INSECTICIDE RESISTANCE ENHANCES MALE REPRODUCTIVE SUCCESS IN A BEETLE

Ludovic Arnaud[1] and Eric Haubruge

Department of Pure and Applied Zoology, Gembloux Agricultural University, 2, Passage des déportés,
B-5030 Gembloux, Belgium
[1]*E-mail: arnaud.l@fsagx.ac.be*

Abstract.—Malathion-specific resistance in the red flour beetle, *Tribolium castaneum*, is widespread and stable in natural populations even in the absence of pesticide exposure. To understand this stability, both resistant and susceptible males were placed in competition for susceptible female fertilization. Females were then isolated and their progeny was tested for malathion susceptibility. Male reproductive success was estimated for populations originating from different geographic areas and for isogenic strains. In most cases, resistant males had a greater reproductive success rate than susceptibles. The results suggest that male reproductive success is not traded against the selection for malathion resistance, even resistant individuals are at an advantage for this fitness trait. This absence of fitness cost may be the result of postselection of (1) modifier gene which ameliorate the fitness of resistant individuals or (2) nondeleterious resistance gene. Resistant phenotype superiority could be due to increased male mating success, improved ability of resistant males in sperm competition, female mate choice, and/or cryptic female choice of resistance gene(s). The effect of male phenotypic frequency on male reproductive success was also examined. We observed that male fertilization success is frequency dependent and inversely related to their frequency. However, this ''rare male'' advantage did not counteract the superiority of the resistant males.

Key words.—Female choice, fitness, malathion, mating competition, selection, sperm competition, *Tribolium castaneum*.

Over the past two decades, there has been the realization that studies of insecticide resistance is not only helpful in improving pest management programs but also allow examination of an evolutionary process (Roush and McKenzie 1987; McKenzie 1996; Raymond et al. 2001). Selection for insecticide resistance is generally accompanied by pleiotropic effects that may place the resistant insects at a competitive disadvantage with the susceptible individuals upon removal of insecticide (Ferrari and Georghiou 1981; Bauerfiend and Chapman 1985; Parello and Trumble 1989; Cochran 1993; McKenzie 1994). Therefore, frequencies of resistant individuals usually decline over time in the absence of pesticide use. However, in some cases, no differences in fitness are observed between resistant and susceptible individuals in the absence of selective pressure or the fitness of resistant individuals is even greater than that of susceptible insects (Varzandeh et al. 1954; Roush and Hoy 1981; Beeman and Nanis 1986; Haubruge and Arnaud 2001; Raymond et al. 2001).

In the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae), malathion-specific resistance is widespread and the resistant phenotype has largely or completely replaced the susceptible (for a review, see Subramanyam and Hagstrum 1996). In addition, it was observed that the frequency of resistance genes was stable in laboratory populations when competing with the susceptible individuals (Beeman and Nanis 1986; Haubruge and Arnaud 2001). To explain this persistence, it was presumed that there is little or no reproductive disadvantage between malathion-specific resistant and malathion susceptible strains.

Although fertilization success can influence inheritance of gene frequencies (Ehrman et al. 1965; Sinnock 1970), it has attracted very little attention in the literature related to insecticide resistance. Understanding the relative reproductive success of susceptible and resistant males is important for the evolution of insecticide resistance and also for the fine tuning of insecticide resistance management programs. The goal of this study was to properly determine the relative fertilization success of susceptible and malathion-specific resistant males of *T. castaneum* when competing for female fertilization.

In the present study, the effect of insecticide resistance on male reproductive success was first examined in eight resistant and four susceptible field strains. In most cases, resistant males had a greater reproductive success than the susceptible. As these populations originated from different geographic areas, they may differ in their genetic backgrounds and selective histories (McKenzie 1996). Thus, the differences of reproductive success between resistant and susceptible individuals may have resulted from other factors than the pleiotropic effects of malathion-specific resistance on male reproductive success. To differentiate between these possibilities, isogenic strains were selected by repeated backcrossing of a resistant strain to a susceptible, resulting in a pair of strains that are genetically identical except for a small region of the chromosome that includes the resistant locus of interest.

Isogenic strains were used to examine the relative reproductive success of malathion resistant *T. castaneum* males when competing with malathion susceptible males for female fertilization. When a single gene is involved in insecticide resistance, the relative fitness estimates of the heterozygote compared to the homozygous susceptible is especially critical because, assuming Hardy-Weinberg equilibrium ($p^2:2pq:q^2$), the resistance gene is carried primarily in heterozygous genotypes when the gene is at low frequency (< 0.5), during the early stages of a resistance episode (Comins 1977; Roush and Plapp 1982). Therefore, the relative reproductive success of homozygous and heterozygous individuals was examined. As with field populations, we observed that malathion resistance was transmitted more successfully than susceptibility.

TABLE 1. Populations of the red flour beetle, *Tribolium castaneum* (Coleoptera, Tenebrionidae), and their origin.

Population	Origin	Year of collection
Argyle	Manitoba, Canada	1992
Asm	Abidjan, Ivory Coast	1989
Ex-Maff	Maff, U.K.	1991
Ga-1	Georgia, U.S.	1980
Kano	Nigeria	1961
Lab-S	Kansas, U.S.	1960
Landmark	Manitoba, Canada	1991
Mozambique	Mozambique	1976
Paulo d'Amico	British Columbia, Canada	1976
PRm	Naphin, Philippines	1976
Rio Desago	New Brunswick, Canada	1976
Waseco	Minnesota, U.S.	1982

MATERIALS AND METHODS

Tribolium Strains

Twelve red flour beetle strains were used in this study (see Table 1). The insects were reared with a mixture of whole wheat flour enriched with brewer's yeast (10/1-w/w) as rearing medium and kept in the dark at 30 ± 3°C and 60 ± 5% relative humidity (rh). These strains were cultured without pesticide exposure for several years. The Asm and PRm strains were used as our references for malathion susceptibility and malathion resistance, respectively.

Malathion Susceptibility and Characterization of the Insecticide Resistance

The following test was conducted to discriminate between malathion-specific resistance and malathion nonspecific resistance (Dyte and Rowlands 1968). A dose of insecticide that would kill all individuals of a susceptible population was used as the discriminating dose, that is, a 1% (w/v) malathion solution as the discriminating dose. Following a 24 h starvation period, fifty adult beetles were placed in contact with treated papers (350 μl per filter paper, Ø 55 mm) in a petri dish. After five hours, adult mortality of every strain was observed and compared to the mortality of the control strain (Asm, 100% mortality; Haubruge et al. 1997). A synergist was used with the strain in which resistant individuals were detected. Papers treated with the synergist solution in acetone (10%, w/v) were placed in a 55 mm petri dish with 50 adult beetles for 24 h. The synergist was triphenyl phosphate (TPP, > 99% purity, Merck-Schuchardt, München, Germany), a carboxylesterase inhibitor (Dyte and Rowlands 1968). Papers treated with TPP were then replaced by filter papers treated with malathion at the discriminating dose. After five hours, adult mortality was controlled. Two replications were performed for every bioassay.

