Genetic relationships between development of insecticide resistance and reduction of egg size as a negative effect on the fitness of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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(Received 15 July 2005; Accepted 5 May 2006)

Abstract

Reciprocal crosses between susceptible (SS) and resistant (RR) lines to fenvalerate were conducted to determine whether or not there are any genetic relationships between insecticide resistance and reduction in egg size, as well to whether or not they affect the offspring fitness of the diamondback moth, *Plutella xylostella*. The dose-mortality regression lines of the F₁ hybrid of susceptible females and resistant males (SR) and resistant females and susceptible males (RS) were roughly intermediate between those of parental lines, i.e., SS and RR. This result indicates that the mode of inheritance of fenvalerate resistance was neither complete dominance nor recessive, with no sex-linkage. The egg size of RR lines was significantly smaller than that of the SS lines. The RS and SR lines have an egg size close to that of their own matrilineage. We considered that the mode of inheritance of resistance and that of egg size were different from each other, although the selection experiment showed that there is a negatively correlated response between these parameters. The survival rate of the RR lines was significantly lower than that of the SS lines. However, the survival rate of the RS lines did not differ from that of the SR lines. Reproductive fitness is discussed with regard to different egg sizes among parental lines and reciprocal crosses.

Key words: Selection; reciprocal cross; fenvalerate; incomplete recessive; maternal effect

INTRODUCTION

The development of insecticide resistance is becoming a very serious problem throughout the world. Over 400 species of insects and mites are now resistant to one or more pesticides (Georghiou and Mellon, 1983). The diamondback moth (DBM) *Plutella xylostella* is a major pest of cabbage and other cruciferous crops throughout the world (Talekar and Shelton, 1993). The DBM is also known to develop resistance to various kinds of insecticides (Miyata et al., 1986). For example, the resistance levels of DBM selected using fenvalerate (a synthetic pyrethroid) were estimated to be 4,500-fold (Tsukahara et al., 2003) and 10,000-fold (Sota et al., 1998) of that before selection.

Fujiwara et al. (2002) reported that the DBM females treated with fenvalerate laid more but smaller eggs than control females did. It is generally known that egg size is one of the maternal effects influencing life history traits. Thus individuals with resistance genes to fenvalerate might have small egg size that would decrease their reproductive fitness. However, it is unclear whether or not egg size is genetically correlated with resistant traits, or whether it directly affects reproductive fitness. Chen and Naka-suji (2004) showed that eggs were miniaturized in a resistant line that was selected successively with a sublethal dose of fenvalerate. This result showed that there is a negatively correlated response between the resistance and egg size (Chen and Nakasuji, 2004). In the present study, we used resistant lines of about 10,000-fold resistant ratio and carried out reciprocal cross experiments between the resistant and susceptible lines. To determine the mode of inheritance of resistant development and reduction in egg size, F₁ offspring of the reciprocal crosses were compared with their parental lines for these traits. Several fitness components were also compared between the F₁ offspring of reciprocal crosses as well as the resistant and susceptible lines, to detect ma-
ternal effects on the components including egg sizes.

MATERIALS AND METHODS

Insect stock culture. A strain of DBM susceptible to insecticide was obtained from a laboratory colony maintained at Sumitomo Chemical Co., Ltd., Japan, from 1998. The strain was then maintained at our laboratory in Okayama University from 1998 to the present without the selection of any insecticide.

Approximately 400 adults were placed in a polypropylene cage (15×26×8 cm) containing radish seedlings for egg laying and absorbent cotton with a 10% honey solution for food. Hatched larvae were fed on radish seedlings in the same cage until pupation. Emerged adults were transferred to a cage of the same size. The fourth-instar larvae were then used for the following experiments. Experimental insects were reared at 25°C under 16L8D light conditions, and fed on radish seedlings and 10% honey solution during larvae and adults stages, respectively, in all of the following experiments.

Determination of insecticide susceptibility. A topical application of fenvalerate was employed to determine the insecticide susceptibility of the DBM. Various concentrations of technical-grade fenvalerate (fenvalerate racemic [(RS)-α-cyano-3-phenoxyl-benyl (RS)-2-(4-chlorophenyl)-3-methyl butyrate] at 96.4% purity) were prepared in acetone. The fourth-instar larvae were anesthetized with carbon dioxide, placed on a filter paper and a 0.5 μl droplet of insecticide was applied to the dorsum with an Arnold Hand Microapplicator (Burkard, UK). Ten larvae treated with insecticide were placed in a plastic cup (6.5 cm diameter, 4 cm depth) containing filter paper and reared. Three or four replicates were prepared for each concentration of insecticide. Larvae in the control plot were treated with acetone. After 24 h, the number of dead (including moribund) larvae and surviving larvae were recorded. Data were analyzed using a probit analysis (Abbott, 1925); where necessary, bioassay data were corrected for control mortality.

