Resistance development and recovery of susceptibility in relation to egg size change in the diamondback moth, *Plutella xylostella*, treated with fenvalerate

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ABSTRACT

The diamondback moth (DBM) treated with sublethal doses of fenvalerate lays more but smaller eggs (insecticide hormoligosis). The smaller eggs and newly hatched larvae exhibit lower viability (Fujiwara et al. 2002). Thus, resistant strains of DBM that are selected by sublethal doses of fenvalerate may lay smaller eggs and have a reproductive disadvantage. Furthermore, such selective disadvantages may allow recovery of susceptibility in the absence of insecticide sprays. Firstly, we established DBM strains resistant to fenvalerate and examined the effects of sublethal doses on egg size and fecundity. The eggs of the selected strains became significantly smaller than those of the non-selected strains within 2 or 3 generations. The survival rate of the selected strains was significantly lower, and the fecundity of females of the selected strains tended to be higher. Secondly, we carried out reciprocal cross experiments between the resistant (RR) and susceptible (SS) strains to determine inheritance mode of resistance development and reduction of egg size. The mode of inheritance of fenvalerate resistance was incompletely recessive with no sex-link. The RS and SR strains have an egg size close to that of their own matrilineage. The inheritable modes of resistance and egg size were different from each other. The survival rate of the RS strains did not differ from that of the SR strains. Fitness disadvantage of the resistant strains in terms of diminished egg size influenced the recovery rate of susceptibility in the absence of insecticide selection. In particular, the recovery rate was higher under harsh environment conditions, e.g. low humidity and high temperature, than under moderate conditions.

INTRODUCTION

Ecology and the life histories of insects may alter the responsiveness of the selection that leads to insecticide resistance (Georghiou and Taylor 1976). Resistant and susceptible strains frequently differ in fitness components, including development time, fecundity and fertility, in addition to their susceptibility to insecticides (Arnaud et al. 2002, Boivin et al. 2004). In general, resistant strains have a reproductive disadvantage in the absence of insecticides (Roush and Plapp 1982, Argentiné et al. 1989, Li et al. 2002). In this case, frequency of resistant individuals may decrease over time (Roush and McKenzie 1987, Arnaud and Haubrure 2002). However, under particular circumstances, the resistant strains may have a fitness advantage and their frequency may remain stable (Arnaud et al. 2002).

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.

The DBM females treated with fenvalerate laid more (Sota et al. 1998) but smaller eggs (Fujiwara et al. 2002) than control females did, and the small egg size often decreased the survival rate of offspring under harsh environmental conditions of low humidity and high temperature (Fujiwara et al. 2002). The DBM individuals probably experience various harsh environments in the field, e.g. during mid-summer. It is necessary to determine whether the environmental conditions affect the dynamics of resistance traits and egg size to elucidate the mechanism of recovery of susceptibility to insecticides in the field. Firstly, we established resistance lines of DBM by selection with fenvalerate and investigated biotic performances, including egg size. Secondly, we carried out reciprocal cross experiments between the resistant and susceptible lines. To determine mode of inheritance of resistant development and reduction of egg size, F1 offspring of the reciprocal crosses were compared with their parental lines for these traits. Finally, using the resistant lines, we examined the recovery rate of susceptibility to fenvalerate and changes in egg size for some generations after freeing them from insecticide selection under both harsh-rearing and optimal-rearing conditions.

**Diminished Egg size in Fenvalerate Resistant Strains of *P. xylostella***

The detailed methods and original data in this section have been reported elsewhere (Chen and Nakasuji 2004, Chen et al. 2006a).

Two populations (Replication 1 and 2) were selected for ten generations with fenvalerate at a LD90 dose. The LD90 values of the SS lines were about 0.3 × 10⁻² µg/larva. The value of the RR line gradually rose during the selection with fenvalerate in both Replication 1 and 2 (Figure 1). The values were 31.1 µg/larva and 36.7 µg/larva for the 10th generation in Replication 1 and 2, respectively. Resistance ratios increased up to about 10 367-fold and 12 233-fold in the 10th generation in Replication 1 and 2. The ratios were similar to those reported by Sota et al. (1998) and Tsukahara et al. (2003).