Selection of Isogenic Strains

To eliminate genetic background as a variable in comparing male phenotype reproductive success, we introduced the malathion resistance alleles into a uniform, susceptible genetic background. The Asm and PRm strains were used to make the isogenic strain. PRm beetles were crossed and back-crossed to the Asm susceptible strain for eight consecutive generations. For the first cross and the four consecutive backcrosses, we used resistant males and susceptible females to replace all field-derived X chromosomes with those of the Asm strain. Resistant females were crossed to susceptible males during the four last backcrosses to replace all field derived Y chromosomes with those of the susceptible strain. Before each backcross, resistant progeny from the previous backcross was selected using a discriminating dose bioassay (Haubruge et al. 1997). Following the last backcross, homozygous resistant beetles were selected using a dose that killed 80% of the adults to eliminate the heterozygous adults. At each new generation, the presence of homozygous susceptible adults was detected with the discriminating-dose bioassay. This procedure was applied until no susceptible adults were detected during four consecutive generations.

Mating Competition and Fertilization Success

Unbiased sex ratio.—The method described by Arnaud et al. (1999) was followed to examine the relative reproductive success of susceptible and malathion-resistant males. Beetles were sexed as pupae and kept individually in rearing medium at 30 ± 3°C and 60 ± 5% rh in a dark incubator. Adults used in the experiment were 15 days old. Ten susceptible females (Asm) were placed in a 90 mm petri dish with 20 g of rearing medium. Ten males of the two different competing phenotypes were added for 48 h, after which males were removed. Three replications were performed for every resistant and susceptible strain.

One proportion of males (five susceptible and five resistant) was tested with the field populations. Asm males were placed in competition with males from the resistant populations and PRm males were used with males from the susceptible populations.

Three proportions of males were tested with the isogenic strains (nine susceptible and one resistant, five susceptible and five resistant, one susceptible and nine resistant), allowing us to examine the effect of phenotype frequency on reproductive success. The relative reproductive success of homozygous resistant and heterozygous resistant males was also studied. To make heterozygous strains, pupae of each strain were sexed and maintained individually with rearing medium. Adults were crossed as follows: $\male_{ss} \times \female_{RR}$ (H1) and $\male_{RR} \times \female_{ss}$ (H2). (SS: homozygous susceptible, Asm; RR: homozygous resistant, ISO.)

Males were marked black with pencil to facilitate their identification. After 48 h of contact with males, females were isolated individually in a 90 mm petri dish with 20 g of rearing medium at 30 ± 3°C and 60 ± 5% rh and allowed to oviposit. Females were not removed until the dish was scored for offspring.

Biased sex ratio.—In this experiment, two males (one susceptible and one resistant, homozygote or heterozygote) were placed in competition for the fertilization of a susceptible female (Asm). Beetles were sexed as pupae and kept individually in rearing medium at 30 ± 3°C and 60 ± 5% rh in a dark incubator. Adults used in the experiment were 15 days old. The males (marked as above) and the female were placed for two days in a glass vial (Ø 30 mm) with 0.5 g of rearing

TABLE 2. Expected proportions of resistant individuals in female progeny if reproductive success of susceptible and malathion resistant males are equal.

Percentage of resistant males in competition with susceptible males	Theoretical rate of resistant phenotype in the F_1 (in %)	
	Resistant male genotype	
	Homozygous (RR)	Heterozygous (Rs or sR)
90	90	45
50	50	25
10	10	5

TABLE 3. Malathion susceptibility of the *Tribolium castaneum* populations with and without synergist (triphenylphosphate-TPP); nd, not determined.

Strain	Mortality (in %) at the discriminating dose of malathion (1%) (after 5 h)	
	without TPP	with TPP
Susceptible		
Asm	100	nd
Ex-Maff	100	nd
Lab-S	100	nd
Mozambique	100	nd
Resistant		
Argyle	0	100
Ga-1	0	100
Kano	0	100
Landmark	0	100
Paulo d'Amico	0	100
PRm	0	100
Rio Desago	0	100
Waseco	0	100

medium. The females were then placed individually in a 90 mm petri dish with 20 g of rearing medium at 30 ± 3°C and 60 ± 5% rh and allowed to oviposit. Females were not removed until the dishes were scored for offspring. Twenty-five replications were performed. Due to disease this experiment was not conducted with the resistant strain Argyle.

In experiments with the field populations, Asm males were placed in competition with males from the resistant populations and PRm males were placed with males from the susceptible populations. With the isogenic strains, the relative reproductive success of homozygous resistant and heterozygous resistant males was examined.

Male reproductive success.—For both experiments (biased and unbiased sex ratio), after 45 days, adult progeny of every single female was treated with malathion using a discriminating dose bioassay (Haubruge et al. 1997). As the malathion-specific resistance is autosomal, dominant, and monofactorial (Haubruge and Arnaud 2001), this test enables us to unambiguously determine the male parent phenotype. The number of adults of each phenotype was observed for every single female. Results were pooled across all female progenies and all replications and the proportion of resistant individuals in female progenies was calculated (Dagnelie 1975). Data were analyzed and compared to the expected percentage using the *u*-test.

The overall relative male reproductive success was first compared between resistant and susceptible male, that is, resistant male and susceptible male relative reproductive success were estimated considering the total number of progeny of every female examined in the experiment. In ''unbiased sex ratio'' experiments, the reproductive success of a male phenotype with respect to another was estimated by comparing the proportion of the female progeny fathered by that male phenotype to a theoretical percentage (see Table 2). In ''biased sex ratio'' experiments, resistant and susceptible male reproductive successes were considered as being equal if they were not different from the theoretical percentage of 50. As malathion-specific resistance is inherited as a Mendelian character, in both experiments, the relative success of heterozygous males (strains H1 and H2) was estimated considering that the progeny of a susceptible female mated to an heterozygous male (strains H1 and H2) is composed of susceptible and resistant individuals in equal proportions.

Last-male precedence is observed in *T. castaneum* (Arnaud et al. 2001a), it is therefore possible to determine the phenotype of the last male mated to a female. In a second step of the analysis, male reproductive success was therefore examined upon the phenotype of the last male to inseminate the female. We considered that a female had been last-mated by a resistant male when more than 50% of her progeny was composed of resistant individuals. The number of females last inseminated by a resistant or a susceptible male and the reproductive success of resistant and susceptible males were compared upon this characteristic, that is, resistant male reproductive success when they are last to mate versus susceptible male reproductive success when they are last to mate. Results were analysed with u-test and chi square analysis.