Selection of resistant line. The procedure for selection is summarized in Fig. 1. Two replications (Replications 1 and 2) of resistant lines (RR lines) of the DBM were established by selection with fenvalerate. To conduct the selection experiment, 200 fourth-instar larvae were tested and the LD50 values were monitored for each generation (“Susceptibility Test” or “Test” in Fig. 1). A LD50-equivalent dose of the insecticide was applied to 400 fourth-instar larvae of the current generation, except for the eleventh, twelfth and thirteenth generations in Replication 1 (“Selection” in Fig. 1). The surviving larvae were reared until adult emergence, and then maintained (RR lines). Susceptible lines (SS lines) were also maintained at a similar population size and density corresponding to each of the RR lines without selection with fenvalerate, but treated with acetone at every generation. The resistant and susceptible lines were selected for 17 (but no selection through 11 to 13) and 10 generations in Replications 1 and 2, respectively.

Comparison of the egg size between resistant
and susceptible lines. The sizes of 100 eggs of each line were measured for every generation using a video micrometer VM-60 (Olympus, Japan) to within 0.01 mm. The major \( (a) \) and minor axes \( (b) \) of the eggs were measured and the volume \( (V) \) was calculated by the formula, \( V = \pi ab^2/12 \), on the assumption that the shape was half-ellipsoidal. Egg size was measured on the first day of oviposition, because the egg size becomes smaller as the female ages irrespective of fenvalerate treatment (Fujiwara et al., 2002).

**Reciprocal cross experiments.** Resistant and susceptible lines were used by reciprocally crossing pairs for the experimental procedure in Fig. 2. There were four combinations in the cross pairs: both female and male were from the resistant lines (RR), a female and male were from the resistant and susceptible lines, respectively (RS), a female and male were from the susceptible and resistant lines, respectively (SR), and both female and male were from the susceptible lines (SS). Thirty pairs were made for each cross. Each pair was introduced into a plastic cup (6.5 cm diameter, 4 cm depth) with food. At least 10 females from each cross actually mated with males and laid eggs. The reciprocal crosses were done in the ninth, tenth and seventeenth generations for Replication 1 and the tenth generation for Replication 2. The eggs and their hatched larvae were used for the experiment of “Comparison of biotic performance” as follows.

**Comparison of biotic performance.** Egg size was measured for 15–20 eggs laid on the first day of oviposition for each of 10 pairs each cross, i.e., RR, SS, RS and SR lines. The eggs of the reciprocal cross lines RS and SR, were actually laid by RR and SS females, respectively. In addition, approximately 100 of the first-instar larvae obtained from each reciprocal cross were individually reared in plastic cups until adult emergence (\( F_1 \) generation). The developmental period, survival rate during the larval and pupal periods, and pupal weight were recorded as biotic performance of the \( F_1 \) generation from the reciprocal crosses. The pupal weight was measured to within 0.01 mg on first day after pupation using a microbalance, BP211D (Sartorius, Germany). The pupae were then introduced into a 20 ml glass vial covered with gauze until emergence. \( F_1 \) males and females that emerged on the same day were paired in a plastic cup and the females were allowed to lay eggs throughout their life-span. The death days of the female and male adults and number of eggs laid by each female were recorded daily. The fertility of the females was the total number of laid eggs through their lifetime. The sizes of 15–20 eggs laid by each female were also measured using the above-described method. The eggs of the RS and SR cross lines, were laid by cross \( F_1 \) RS and SR, respectively. To determine whether the resistant gene(s) negatively affects the above biotic performance fitness components, the RR lines were compared with the SS lines. Furthermore, to determine whether or not maternal effects influence differences, the components of the \( F_1 \) generation from the RS lines were compared with those of the SR lines. We also analyzed differences between these components for RR and RS, and SS and SR, to determine whether or not any sex linkage genes affect them.

