Inheritance Patterns, Dominance, Stability, and Allelism of Insecticide Resistance and Cross-Resistance in Two Colonies of *Culex quinquefasciatus* (Diptera: Culicidae) Selected With Cry Toxins From *Bacillus thuringiensis* subsp. *israelensis*

MARGARET C. WIRTH,1,2 WILLIAM E. WALTON,1 AND BRIAN A. FEDERICI1,3


**ABSTRACT** Mendelian crosses were used to analyze the patterns of inheritance of Cry-toxin resistance in two colonies of *Culex quinquefasciatus* Say larvae resistant to bacterial toxins produced by *Bacillus thuringiensis* subsp. *israelensis* de Barjac. Resistance levels exceeded 1000-fold at 95% lethal concentration of the Cry11Aa-resistant colony (Cq11A). F1 offspring of reciprocal crosses to a susceptible colony revealed autosomal inheritance and offspring were intermediate in resistance to the susceptible and resistant parent strains. Dose-response tests on backcross offspring were consistent with polyfactorial inheritance of resistance toward Cry11Aa and Cry4Aa + Cry4Ba, whereas cross-resistance toward Cry11Ba best fit a monofactorial model. Resistance was 600-fold at 95% lethal concentration in the colony selected with Cry4A + Cry4B (Cq4AB). Inheritance of resistance in F1 offspring was autosomal and intermediate to the susceptible and resistant parents. Inheritance of Cry4Aa + Cry4Ba and Cry11Ba resistance best fit a polyfactorial model in offspring of the Cq4AB backcross, whereas Cry11Aa-resistance inheritance fit a monofactorial model. Dominance values were calculated at different Cry-toxin concentrations for F1 offspring of both resistant colonies; dominance generally decreased as treatment concentration increased. Resistance and cross-resistance remained stable in Cq11A and Cq4AB in the absence of insecticide pressure. Allelic complementation tests were complementary and suggested that Cq11A and Cq4AB evolved resistance to Cry toxins at common loci. The patterns of cross-resistance suggest cross-recognition of binding moieties by Cry11Aa, Cry4Aa + Cry4Ba, and Cry11Ba in these *Culex*, which may be partly responsible for the toxin synergy characteristic of *B. thuringiensis* subsp. *israelensis* de Barjac.

**KEY WORDS** *Bacillus thuringiensis*, Cry toxins, genetics, mosquitoes, resistance

The bacterium *Bacillus thuringiensis* Berliner is characterized by the production of crystalline proteinaceous inclusions during sporulation (Whiteley and Schnepf 1986). In some strains of *B. thuringiensis*, the inclusion proteins are toxic when eaten by sensitive insect species, and this trait has been exploited to produce insecticides to control pests of agricultural and medical importance (van Frankenhuyzen 1993). Strains such as *B. thuringiensis* subsp. *kurstaki* that are active against lepidopterans are used as insecticidal sprays on crops, and several of the insecticidal proteins of these have been genetically engineered into various crop plants to target susceptible pest species (Whalon and Wingerd 2003). *B. thuringiensis* subsp. *israelensis* de Barjac is primarily active against mosquitoes and black flies and is used to reduce larval population densities and disrupt the spread of various diseases, including West Nile virus, dengue fever, and malaria in human populations (Porter et al. 1993).