When 10 males were placed in competition for the fertilization of 10 females, it is likely that males of the same phenotype had the opportunity to inseminate successively the same female, with a male of that phenotype being the last to copulate with the considered female. ''Last-male reproductive success'' sensu stricto was thus not determinable unequivocally. The male fitness trait estimated here was thus the ''*last-phenotype reproductive success*.'' Nevertheless, the term ''last-male reproductive success'' was adopted throughout the text.

RESULTS

Malathion Susceptibility and Characterization of the Insecticide Resistance

The malathion susceptibility of the different flour beetle populations, and the characterization of the insecticide resistance mechanism involved in the resistant populations are presented in Table 3. We observed that the mechanism of resistance was malathion specific in every resistant strain. All the malathion-resistant beetles tested died when they had previously been in contact with TPP. These populations were thus retained for the experiments of mating competition and fertilization success.

Mating Competition and Fertilization Success

Unbiased sex-ratio.—When 10 males competed for the fertilization of 10 females, the relative reproductive success of the resistant males from the field populations was greater

TABLE 4. Male reproductive success (in % ± SE) of malathion-specific resistant *Tribolium castaneum* males when five resistant males compete with five susceptibles for female fertilization.

Competing males	Reproductive success[1]	U-test		
		n[2]	u	P
Resistant against Asm				
Argyle × Asm	71.5 ± 0.8	3173	21.18	<0.001
Ga-1 × Asm	64.0 ± 0.9	2919	15.09	<0.001
Kano × Asm	61.1 ± 0.9	3028	12.18	<0.001
Landmark × Asm	60.7 ± 0.9	3202	12.12	<0.001
Paul d'Amico × Asm	66.6 ± 0.9	2762	17.43	<0.001
PRm × Asm	59.4 ± 0.8	3639	14.82	<0.001
Rio Desago × Asm	49.9 ± 0.9	3072	0.072	0.942
Waseco × Asm	58.9 ± 0.8	3476	10.48	<0.001
Susceptible against PRm				
Ex-Maff × PRm	54.4 ± 0.9	3342	5.01	<0.001
Lab-S × PRm	55.0 ± 0.9	3175	5.66	<0.001
Mozambique × PRm	54.7 ± 0.9	3402	5.52	<0.001

[1] Percentage of malathion-specific resistant individuals in female progenies.
[2] Total number of individuals observed across three replications.

than that of susceptible ones. The F_1 progenies are constituted with more resistant phenotypes than expected (see Table 4). In one case, Rio Desago against Asm, male reproductive success was equal between the resistant and the susceptible insects.

The number of females last inseminated by a resistant male and the reproductive success of susceptible and resistant males when they are the last to inseminate the female are presented in Table 5. In most cases, excepted when Rio Desago males competed with Asm males, we observed that females were mostly last inseminated by a resistant male, but the differences were generally not significant. The last-male reproductive success of resistant males was not different or was significantly higher then that of susceptible males. In only one experiment (Asm against Paulo d'Amico), susceptible males were found to be significantly fitter than resistant individuals at this fitness component (Table 5).

In experiments with the isogenic strains, homozygous and heterozygous resistant males had also a relative reproductive success equal or even higher than that of susceptible ones (see Table 6). The fertilization success of a male phenotype relative to its frequency is presented in Table 6. Males are more successful when they are relatively rare, however, the rare male advantage of susceptible males is not sufficient to counteract the resistant male fitness advantage at fertilization. The number of females last inseminated by a resistant male was not different than expected (Table 7). When resistant and susceptible males were placed in the same proportion (5:5), the reproductive success of resistant males was the highest. As expected, when nine resistant males competed with one susceptible, the reproductive success of resistant males was higher than that of the susceptible. The reverse was observed when resistant males were in minority. The highest reproductive success of the phenotype in majority may be ex-

TABLE 5. Number of females (in % ± SE) last inseminated by a resistant male and last-male reproductive success (in % ± SE) of malathion-specific resistant and malathion susceptible *Tribolium castaneum* males when five resistant males compete with five susceptibles for female fertilization.

Competing males	Number of females last inseminated by a resistant male[1]					Last-male reproductive success[3]				Chi square test[6]	
		U-test									
	%	n[2]	u	p	Resistant	n[4]	Susceptible	n[5]	χ^2	P
Resistant against Asm											
PRm × Asm	57.7 ± 9.7	26	0.78	0.433	76.5 ± 0.9	2262	61.9 ± 1.3	1312	86.08	<0.001
Argyle × Asm	83.3 ± 6.8	30	3.65	<0.001	73.0 ± 0.8	2744	58.3 ± 2.4	429	39.27	<0.001
Ga-1 × Asm	64.3 ± 9.1	28	1.51	0.131	82.8 ± 0.9	1789	65.8 ± 1.4	1130	109.70	<0.001
Kano × Asm	67.9 ± 8.8	28	1.89	0.059	69.9 ± 1.0	2292	66.4 ± 1.7	736	3.11	0.078
Landmark × Asm	72.4 ± 8.2	30	2.41	0.106	68.9 ± 1.0	2325	61.1 ± 1.6	877	15.60	<0.001
Paulo d'Amico × Asm	90.0 ± 5.5	30	4.38	<0.001	71.8 ± 0.9	2487	80.4 ± 2.4	275	9.19	0.002
Rio Desago × Asm	46.7 ± 9.1	30	0.37	0.175	64.6 ± 1.3	1453	63.2 ± 1.2	1619	0.63	0.428
Waseco × Asm	60.0 ± 8.9	30	1.10	0.273	79.4 ± 0.9	2014	53.4 ± 1.3	1462	265.99	<0.001
Susceptible against PRm											
Ex-Maff × PRm	58.6 ± 9.1	29	0.93	0.353	69.2 ± 1.0	2098	66.7 ± 1.3	1244	2.23	0.135
Lab-S × PRm	58.6 ± 9.1	29	0.93	0.353	76.1 ± 1.0	1713	69.6 ± 1.2	1462	16.61	<0.001
Mozambique × PRm	63.3 ± 8.8	30	1.46	0.144	69.4 ± 1.0	2170	71.2 ± 1.3	1232	1.13	0.287

[1] In percents; [2] Female sample size (some females did not give progeny, sample size is thus lower or equal to 30), percentage of malathion-specific resistant individuals in female progenies; [3] In percent; [4] Total number of individuals in the progeny of females last inseminated by a resistant male; [5] Total number of individuals in the progeny of females last inseminated by a susceptible male; [6] 1 df.