**Statistical analysis.** All of the data for the RR and SS lines, and RS and SR lines were analyzed...
with the use of nested ANOVA, in which Replications 1 and 2 (random effect) were nested within a selection or reciprocal cross (fixed effect). When a trait of biotic performance was significantly different among the four lines, it was then analyzed as a pair combination between parental lines and reciprocal lines according to significant effects followed by the multiplied analysis, sequential Bonferroni test (Rice, 1989). These statistical analyses were performed separately for females and males. All of the statistical analyses were done for only the ninth generation in Replication 1 and the tenth generation in Replication 2. This was because the data of the ninth, tenth and seventeenth generations were detected repeatedly from a single resistance population, Replication 1, and thus these data were statistically not independent among each other. We selected the data of the ninth generation since all of the data relating to biotic performance was completely measured in only the ninth generation out of the three generations in Replication 1. All of statistical analyses were using SPSS ver. 11 (SPSS Japan Inc., Japan) except survival rate. Survival rate was analyzed using logistic regression analysis in R 2.0.2.

RESULTS

Changes in susceptibility level
The LD_{50} values of the SS lines were about 0.3×10^{-2} \mu g/larva in both Replications 1 and 2. The LD_{50} value of the RR line gradually rose during the selection with fenvalerate in both replications (Fig. 3). The values were 28.5 \mu g/larva for the ninth generation, 31.1 \mu g/larva for the tenth generation and 24.3 \mu g/larva for the seventeenth generation in Replication 1 and 36.7 \mu g/larva for the tenth generation in Replication 2. Resistance ratios increased up to about 9,500-fold in the ninth generation and, 10,367-fold in the tenth generation and, 8,100-fold in the seventeenth generation in Replication 1, and 12,233-fold in the tenth generation in Replication 2. The ratios were similar to those reported by Sota et al. (1998) and Tsukahara et al. (2003).

Inheritance of resistance to fenvalerate
The dose-mortality regression lines of the SS lines, RR lines and their crosses, and the RS and SR lines are given in Fig. 4. The regression lines of the F_1 hybrids, SR and RS, were roughly intermediate between those of the SS and RR, although the SR lines were close to that of the RR line and the RS lines were close to that of the SS lines in all generations of both replications. Resistance was therefore neither complete recessive or dominant, nor sex-linked inheritance.

Inheritance of egg size of resistance line
The egg size of the RR lines was drastically decreased at the first selected generation and maintained a small size through successive generations compared with that of the SS lines (Fig. 5). The egg sizes were compared among the lines for the parental (Fig. 6) and F_1 generations (Fig. 7) of the ninth generation in Replication 1 and tenth generation in Replication 2. In the parental generation, egg sizes were significantly different among the four lines (F=29.7, p<0.01) (Fig. 6). The egg sizes of the RR lines were significantly smaller...
than those of the SS lines ($F=263.7, p<0.001$). Those of the RS lines were also significantly smaller than those of the SR lines ($F=78.5, p<0.001$). The RR and RS lines had consistently smaller egg sizes than the SR and SS lines did. In the F₁ generation, egg sizes were also significantly different among the four lines ($F=29.0, p<0.01$) (Fig. 7). The egg size of the RR lines was significantly smaller than that of the SS lines ($F=4.81, p<0.01$), and the egg size of the RS lines was significantly smaller than that of the SR lines ($F=66.2, p<0.001$). Furthermore, that of the RR lines was not different from that of the RS lines ($F=10.28, p>0.05$). This is true for the case between SS and SR lines ($F=0.0007, p>0.05$). These results show that adult females emerging from large eggs laid large eggs, while adult females from small eggs laid small eggs. Thus, egg size is a maternally inherited trait in the DBM.

**Comparison of biotic performance of reciprocal crossing**

**Survival rate from hatching to adult emergence**

![Dose-mortality relationships among the RR and SS lines, and reciprocal crosses of RS and SR lines in the ninth (a), tenth (b) and seventeenth (c) generations in Replication 1, and the tenth (d) generation in Replication 2.](image)

![Egg size of females from reciprocal crosses between RR and SS lines in Replications 1 and 2. Error bars indicate SE.](image)

![Egg size of selected (RR) and non-selected (SS) lines in each selected generation in Replications 1 and 2. Error bars indicate SE.](image)
was compared among the lines of the ninth generation in Replication 1 and tenth generation in Replication 2 (Fig. 8). It was significantly different among the four lines (\(z = 1.99, p < 0.05\)). The survival rates of the RR lines were significantly lower than those of the SS lines. The survival rates of the RS lines were not significantly different from those of the SR lines (\(z = -0.12, p > 0.05\)). The remaining fitness components (i.e., adult longevity, development time, pupal weight and fecundity) were not significantly different among the four lines (i.e., the parental lines and the reciprocal crosses in both females and males) (Table 1).