The egg size in each generation was compared between the RR and SS lines (Figure 2). The eggs of the RR line became smaller within 2 or 3 generations. Thereafter, the size stabilized at around 1.15 × 10⁻² mm³ in both Replication 1 and 2. The egg size of the SS line fluctuated at around 1.35 × 10⁻² mm³ in both replications. The eggs of the RR line were significantly smaller than those of the SS line in all generations (P <0.001). Chen and Nakasuji (2004) also reported that selection by fenvalerate favors diminished egg size in DBM and the smaller-sized egg is a genetic trait.

![Figure 1 Susceptibility to fenvalerate under selection at LD₉₀ in the RR lines in Replication 1 and 2 (Chen et al. 2006a)](image-url)
It is generally considered that a trade-off between fitness and insecticide resistance may result from pleiotrophic effects of the genes involved in resistance (McKenzie and Battenham 1994, Arnaud et al. 2002). However, in some cases, the strains may differ in fitness for reasons independent of resistance (Roush and McKenzie 1987).

In the present study, RR strain females laid smaller eggs and the survival rate of immature stages hatched from the smaller eggs was lower than that from normal-sized eggs of the SS strain (Chen et al. 2006a). These results suggest that the RR strain has a lower fitness level than the SS strain in the absence of selection pressure.

A similar disadvantage in life history traits has been reported in DBM resistant to Bacillus thuringiensis (Groeters et al. 1994, Shirai et al. 1998). Those studies reported that resistant strains exhibited lower hatchability, a prolonged developmental period, lower survival rate and less fecundity. To examine genetic backgrounds of resistance and egg size change, a crossing experiment was carried out in the next section.

Genetic Relationships Between Development of Insecticide Resistance and Reduction of Egg size as a Negative Effect on the Fitness of P. xylostella

The detailed methods and original data in this section have been reported elsewhere (Chen et al. 2006a)

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 Figure 3. The regression lines of the F1 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 lines and the RS lines were close to that of the SS lines in all generations of both replications. Resistance was therefore neither completely recessive or dominant, nor sex-linked.
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, Tanaka and Noppun 1989). 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.

![Graph showing dose-mortality relationships](image)

**Figure 3** Dose-mortality relationships among the RR and SS lines, and reciprocal crosses of RS and SR lines in the 9th generation in Replication 1, and the 10th generation in Replication 2 (Chen et al. 2006a)

*Inheritance of egg size of resistance line*

The egg sizes were compared among the lines for the parental and F1 generations of the 9th generation and 10th generation (selected data are shown in Figure 4). In the parental generation, egg sizes were significantly different among the four lines (nested ANOVA, \( P < 0.01 \)). The egg sizes of the RR lines were significantly smaller than those of the SS lines (\( P < 0.001 \)). Those of the RS lines were also significantly smaller than those of the SR lines (\( P < 0.001 \)). The RR and RS lines had consistently smaller egg sizes than the SR and SS lines did. Furthermore, egg size of the RR lines was not different from that of the RS lines (\( P > 0.05 \)). 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 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 also 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 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).

![Graph showing egg size comparisons](image)

**Figure 4** Egg size of F1 generation female offspring from reciprocal crosses between RR and SS lines in the 9th generation in Replication 1 and in the 10th generation in Replication 2. Error bars indicate SE (Chen et al. 2006a)

*Comparison of biotic performance of reciprocal crossing*

Survival rate from hatching to adult emergence was compared among the lines of the 9th generation and 10th generation (data not shown). It was significantly different among the four lines (Logistic regression analysis, \( P < 0.05 \)). 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 (\( P > 0.05 \)).

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 (Chen et al. 2006a). 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 rates 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 generation 9 and generation 10.

**Rapid Recovery of Susceptibility Under Harsh Environmental Conditions in Fenvalerate-resistant Strains of P. xylostella**

The detailed methods and original data in this section have been reported elsewhere (Chen et al. 2006b).
Recovery of susceptibility after release selection in Harsh and Optimal lines

Recovery speed of susceptibility in RR lines (Replication 1 and 2, Figure 1) was compared between two different experimental lines (Harsh line and Optimal line), reared under harsh (Replication 1-1 and Replication 2-1) and optimal (Replication 1-2 and Replication 2-2) environmental conditions (Figure 5). The insects of the Harsh line were exposed to low humidity in the egg stage and high temperature in the larval stage in every other (odd) generation. The LD₅₀ values of Harsh lines declined more rapidly than those of Optimal lines through all 10 generations in both Replication lines 1 and 2. Finally, the LD₅₀ value of Harsh lines declined from 31.1 μg per larva and 36.7 μg per larva to 0.03 μg per larva and 0.02 μg per larva, respectively, in Replication 1-1 and 2-1 at the tenth generation (Figure 5). Those of Optimal lines declined to 0.20 μg per larva and 0.29 μg per larva in Replication 1-2 and 2-2.