TABLE 6. Theoretical and observed reproductive success (in % ± SE) and individual reproductive success (in %) of malathion-specific resistant *Tribolium castaneum* males when competing with susceptible males for female fertilization.

Competing males	Theoretical reproductive success[1]	Observed reproductive success[1]	U-test			Individual male success[3]
			n^2	u	P	
Homozygous						
9 Iso × 1 Asm	90	89.1 ± 0.5	3704	1.83	0.067	9.9
5 Iso × 5 Asm	50	50.8 ± 0.8	3666	0.99	0.322	10.2
1 Iso × 9 Asm	10	12.9 ± 0.6	3704	5.95	<0.001	12.9
Heterozygous						
9 H1 × 1 Asm	45	48.2 ± 0.8	3516	3.89	<0.001	5.4
5 H1 × 5 Asm	25	27.2 ± 0.8	3388	2.94	0.003	5.4
1 H1 × 9 Asm	5	7.6 ± 0.5	3243	6.76	<0.001	7.6
9 H2 × 1 Asm	45	44.8 ± 0.8	3434	0.22	0.829	5.0
5 H2 × 5 Asm	25	25.8 ± 0.7	3514	1.07	0.284	5.2
1 H2 × 9 Asm	5	7.5 ± 0.4	3795	6.95	<0.001	7.5

[1] Percentage of malathion-specific resistant individuals in female progenies; [2] Total number of individuals observed across three replications; [3] Ratio between the percentage of resistant individuals in female progenies and the number of resistant males in competition.

plained by the opportunity of this phenotype to be transmitted to more than one female at a time. Even if last-male sperm precedence occurs in *T. castaneum*, males in majority achieved a better reproductive success because the spermatheca of most females may contain much more of their sperm than that of the male in minority. In addition, when a female is successively inseminated by different males of the same phenotype, which is more likely to occur when one phenotype is in majority, sperm of every male participates to egg fertilization. The reproductive success of the phenotype examined is therefore increased compared to that of the lonely male inseminating a female after several rivals of the opposite phenotype. This phenomenon could also occur when five resistant and five susceptible males competed for female fertilization, but in a less extended manner.

Biased sex ratio.—When two males were competing for fertilization of the same female, we also found that malathion resistance is not a trade against male reproductive success (see Table 8). In most experiments, resistant males had better

reproductive success than susceptible males. In contrast with the ''unbiased sex ratio'' experiments, males from the Rio Desago populations had better reproductive success than the susceptible males (Asm). In only one case (PRm against Ex-Maff), the resistant males were less fit than the susceptible males, with female progenies being mostly composed of susceptible individuals. As expected from the previous observations, the reproductive success of the homozygous and the heterozygous resistant males of the isogenic strains was better than that of the susceptible strain Asm (see Table 8).

When results are considered upon the phenotype of the last male to inseminate the females, we observed that females were significantly more last inseminated by resistant males than by susceptible (see Table 9). In two experiments (PRm against Mozambique and H1 against Asm), susceptible males achieved a better last-male reproductive success than the resistant. However, in both cases, females were more frequently last inseminated by resistant males than by susceptible ones, and the overall reproductive success of resistant males was

TABLE 7. Number of females (in % ± SE) last inseminated by a resident male and last-male reproductive success (in % ± SE) of malathion-specific resistant and malathion-susceptible *Tribolium castaneum* males when resistant and susceptible males compete for female fertilization (ten males are placed with ten females, three proportions of resistant males are tested).

Competing males	Number of females last inseminated by a resistant male[1]				Last male reproductive success[3]				Chi-square test[6]	
	%	U-test			Resistant	n^4	Susceptible	n^5	χ^2	P
		n^2	u	P						
Homozygous										
9 Iso × 1 Asm	96.7 ± 3.3	30	1.22	0.224	90.1 ± 0.5	3228	62.3 ± 4.5	114	98.94	<0.001
5 Iso × 5 Asm	44.8 ± 9.2	29	0.56	0.578	68.0 ± 1.1	1747	64.8 ± 1.1	1919	4.13	0.042
1 Iso × 9 Asm	3.3 ± 3.3	30	1.21	0.224	54.5 ± 4.5	121	88.5 ± 0.5	3583	122.06	<0.001
Heterozygous										
9 H1 × 1 Asm	100.0	30	1.82	0.068	96.5 ± 0.3	3516	0.0	0	—	—
5 H1 × 5 Asm	53.3 ± 9.1	30	0.37	0.715	71.7 ± 1.0	1884	67.3 ± 1.2	1504	7.56	0.006
1 H1 × 9 Asm	0.0	30	1.83	0.068	0.0	0	92.4 ± 0.5	3243	—	—
9 H2 × 1 Asm	96.7 ± 3.3	30	1.22	0.224	91.2 ± 0.5	3336	82.7 ± 3.8	98	74.56	<0.001
5 H2 × 5 Asm	56.7 ± 9.0	30	0.73	0.465	62.8 ± 1.1	2106	65.2 ± 1.3	1408	2.15	0.143
1 H2 × 9 Asm	3.3 ± 3.3	30	1.22	0.224	60.5 ± 3.8	162	87.1 ± 0.6	3633	91.21	<0.001

[1] In percents; [2] Female sample size (some females did not give progeny, sample size is thus lower or equal to 30), percentage of malathion-specific resistant individuals in female progenies; [3] In percents; [4] Total number of individuals in the progeny of females last inseminated by a resistant male; [5] Total number of individuals in the progeny of females last inseminated by a susceptible male; [6] 1 df.

TABLE 8. Reproductive success (in % ± SE) of malathion-specific resistant *Tribolium castaneum* males of different field populations and of isogenic strains when one resistant and one susceptible male compete for the fertilization of the same female.

Competing males	n^1	Reproductive success[2]	*U*-test		
			n^3	u	P
Field populations					
Ga-1 × Asm	24	62.1 ± 1.0	2604	12.35	<0.001
Kano × Asm	23	67.2 ± 0.9	2477	17.14	<0.001
Landmark × Asm	25	73.2 ± 0.8	3090	7.05	<0.001
Paulo d'Amico × Asm	25	61.6 ± 0.9	2864	12.86	<0.001
PRm × Asm	24	67.8 ± 0.8	3091	19.84	<0.001
Rio Desago × Asm	25	59.5 ± 0.9	3202	1.98	0.048
Waseco × Asm	24	63.6 ± 1.0	2120	12.51	<0.001
Ex-Maff × PRm	24	47.2 ± 0.9	2964	2.90	0.004
Lab-S × PRm	24	53.5 ± 0.9	3202	3.99	<0.001
Mozambique × PRm	25	56.3 ± 0.9	3090	7.05	<0.001
Isogenic strains					
Iso × Asm	25	52.4 ± 0.9	2813	2.55	0.011
H1 × Asm	25	59.1 ± 0.9	2909	5.64	<0.001
H2 × Asm	25	60.7 ± 0.9	3092	6.85	<0.001

[1] Some females did not give any progeny, the sample size is thus lower or equal to 25.
[2] Percentage of malathion-specific resistant individuals in female progenies.
[3] Total number of individuals observed across three replications.

significantly higher than that of susceptibles (see Table 8). In the other experiments, we found that resistant male reproductive success was not different or was significantly higher than that of susceptible males (see Table 9).