**DISCUSSION**

The selection experiments showed that the induced resistance to fenvalerate in the DBM was correlated with the reduction in egg size. However, the reciprocal crosses suggest that the mode of inheritance of the resistance was different from that of egg size. The typical response to insecticides by the genotypes of reciprocal crosses showed that fenvalerate resistance is neither a completely recessive nor a dominant trait. Furthermore, although it is likely that resistances of reciprocal crosses are closer to the patrilineal lines, it should not be sex linked, since females, not males in the lepidopteran insect group have heterogametic chromosomes including the DBM (Resh and Cardé, 2003). Several authors have suggested that fenvalerate resistance is inherited through partially recessive genes with no sex linkage in DBM (Liu et al., 1981; Noppun et al., 1986; Hama, 1989; Tanaka and Noppun, 1989). Since we have performed neither backcross experiments nor crossing between \(F_1\), we did not determine unequivocally whether resistance to fenvalerate was conferred by a single gene or poly-

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**Fig. 7.** Egg size of \(F_1\) generation female offspring from reciprocal crosses between RR and SS lines in Replications 1 and 2. Error bars indicate SE.

**Fig. 8.** Survival rate during larval and pupal periods of female and male offspring from reciprocal crosses between RR and SS lines in Replications 1 and 2.
gene in the DBM. Consistent with most of the previous genetic studies, however, the resistance to fenvalerate might also be conferred by incompletely recessive and autosomal gene(s) in the DBM.

Comparison between the RR and SS lines showed that the egg size of the RR lines was smaller than that of the SS lines in all selected generations. In the parental generation, the egg size of the RR lines was not different from that of the RS lines. The egg size of the SS lines was not different from that of the SR lines. This is because the mother's genotype in both RS and RR lines was RR. This is true of the case for SR and SS lines in parental generation. It is necessary to compare the size of eggs laid by F1 females from reciprocal crosses for determining whether maternal genotype affects their egg size. Our reciprocal cross experiments showed that egg size laid by F1 females of the RS and SR lines was strongly influenced by matrilineage. This result indicates that maternal effects and/or sex-linked genes strongly influence the egg size of offspring. Cross experiments among strains of Drosophila melanogaster (Warren, 1924) also suggest that at least one autosomal and sex-linked gene affects the variation in egg size. Egg size variation among Choristoneura species (Campbell, 1962) and among breeding lines of the silkworm Bombyx mori (Kawamura, 1990) is also partially sex linked. Azevedo et al. (1997) also detected genetic effects of egg size in D. melanogaster. Egg size is an important pathway for the expression of maternal effects (e.g., egg yolk, with high quality from the large body size of the mother) (Kaplan, 1998). In the present study, since the pupal weight of females was not different between the RS and SR lines, and SS and RR lines, we did not detect maternal physiological effects such as body weight. It is necessary to rigorously examine cross experiments with more complicated design and a large sample size to achieve a segregation of maternal physiological and sex-linked effects in DBM.

Resistant strains of insects are often reported to show disadvantages in life-history characteristics (Roush and Plapp, 1982; Chevillon et al., 1997; Shirley and Sibly, 1999). The present study also showed that the survival rate of the RR line was significantly lower than that of the SS line, while other measured fitness components did not differ
among them. Our results indicate that resistant genes give the resistant insects some fitness costs. Since the RR line has a significantly smaller egg size than the SS line does, it is most likely that the smaller size affects the survival rates of offspring. However the survival rate of the RS lines with smaller egg size were not significantly different from those of SR lines with larger egg size, although the survivability tended to be lower in RS than SR lines in both Replications 1 and 2. Fujiwara et al. (2002) showed that small egg size induced by spraying with insecticide often decreases the survival rate of offspring under harsh environmental conditions (e.g., low humidity and high temperature). Thus it is possible that insects with the genotypes of RR and RS have more remarkable decreases in survivability than those with the genotypes of SS and SR, and are eliminated more quickly under these harsh environmental conditions in the field. This phenomenon might influence the recovery of susceptibility in the absence of insecticide spray in the field. A more detailed understanding of relationships among insecticide resistance, egg size and biotic performance of pest insects should be required for control programs that use of insecticides in the field.

ACKNOWLEDGEMENTS

We thank Professor H. Tsumuki, Dr. T. Miyatake and colleagues at Okayama University for their assistance with this work. Thanks are also due to Sumitomo Chemical Co., Ltd., for providing technical-grade fenvalerate. This work was supported partly by Grants-in-Aid for Scientific Research (Kakenhi) (14360029, 14606001).

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