Some pest insects recover susceptibility to insecticides without insecticide sprays (Georghiou 1963, Strong et al. 1990, Sayed et al. 2000, Lenormand and Raymond 1998, Sawicki, et al. 1980). In the present study, the LD₅₀ values of Harsh lines decreased more rapidly than those of Optimal lines through 10 generations. This result indicates that resistant individuals were eliminated intensively by harsh environmental pressure.

![Graph](image)

**Figure 5  LD₅₀ value of fenvalerate of Harsh and Optimal lines by generation after release from insecticide selection (Chen et al. 2006b)**

Recovery of egg size after selection release in Harsh and Optimal lines.

As stated in the previous section, resistant females laid smaller eggs than those of susceptible females under selection by sub-lethal doses of fenvalerate. After release of fenvalerate selection, the size of eggs laid by females increased gradually through 10 generations in both Harsh and Optimal lines in Replication 1 and 2 (Figure 6). Since the line × generation (P < 0.01), the generation × replication (P < 0.01), the line × replication (P < 0.05) interactions were significant in odd generations, when Harsh lines were under harsh environmental conditions, the egg size was compared for Harsh and Optimal lines at each treatment and generation. In the first generation, egg size was not significantly different between Harsh and Optimal lines in each replication. In the third generation, egg size was significantly larger in Harsh lines than in Optimal lines in both Replication 1 (P < 0.01) and 2 (P < 0.01). In the fifth generation, egg size was significantly larger in Harsh lines than in Optimal lines in Replication 2 (P < 0.01) but not in Replication 1. In the seventh and ninth generations, egg sizes were not different between Harsh and Optimal lines in both
Replications 1 and 2. The egg size was also compared for Harsh and Optimal lines in even generations, when Harsh lines were not reared under harsh environmental conditions. In the second and fourth generations, egg sizes were significantly larger in Harsh lines than in Optimal lines in both Replication 1 and 2.

Comparison of biotic performance in Harsh and Optimal lines

Survival rates and hatchabilities were significantly lower for Harsh lines than for Optimal lines at all odd generations (logistic regression analysis: P < 0.001 in all cases) (Chen et al. 2006b). On the other hand, survivability was significantly higher for Harsh lines than for Optimal lines at all even generations (logistic regression analysis: P < 0.05 in all cases). Egg size of females reared individually in Harsh lines was significantly larger than that of Optimal lines in second (P < 0.01) and fourth generations in Replication 3 (P < 0.01) (Figure 7). Furthermore, egg size of Harsh lines was larger than that of Optimal lines in the sixth generation although its statistical significance was not detected (P = 0.058).

Resistance gene(s) are known to act in antagonistic pleiotropy for fitness traits in insects (McKenzie and Batterham 1994, Arnaud et al. 2002). For instance, resistance genes of Bacillus thuringiensis (Bt) toxins negatively affect over winter survivorship in the southern house mosquito Culex pipiens (Chevillon et al. 1997).

Fitness costs associated with resistant genes in the absence of insecticides are observed for numerous characteristics, including life-history traits (Carrière et al. 1994, Hollingsworth et al. 1997, Alyokhin and Ferro 1999), diapause induction (Carrière et al. 1995), mating ability (Alyokhin and Ferro 1999), and predator avoidance (Rowland 1991). As stated in the previous section, eggs were miniaturized by insecticide selection in DBMs. Furthermore, egg size was a maternal inheritable trait. Reportedly small eggs correlated with low viability of the offspring under harsh environmental conditions such as low humidity and high temperature (Fujisawa et al. 2002). In this study, the line x generation interactions of the egg size were shown to be significant in both odd and even generations. The results showed that the rate in size recovery were different between Harsh and Optimal lines.

![Graph showing egg size of Harsh and Optimal lines by generation after release from insecticide selection (Chen et al. 2006b)](image)

Figure 6 Egg size of Harsh and Optimal lines by generation after release from insecticide selection (Chen et al. 2006b)
**REFERENCES**

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