DISCUSSION

Relative fitness comparisons between resistant and susceptible individuals are often studied in terms of development time, fecundity, and egg fertility (Georghiou and Taylor 1986). Although mating competition, sperm competition, pre- and post-copulatory female choice, and fertilization success differences may be critical for insecticide resistance gene(s)

transmission, these parameters are rarely considered in studies of insecticide resistance fitness. In the present paper, we proposed to examine the pleiotropic effects of malathion-specific resistance gene(s) on the reproductive success of *T. castaneum*.

First, we observed that malathion-specific resistant males of field populations had a greater reproductive success than susceptible ones. This phenomenon was widespread across eight resistant and four susceptible field strains. Therefore, it seems likely that malathion resistance is not traded against male reproductive success in *T. castaneum*. However, because these populations originated from different geographic areas,

TABLE 9. Number of females (in % ± SE) last inseminated by a resistant male and last-male reproductive success (in % ± SE) of malathion-specific resistant and malathion susceptible *Tribolium castaneum* males when one resistant and one susceptible male compete for the fertilization of the same female.

Competing males	Number of females last inseminated by a resistant male[1]				Last-male reproductive success[3]				Chi-square test[6]	
	%	*U*-test			Resistant	n^4	Susceptible	n^5	χ^2	P
		n^2	u	P						
Field populations										
PRm × Asm	76.0 ± 8.5	25	2.60	0.009	78.4 ± 0.8	2385	65.0 ± 1.9	652	49.22	<0.001
Ga-1 × Asm	62.5 ± 9.9	24	1.22	0.221	78.0 ± 1.0	1892	78.9 ± 1.6	673	0.26	0.612
Kano × Asm	78.3 ± 8.6	23	2.71	0.007	78.9 ± 0.9	1865	68.5 ± 1.9	612	27.93	<0.001
Landmark × Asm	80.0 ± 8.0	25	3.00	0.003	80.0 ± 0.8	2240	55.3 ± 2.1	535	141.78	<0.001
Paulo d'Amico × Asm	76.0 ± 8.5	25	2.60	0.009	72.2 ± 0.9	2232	73.9 ± 1.7	632	0.73	0.394
Rio Desago × Asm	70.8 ± 9.3	24	2.04	0.041	73.1 ± 1.0	1851	76.6 ± 1.6	700	3.10	0.078
Waseco × Asm	83.3 ± 7.6	24	3.27	0.001	71.6 ± 1.1	1653	64.7 ± 2.2	467	8.26	0.004
Ex-Maff × PRm	36.0 ± 9.6	25	1.40	0.162	74.5 ± 1.2	1251	74.2 ± 1.1	1685	0.04	0.848
Lab-S × PRm	52.0 ± 10.0	25	0.20	0.842	72.1 ± 1.2	1497	62.8 ± 1.2	1705	31.46	<0.001
Mozambique × PRm	79.2 ± 8.3	24	2.86	0.004	71.1 ± 1.0	2158	77.8 ± 1.4	932	14.89	<0.001
Isogenic strains										
Iso × Asm	52.0 ± 10.0	25	0.20	0.842	78.7 ± 1.1	1459	75.9 ± 1.2	1354	3.06	0.800
H1 × Asm	76.0 ± 8.5	25	2.60	0.009	71.8 ± 1.0	2126	75.5 ± 1.5	783	3.96	0.047
H2 × Asm	76.0 ± 8.5	25	2.60	0.009	71.2 ± 0.9	2380	74.4 ± 1.6	712	2.89	0.089

[1] In percents; [2] Female sample size (some females did not give progeny, sample size is thus lower or equal to 25), percentage of malathion-specific resistant individuals in female progenies; [3] In percents; [4] Total number of individuals in the progeny of females last inseminated by a resistant male; [5] Total number of individuals in the progeny of females last inseminated by a susceptible male; [6] 1 df.

they must have different genetic backgrounds which may explain the advantage of the resistant individuals. To differentiate between these possibilities, isogenic strains for malathion resistance were selected and the reproductive success of homozygous and heterozygous resistant males was observed to complement the observations made with the field derived populations. Similar results were obtained.

Experiments on the effects of resistance to dieldrin showed that in *Anopheles albimanus* mosquitoes, resistant males are more competitive at mating than the susceptible ones. The insemination rate of the resistant males was 17% higher than that of susceptibles. This difference was explained by a greater aggressiveness of the dieldrin-resistant males (Gilotra 1965). However, Gilotra's strains had different geographic origin and were not isogenic. Thus, it is likely that the better reproductive success of the resistant males has resulted from a difference of genetic background between the resistant and the susceptible populations and not because of pleiotropic effects of the resistance genes. In other respects, homozygous and heterozygous dieldrin resistant males of *Anopheles* spp. mosquitoes were found to be less successful at mating competition than susceptible ones (Rowland 1991). In addition, it was found recently that susceptible *Culex pipiens* mosquitoes had a mating advantage when competing with males resistant to organophosphorus insecticides, suggesting a substantial cost of resistance genes to this fitness trait (Berticat et al. 2002). However, in the mite *Metaseiulus occidentalis*, no differences in mating ability or fertilization success has been observed between susceptible and artificially selected carbaryl resistant mites (Roush and Hoy 1981; Roush and Plapp 1982).

Contrary to these previous findings, we observed that, in most competition experiments, malathion-specific resistant males of *T. castaneum* had a higher relative fertilization success than susceptible males. Resistant phenotypes were observed at higher percentages than expected in the female progenies. In addition, females were observed to be more often last inseminated by resistant males than by susceptible ones. And last-male reproductive success of resistant males was mostly equal or even greater than that of susceptible. It is therefore clear that, in *T. castaneum*, malathion resistance is not traded against male reproductive success. These observations are observed with 12 field populations and isogenic strains. This phenomenon may have contributed to the spread of malathion-specific resistance in flour beetle populations.