Insecticide resistance is considered a significant barrier to the long-term success of *B. thuringiensis*-based insect control strategies. Resistance to *B. thuringiensis* has evolved in field populations of *Plutella xylostella* (L.) in response to foliar sprays (Tabashnik et al. 1990, Shelton et al. 1993) and in greenhouse populations of *Trichoplusia ni* (Hubner) (Janmaat and Meyers 2003). Furthermore, other important insect pests have demonstrated the capacity to evolve resistance to *B. thuringiensis* under laboratory selection pressure (for a review, see Tabashnik 1994). In contrast, *B. thuringiensis* subsp. *israelensis* has been successfully used to control mosquito and black fly larvae for >20 yr, with no evidence of field control failure (Becker and Ludwig 1993, Becker 1997). Unlike the *B. thuringiensis* strains active against lepidopteran pests, *B. thuringiensis* subsp. *israelensis* synthesizes a diverse spectrum of four major toxins, Cry4Aa, Cry4Ba, Cry11Aa, and Cyt1Aa, which interact synergistically with each other, and specifically with the latter cyto-
lytic toxin, to greatly enhance toxicity (Crickmore et al.
1995, Poncet et al. 1995) and suppress insecticide
resistance (Wirth et al. 1997). Despite this advantage,
laboratory studies with Culex quinquefasciatus Say
have shown that mosquitoes can evolve high levels of
resistance to the various component toxins of B. thur-
ingiensis subsp. israelensis in the absence of Cyt1Aa
(Georghiou and Wirth 1997). Consequently, it would
be shortsighted to assume that resistance will never
occur.
Understanding the genetic basis of resistance to B.
thurigniensis is key to developing predictive models
and resistance-monitoring strategies, as well as man-
aging resistance. On a fundamental level, knowledge
of the mode of inheritance of B. thurigniensis resis-
tance can provide tools to elucidate the mode of action
of B. thurigniensis toxins and facilitate the design of
engineered bacterial strains with enhanced host range
and refractoriness to resistance. Genetic studies of B.
thurigniensis resistance have been undertaken for ma-
jor agricultural pest species, but no information is
available for mosquitoes. In this study, we examined
the inheritance of resistance and cross-resistance to
Cry toxins from B. thurigniensis subsp. israelensis in
two laboratory-selected colonies of C. quinquefascia-
tus. We used Mendelian crosses to evaluate maternal
effect, sex linkage, and dominance. Backcrosses were
used to estimate the number of loci involved in resis-
tance and cross-resistance. The stability of resistance
in the absence of selection was studied. Finally, allelic
complementation tests examined whether the two re-
sistant lines shared loci for resistance and cross-resis-
tance.

Materials and Methods
Mosquito Colonies. Three laboratory colonies of C.
quintquefasciatus were used for these studies. The
Cq11A- and Cq4AB-resistant colonies were each es-
abled in 1990 and derived from a large synthetic
population formed by pooling multiple field collec-
tions. Both colonies have been maintained under se-
lection pressure with Cry11Aa or Cry4Aa + Cry4Ba,
respectively, since that time (Georghiou and Wirth
1997). Resistance levels to Cry11Aa in Cq11A reached
>913-fold by generation 28 and exceeded 7,000-fold 1
yr later (Wirth et al. 1998). Cry4AB resistance reached
122-fold in generation 28 (Georghiou and Wirth 1997)
and 290-fold after an additional year of selection
(Wirth et al. 1998). Subsequent selection has in-
creased and stabilized the levels of resistance. Colony
CqSyn was a synthetic population established from
a large synthetic population formed by pooling multiple Þeld collec-
tions and bioassay tests on CqSyn and the resistant colonies using
the same bacterial stocks and dilutions, and were cal-
culated by dividing the 50% lethal concentration (LC50)
or the 95% lethal concentration (LC95) of the selected colony by the LC50
or LC95 of CqSyn. Dose-
response values with overlapping Þducial limits were not
considered signiÞcantly different.
The two resistant colonies were reared under weekly insecticide pressure with either Cry11Aa or
Cry4Aa + Cry4Ba (Georghiou and Wirth 1997).
Groups of 1,000 early fourth instars were placed in
enamel metal pans containing 1 liter of deionized
water and exposed to their respective recombinant
bacterial powder suspension for 24 h. Survivors are
removed to clean water, fed, and used to continue the
colony. Initially, selection pressure ensured 70–90%
mortality, and generations were maintained sepa-
ately. To avoid population bottlenecks, 500–1,000
adults were used to establish any single generation.
However, generations have been allowed to overlap
recently. Resistance has reached very high levels and
has been very stable; therefore, resistance has been
maintained in Cq11A and Cq4AB using a selection
concentration of 40 μg/ml of either Cry11Aa or
Cry4Aa + Cry4Ba powders. This concentration is 1,000-
fold higher than the original selection concentration
and generally kills <10% of the larvae.
Genetic Crosses. Reciprocal mass crosses were car-
rried out between CqSyn and the respective resistant
colonies (Cq11A or Cq4AB). Virgin males and females
were obtained by isolating pupae in scintillation vials.
A minimum of 300 males and 300 females was used for
each mass cross. The following crosses and back-
crosses were made, with the female parent listed Þrst:
1) CqSyn × Cq11A; 2) Cq11A × CqSyn; 3) CqSyn ×
Cq4AB; 4) Cq4AB × CqSyn; 5) [Cq11A × CqSyn]
Table 1. Dose-response values and resistance ratios for Cry11Aa, Cry11Ba, or Cry4Aa + Cry4Ba toward parental and F1 offspring of reciprocal crosses between Cq11A and CqSyn

