible or resistant alleles in the population (Wool and Manhein, 1980). The dispersion and competitive mating of pests of differing resistance status has attracted very little attention and it is therefore important to study the influence of such introductions on the development of resistance in pests of stored products.

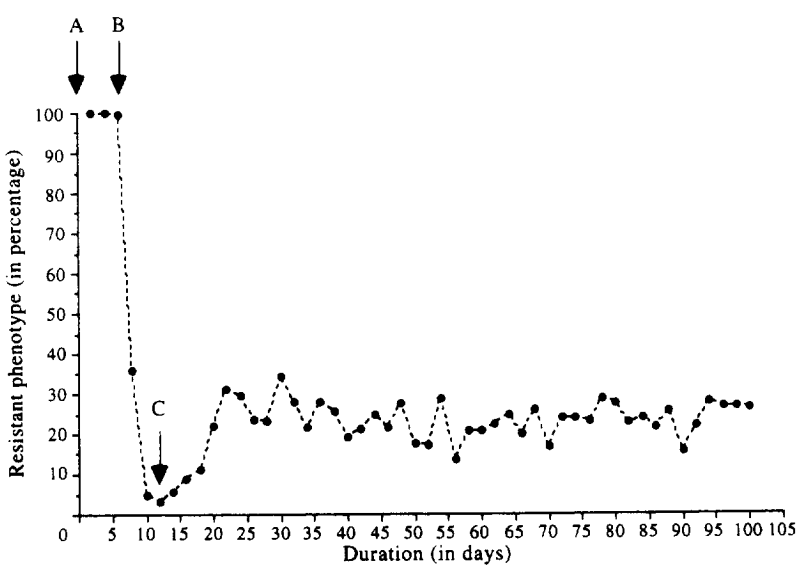

Fig. 2. The rate of malathion-resistant sperm utilization (percentage of resistant adults) by susceptible females of *Tribolium castaneum* during 102 days. A, susceptible females with resistant males; B, susceptible females with susceptible males; C, susceptible females without male.

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