The reproductive advantage of resistant males observed here is unexpected. If a gene associated with resistance to insecticide was also advantageous for the fitness of the individuals in absence of selective pressure, we can addressed the following question: Why has not this advantageous gene occurred in the first place? The most likely answer is because it is costly to fitness. Studies of the impact of insecticide resistance genes on the fitness generally showed that, at least, a substantial fitness cost is associated with resistance, in absence of insecticide. Thus, the stability of the resistance gene observed in some insect species may be the result of posterior modification of the insect genome (Roush and McKenzie 1987; McKenzie 1996; Raymond et al. 2001).

In the sheep blow-fly, *Lucilia cuprina*, there is a fitness cost associated with gene coding for resistance to diazinon. However, following the intensive use of diazinon insecticide, a modifier gene has been selected in the field to completely suppress this cost. As a result, resistant insects carrying the modifier are equally as fit as the susceptible insects (for a review of the phenomenon, see McKenzie 1996). Organophosphorus insecticides are widely used to control mosquito populations. Within a few years, populations of the mosquito *Culex pipiens* have developed resistance against these insecticides. The genes involved in the resistance are costly to fitness, however, at one resistant locus, a reduction of cost driven by allele replacement and not by selection of modifiers has been observed (Raymond et al. 2001).

Malathion has been intensively used in grain storage since the late fifties and the first case of malathion resistance in the red flour beetle, *Tribolium castaneum*, was reported in Kenya in 1961 (Parkin et al. 1962). By 1974, this phenomenon was regarded as a common attribute of the species (Champ and Dyte 1976) and malathion usage has declined or even been abandoned in some countries in the early seventies (Champ 1984). In addition, during the field surveys achieved between the 1960s and the 1990s about 90% of the *T. castaneum* populations tested were found to be resistant to malathion (for a review, see Subramanyam and Hagstrum, 1996). However, malathion has been continuously used and is still in usage in many countries to control insects in stored products (White and Leesch, 1996), leading to continuous selective pressure against the susceptible insects. As a result of intensive selective pressure lasting for many years after the selection of the first resistant individuals, the deleterious effect of the resistance allele selected at first may have been suppressed. This could have been achieved by the selection of a modifier that ameliorates the fitness of resistant individuals as observed in *L. cuprina*, or of a new resistance allele without deleterious effect as in *C. pipiens*. It is therefore likely that, at first, the individuals selected for malathion-specific resistance were at a fitness disadvantage in absence of insecticide. Because of the continuous use of malathion to protect stored food, is it probable that a modifier gene or a not costly resistance allele has been selected reducing the fitness cost of malathion-specific resistance gene in this species. In both hypotheses, the modifier or the allele of substitution not only suppress the cost of the resistance allele, but also increases the reproductive success of resistant individuals given them a reproductive advantage compared to the susceptible. A more speculative hypothesis could be that both the modifier and the nondeleterious allele were selected in the resistant populations. The highest reproductive success of resistant males would result in a synergetic effect of both gene modifications.

In most experiments, the same susceptible genetic background was used (strain Asm). A third hypothesis explaining the highest reproductive success of resistant insects could be that this susceptible population possess a deleterious gene near the resistant locus. Because only eight backcrosses were performed to select isogenic strains, it is unlikely that such a deleterious gene is shared by both the susceptible and the isogenic resistant strains. The comparison of the reproductive success of these two strains therefore indicates that resistant insects have a higher reproductive success than susceptibles,

even if the resistance gene is costly, if the cost of resistance for the traits examined is lower than the one given by the deleterious gene in question.

In the absence of selection, average fitness of individuals has been observed to decline due to the accumulation of deleterious mutations (Lynch et al. 1999; Schultz et al. 1999; Vassielieva et al. 2000). In the confused flour beetle, *Tribolium confusum*, Lomnicki and Jasienski (2000) found that the fitness of beetles from a selection-free population was reduced compared to beetles from stock population where selection is more intense (nevertheless, this phenomenon was observed in only one of the two selection-free populations tested). The fitness erosion observed in *T. confusum* could be attributed to the selection of the deleterious gene during the 22 generations of relaxation of selective pressure.

Despite the use of the susceptible strain Asm as the ''susceptible control'' in most experiments, other field-derived susceptible populations were used for fertilization competition experiments, with resistant insects being usually more successful than the susceptible insects. If deleterious genes occur in *T. castaneum*, it seems unlikely that these mutations were selected only in susceptible populations. It is therefore more probable that the reproductive advantage of resistant males observed here is due to the positive effect of a modifier gene associated with the resistance gene or simply to the resistance gene itself, via the postselection of a nondeleterious resistance gene, than on deleterious genes present mainly in susceptible populations. Moreover, the *T. castaneum* populations used in our study have been in isolation for between eight and 39 years at the beginning of the experiments. They have been maintained in the same rearing conditions for about 5 years (i.e. at least 30 generations), in high density conditions (i.e. about 500 to 1000 individuals per 80 g of medium, ≈150 ml) where natural selection is likely to occur. Deleterious mutations may therefore not have the opportunity to be selected. Nevertheless, if our rearing conditions are not selective enough to prevent fitness erosion, deleterious mutations had the opportunity to be selected in susceptible as well as in resistant populations.

The second question that may be addressed here is about the biological changes given a reproductive advantage to the resistant males. Several hypothesis could be advanced to explain our observations. There is now widespread evidence that spermatozoa from several males could compete with each other in the female sperm storage organ (Parker 1970; Smith 1984; Birkhead and Möller 1998). However, females are not only a receptacle in which sperm competition is played out. In the past few years, increasing attention has been paid to the possibility that females of many species are active not only in (pre-)copulatory choice but also in controlling the process of sperm storage and use, and in the possibility of postcopulatory cryptic choice. By the existence of the sperm storage organ, females may thus control more subtle events both during copula and thereafter (Eberhard 1996; Pizzari and Birkhead 2000).

In most insect species, the last male to mate achieves fertilization precedence (Simmons and Siva-Jothy 1998; Simmons 2001). In *T. castaneum*, the high degree of last-male sperm precedence confers a fertilization advantage to the last male to copulate (Arnaud et al. 2001a). Males doing better at mating competition will be more often the last to mate with a female. Thus, their fertilization success will be favored. Lewis and Austad (1994) observed that, in *T. castaneum*, more attractive males achieve higher last-male reproductive success. Resistant males may be more attractive to females than susceptible.