<table>
<thead>
<tr>
<th>Toxin(s)</th>
<th>Colony</th>
<th>N</th>
<th>LC50 (fidelucial limit) μg/ml</th>
<th>LC95 (fidelucial limit) μg/ml</th>
<th>Resistance ratio</th>
<th>χ²</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cry11Aa</td>
<td>CqSyn</td>
<td>1,100</td>
<td>1.11 (0.442-2.78)</td>
<td>84.6 (15.0-481)</td>
<td>1.0</td>
<td>1.0</td>
<td>92.3</td>
</tr>
<tr>
<td></td>
<td>Cq11A</td>
<td>900</td>
<td>493 (251-1.394)</td>
<td>240.000 (39.493-443.000)</td>
<td>444</td>
<td>2.536</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>S × R</td>
<td>1,200</td>
<td>84.9 (25.2-305)</td>
<td>70.388 (805-›×10⁵)</td>
<td>76.4</td>
<td>832</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>R × S</td>
<td>1,100</td>
<td>139 (30.4-680)</td>
<td>79.023 (536-›×10⁵)</td>
<td>125</td>
<td>934</td>
<td>25.9</td>
</tr>
<tr>
<td>Cry11Ba</td>
<td>CqSyn</td>
<td>700</td>
<td>0.0390 (0.0341-0.0444)</td>
<td>0.216 (0.173-0.282)</td>
<td>1.0</td>
<td>1.0</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>Cq11A</td>
<td>1,200</td>
<td>0.177 (0.106-0.297)</td>
<td>4.77 (1.86-12.4)</td>
<td>4.5</td>
<td>22.0</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>S × R</td>
<td>900</td>
<td>0.232 (0.169-0.318)</td>
<td>2.57 (1.46-4.71)</td>
<td>5.9</td>
<td>11.9</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>R × S</td>
<td>800</td>
<td>0.184 (0.156-0.217)</td>
<td>2.13 (1.61-3.02)</td>
<td>4.7</td>
<td>9.9</td>
<td>9.2</td>
</tr>
<tr>
<td>Cry4Aa + Cry4Ba</td>
<td>CqSyn</td>
<td>900</td>
<td>0.199 (0.115-0.344)</td>
<td>2.80 (1.01-7.83)</td>
<td>1.0</td>
<td>1.0</td>
<td>49.5</td>
</tr>
<tr>
<td></td>
<td>Cq11A</td>
<td>1,000</td>
<td>6.98 (3.77-12.9)</td>
<td>731 (158-3.324)</td>
<td>35</td>
<td>261</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>S × R</td>
<td>1,000</td>
<td>2.31 (1.95-2.74)</td>
<td>37.1 (27.6-52.9)</td>
<td>11.6</td>
<td>13.2</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>R × S</td>
<td>900</td>
<td>2.41 (1.41-4.1)</td>
<td>74.0 (25.1-223)</td>
<td>12.1</td>
<td>26.4</td>
<td>32.9</td>
</tr>
</tbody>
</table>

F1 × CqSyn; 6) Cq4AB × CqSyn; 7) Cq11A × Cq4AB; and 8) Cq4AB × Cq11A.

The standard backcross method was used to estimate the number of alleles involved in resistance. We accepted the assumption that all parental lines are homogeneous for resistance or susceptibility based on several lines of evidence. First, the fiducial limits of the dose-response lines to Cry11Aa, Cry4Aa, and Cry11Ba were not overlapping. Second, the fiducial limits to the three toxin powders in the susceptible colony and any of the F1 offspring of the reciprocal crosses did not overlap. Third, resistance was stable to the three different toxin powders in the absence of the insecticide for 18 generations. These data are consistent with a high proportion of homozygosity in both Cq11A and Cq4AB. Because the determination of monofactorial or polyfactorial inheritance must be inferred from dose-response lines on backcross offspring, the technique is prone to error. However, we increased the number of replicates in the bioassays from 5 to 10 or more for each cross and included 10 or more test concentrations to improve the estimation of the slope of the dose-response line. The proportion of survivors of exposure to the concentrations 0.2, 2, 20, and 200 μg/ml for each insecticide was used to calculate the change in frequency of resistant genotypes in the population. The proportion of larvae surviving exposure to insecticide in the absence of selection pressure, and susceptibility was evaluated in generations 1, 2, 3, and 10.