Tribolium castaneum females have a single spermatheca but this consists of multiple tubules (Sinha 1953) which could provide a mechanistic opportunity for preferential sperm storage and usage, as is claimed for other insects (Ward 1993; Yuval et al. 1996, Otronen 1997; Otronen et al. 1997). Females of the yellow dung fly, *Scatophaga stercoraria*, differentially sort the ejaculates from different males across their three spermatheca allowing them to use sperm differentially over time (Ward 1993). Moreover, female dung flies adapt their storage decisions upon male characteristics accepting more sperm when her last mate is superior to the previous ones. Also, they may exercise a control by selectively opening or closing their spermathecal ducts (Otronen et al. 1997). Similar behavior is observed in the fly *Dryomyza anilis* (Otronen 1997). In *T. castaneum* male fertilization success in sperm competition is influenced by the rate that males rub the female elytra with their tarsi during copula (Edvardsson and Arnqvist 2000). Leg rubbing may therefore provide honest signals of some components of male fitness that females subsequently base their sperm selection decisions upon. Genital stimulation during mating may also provide such signals to the female (Arnaud et al. 2001b). Malathion-specific resistant males may be more fit at leg rubbing or genital stimulation than susceptible males, resulting in the selection of their sperm for egg fertilization.

Evidence of mate choice mediated by cuticular pheromones has been widely demonstrated in insects (e.g., Tompkins et al. 1993; Tregenza and Wedell 1997). In *Anopheles* spp. mosquitoes, there are significant differences in the cuticular hydrocarbons of susceptible and insecticide resistant insects. This hydrocarbon segregation might be associated with geographical variation, but also with genetic alterations resulting from prior insecticide exposure (Anyanwu et al. 1997a, b). As in *Anopheles*, the composition of the cuticular hydrocarbons of malathion resistant *T. castaneum* males may differ from susceptible ones. Females may therefore assess male quality through cuticular hydrocarbon composition. Despite *T. castaneum* males initiating most of the copulations (Arnaud and Haubruge 1999), females may exert copulatory choice while males enter into contact with them and try to copulate. This choice could be based on the qualitative and/or quantitative composition of the male cuticular hydrocarbons.

Our results show that the male fertilization success is higher when they are rare. Rare-male advantage could bring about the maintenance of a polymorphism in a population (Sinnock 1970). However, models suggest that any net selective advantage of one phenotype will eventually result in extinction of alleles coding for alternative phenotype(s), and that polymorphisms are likely to be maintained only under specific conditions (Forsman and Shine 1995). Sinnock (1970) and Ehrman et al. (1965) observed that in *T. castaneum* and *Drosophila pseudoobscura*, respectively, the fertilization success of the genotype in the minority is higher than expected, independently of the male genotype. Moreover, populations of

Drosophila melanogaster perturbed for alcohol dehydrogenase gene frequencies returned to preperturbation levels in a predictable manner (McKenzie et al. 1994). We observed that when susceptible males are rare, their fertilization success is not higher than that of malathion-resistant males. Resistant males seem to have a better mating competitiveness or they do better at sperm competition than susceptible ones independently of their frequency. The fact that resistant males have, in most cases, a greater fertilization success suggests that if cryptic female choice for genes in the minority occurs, it could not counteract the resistant males superiority at sperm competition. This phenomenon may have played in favor of the development of malathion-specific resistance in flour beetle populations when the resistance genes were at low frequency in the early stages of resistance selection or further in the selection of the hypothetical modifier gene or the nondeleterious resistance allele.

Literature Cited

Anyanwu, G. I., D. H Davies, D. H. Molyneux, and A. Phillips. 1997a. Hydrocarbon variation/discrimination between two strains of *Anopheles albimanus* Wied. from l Salvador. Ann. Trop. Med. Parasitol. 91:493–497.

———. 1997b. Variation in cuticular hydrocarbons among strains of *Anopheles* (*Cellia*) *stephensi* Liston possibly related to prior insecticide exposure. Ann. Trop. Med. Parasitol. 91:649–659.

Arnaud, L., and E. Haubruge. 1999. Mating behaviour and male mate choice in *Tribolium castaneum* (Coleoptera: Tenebrionidae). Behaviour 136:67–77.

Arnaud, L., E. Haubruge, and C. Gaspar. 1999. La résistance au malathion augmente le succès reproducteur des mâles de *Tribolium castaneum* (Coleoptera, Tenebrionidae). Actes de la IVème Conférence Internationale Francophone d'Entomologie, Saint-Malo (France), 5–9 juillet 1998. Ann. Soc. Entomol. France 35:65–70.

Arnaud, L., E. Haubruge, and M. J G Gage. 2001a. The dynamics of second- and third-male fertilization precedence in *Tribolium castaneum*. Entomol. Exp. Appl. 99:55–64.

———. 2001b. Morphology of *Tribolium castaneum* male genitalia and its possible role in sperm competition and cryptic female choice. Belg. J. Zool. 131:93–97.

Bauerfiend, R. J. and R. K. Chapman. 1985. Non-stable parathion and endosulfan resistance in green peach aphids (Homoptera: Aphididae). J. Econ. Entomol. 78:516–522.

Beeman, R. W. and S. M. Nanis. 1986. Malathion resistance alleles and their fitness in the red flour beetle (Coleoptera: Tenebrionidae). J. Econ. Entomol. 79:580–587.

Berticat, C., G. Boquien, M. Raymond and C. Chevillon 2002. Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes. Genet. Res. Camb. 79:41–47.

Birkhead, T. R. and A. P. Möller, eds. 1998. Sperm competition and sexual selection, Academic Press, London, U.K.

Champ, B. R., ed. 1984. Pesticide resistance in stored product insects. Pp. 681–690. *in* Proc. Aust. Dev. Assist. Course on Preservation of Stored Cereals, 1981. Commonwealth Scientific and Industrial Research Organization, Division of Entomology, Canberra, Australia.

Champ, B. R., and C. E. Dyte. 1976. Report of the FAO global survey of pesticides susceptibility of stored grain pests. Food and Agriculture Organization of the United Nations, Plant Production and Protection, series no. 5. FAO, Rome.

Cochran, D. G. 1993. Decline of pyrethroid resistance in the absence of selection pressure in a population of German cockroaches (Dictyoptera: Blattelidae). J. Econ. Entomol. 86:1639–1644.

Comins, H. N. 1977. The management of pesticide resistance. J. Theor. Biol. 65:399–420.

Dagnelie, P. 1975. Théorie et méthodes statistiques, Vol. II. Les Presses Agronomiques de Gembloux, Belgium.

Dyte, C. E., and D. G. Rowlands. 1968. The metabolism and synergism of malathion in resistant and susceptible strains of *Tribolium castaneum*. J. Stored Prod. Res. 4:157–173.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton Univ. Press, Princeton, NJ.

Edvardsson, M., and G. Arnqvist. 2000. Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. Proc. R. Soc. Lond. B 267:559–563.

Erhman, L., B. Spassky, O. Pavlovsky, and Th. Dobzansky. 1965. Sexual selection, geotaxis and chromosomal polymorphism in experimental populations of *Drosophila pseudoobscura*. Evolution 19:337–346.

Ferrari, J. A., and G. P. Georghiou. 1981. Effects on insectidal selection and treatment on reproductive potential of resistant, susceptible, and heterozygous strains of the southern house mosquito. J. Econ. Entomol. 74:323–327.

Forsman A., and R. Shine. 1995. The adaptative significance of colour pattern polymorphism in the australian scincid lizard *Lampropholis delicata*. Biol. J. Linn. Soc. 55:273–291.

Georghiou, G. P., and C. E. Taylor. 1986. Factor influencing the evolution of resistance. Pp. 157–169 *in* Pesticide resistance: strategies and tactics for management, Committee on Strategies for the Management of Pesticide Resistant Pest Populations, National Research Council, National Academy Press, Washington, DC.

Gilotra, S. K. 1965. Reproductive potential of dieldrin-resistant and susceptible populations of *Anopheles albimanus* Wiedemann. Am. J. Trop. Med. Hyg. 14:165–169.

Haubruge, E., L. Arnaud, and J. Mignon. 1997. The impact of sperm precedence in malathion resistance transmission in population of the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). J. Stored Prod. Res. 33:143–146.

Haubruge, E., and L. Arnaud. 2001. Fitness consequences of malathion-specific resistance in the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), and selection for resistance in the absence of insecticide. J. Econ. Entomol. 94:552–557.

Lewis, S. M., and S. N. Austad. 1994. Sexual selection in flour beetles: the relationship between sperm precedence and male olfactory attractiveness. Behav. Ecol. 5:219–224.

Lomnicki, A., and M. Jasienski. 2000. Does fitness erode in the absence of selection? An experimental test with *Tribolium*. J. Hered. 91:407–411.

Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva, and J. Willis. 1999. Perspective: spontaneous deleterious mutation. Evolution 53:645–663.

McKenzie, J. A. 1994. Selection at the diazinon resistance locus in overwintering populations of *Lucilia cuprina* (the Australian sheep blowfly). Heredity 73:57–64.

McKenzie, J. A., ed. 1996. Ecological and evolutionary aspects of insecticide resistance. R. G. Landes, Austin, TX and Academic Press, San Diego, CA.

McKenzie, J. A., S. W. McKechnie, and P. Batterham. 1994. Perturbation of gene frequencies in a natural population of *Drosophila melanogaster*: evidence for selection at the ADH locus. Genetica 92:187–196.

Otronen, M. 1997. Sperm numbers, their storage and usage in the fly *Dryomyza anilis*. Proc. R. Soc. Lond. B 264:777–782.

Otronen M., P. Reguera, and P. I. Ward. 1997. Sperm storage in the yellow dung fly *Scatophaga stercoraria*: identifying the

sperm of competing males in separate female spermathecae. Ethology 103:844–854.

Parello, M. P., and J. T. Trumble. 1989. Decline in resistance in *Liriomyza trifolii* (Diptera: Agromyzidae) in the absence of insecticide selection pressure. J. Econ. Entomol. 82:365–368.

Parker G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–567.

Parkin, E. A., E. I. C. Scott and R. Forester. 1962. Increase resistance of stored-product insects to insecticides. The resistance of field strains of beetles. (c) *Tribolium castaneum*. Pest Infest. Res. 21:34–35.

Pizzari, T., and T. Birkhead. 2000. Female feral fowl eject sperm of subdominant males. Nature 405:787–789.

Raymond, M., C. Berticat, M. Weill, N. Pasteur, and C. Chevillon. 2001. Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation? Genetica 112–113:287–296.

Roush, R. T., and M. A. Hoy. 1981. Laboratory, glasshouse, and field study of artificially selected carbaryl resistance in *Metaseiulus occidentalis*. J. Econ. Entomol. 74:142–147.

Roush, R. T., and F. W. Plapp. 1982. Effects of insecticide resistance on biotic potential of the house fly (Diptera: Muscidae). J. Econ. Entomol. 75:708–713.

Roush, R. T. and J. A. McKenzie. 1987. Ecological genetics of insecticide and acaricide resistance. Annu. Rev. Entomol. 32: 361–380.

Rowland, M. 1991. Activity and mating competitiveness of HCH\dieldrin resistant and susceptible male *Anopheles gambiae* and *A. stephensi* and the prospect of resistance management by rotations. Med. Vet. Entomol. 5:207–222.

Schultz, S. T., M. Lynch, and J. H. Willis. 1999. Spontaneous deleterious mutation in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 96:11393–11398.

Simmons, L. W., ed. 2001. Sperm competition and its evolutionary consequences in the insects. Monographs in behavior and ecology. Princeton Univ. Press, Princeton, NJ.

Simmons, L. W. and M. T. Siva-Jothy. 1998. Sperm competition in insects: mechanisms and the potential for selection. Pp. 341–434 *in* T. Birkhead and A. Moller, eds, Sperm competition and sexual selection, Academic Press, London.

Sinha, R. N. 1953. The spermatheca in the flour beetle (*Tribolium castaneum* Herbst). J. N. Y. Entomol. Soc. 61:131–134.

Sinnock, P. 1970. Frequency dependence and mating behaviour of *Tribolium castaneum*. Am. Nat. 939:469–476.

Smith, R. L., ed. 1984. Sperm competition and the evolution of animal mating systems. Academic Press, London.

Subramanyam, B., and D. W. Hagstrum. 1996. Resistance measurement and management. Pp. 331–397. *in* B. Subramanyam and D. W. Hagstrum, eds. Integrated management of insects in stored products. Marcel Dekker, Inc., New York, USA.

Tompkins, L., S. P. McRobert, and K. Y. Kaneshiro. 1993. Chemical communication in Hawaiian *Drosphila*. Evolution 47:1407–1419.

Tregenza, T., and N. Wedell. 1997. Defenitive evidence for cuticular pheromones in a cricket. Anim. Behav. 54:979–984.

Varzandeh, M., W. N. Bruce, and G. C. Decker. 1954. Resistance to insecticides as a factor influencing the biotic potential of the house fly. J. Econ. Entomol. 47:129–134.

Vassilieva, L. L., A. M. Hook, and M. Lynch. 2000. The fitness effects of spontaneous mutations in *Caenorhabditis elegans*. Evolution 54:1234–1246.

Ward, P. I. 1993. Females influence sperm storage and use in the yellow dung fly *Scatophaga stercoraria* (L.). Behav. Ecol. Sociobiol. 32:313–319.

White, N. D. G., and J. G. Leesch. 1996. Chemical control. Pp. 287–330 *in* B. Subramanyam and D. W. Hagstrum, eds. Integrated management of insects in stored products, Marcel Dekker, New York.

Yuval, B., S. Blay and R. Kaspi. 1996. Sperm transfer and storage in the Mediterranean fruit fly (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 89:486–492.

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