The proportion of survivors of exposure to the concentrations 0.2, 2, 20, and 200 μg/ml for each insecticide was used to calculate the change in frequency of resistant genotypes in the populations. The proportion of larvae surviving exposure to insecticide in the absence of selection pressure, and susceptibility was evaluated in generations 1, 2, 3, and 10.

The Cry11A-selected colony had LC50 and LC95 values of 493 and 3,382 for Cry11Aa, respectively (Table 1). LC50 and LC95 values were significantly different from those of the susceptible colony, CqSyn, because of the lack of overlap in their fiducial limits. The F1 offspring from reciprocal crosses between Cq11A and
Cq4AB showed LC50 and LC95 values generally intermediate to their resistant and susceptible parents, and these values were also significantly different from those of CqSyn. RR50 of the F1 offspring were 76.4 and 934, whereas RR95 were 832 and 934. The lethal concentration values (LC50, LC95) for the F1 offspring of the reciprocal crosses were not significantly different from each other.

Using Cry11Ba toxin powder, the Cq11A colony showed low, but significant resistance ratios of 4.5 and 22 at the LC50 and LC95, respectively (Table 1). Lethal concentration values for Cq11A and the F1 offspring of the reciprocal crosses were significantly different from those for CqSyn. The LC values of F1 offspring were more similar to the Cq11A parent at test concentrations below the LC50, but were intermediate to the respective parental lines at the higher treatment concentrations. RR50 were 5.9 and 4.7, and RR95 were 31.3 and 18.6. Cq4AB and the F1 offspring were not significantly different from each other.

Cross-resistance levels to Cry11Aa in Cq4AB were lower than the resistance levels observed with colony Cq11A, with resistance ratios of 156 and 71 at the LC50 and LC95. The F1 offspring of the reciprocal crosses showed low, but significant cross-resistance to Cry11Aa, and resistance ratios at the LC50 were 7.2 and 7.6, and at the LC95 were 2.9 and 6.3. Both Cq4AB and the F1 offspring of the reciprocal crosses were significantly different from CqSyn in susceptibility to Cry11Aa. The F1 offspring of the reciprocal crosses were not significantly different from each other.

Cross-resistance ratios for Cry4AB toward Cry11Ba were 34.4 and 321 at the LC50 and LC95, and were considerably higher than observed for Cq11A. F1 offspring of reciprocal crosses were intermediate in resistance to their respective parents with resistance ratios of 6.2 and 7.1 at the LC50, and 36 and 45 at the LC95. Both Cq4AB and the offspring of the reciprocal crosses were significantly different from CqSyn in susceptibility to Cry11Ba. The F1 offspring of the reciprocal crosses were not significantly different from each other.

The dose-response data for offspring of the (Cq4AB × CqSyn) F1 × CqSyn backcross with Cry4Aa + Cry4Ba (χ² = 28.6, df = 11, P < 0.05) (Fig. 1A) and Cq11Ba powders (χ² = 47.5, df = 11, P < 0.05) (data not shown) were not consistent with the monofactorial model because of significant deviations at multiple treatment concentrations. However, the (Cq4AB × CqSyn) F1 × Cq4AB backcross offspring fit the monofactorial model when tested with Cq11Ba (χ² = 5.5, df = 6, P > 0.05) (Fig. 1B).

Dominance estimates at various treatment concentrations showed that resistance to Cry11Ba toxin in Cq11A ranged from semidominant to dominant, depending on the cross and the treatment concentration (Table 3). For example, h values for the
CqSyn × Cq11A cross were one (complete dominance) at 2.0 μg/ml, 0.70 (semidominant) at 20 μg/ml, and 0.89 (semidominant) at 200 μg/ml. Cross-resistance to Cry4Aa + Cry4Ba and to Cry11Ba ranged from dominant \( h = 1 \) to recessive \( h = 0 \), and dominance declined as the treatment concentration increased.

Colony Cq4AB showed patterns of dominance different than those observed in colony Cq11A. Cross-resistance to Cry11Aa ranged from dominant to semirecessive, and declined as treatment concentration increased. Resistance to Cry4Aa + Cry4Ba and cross-resistance to Cry11Ba ranged from semidominant to semirecessive, and also declined with increasing treatment concentration.

In the absence of insecticide exposure, resistance remained stable in both selected colonies, particularly at the lower treatment concentrations. R values were calculated at 0.2, 2, 20, and 200 μg/ml for each colony/insecticide combination (Table 4). R values for colony Cq11A were generally positive for Cry11Aa and <0.01. R values for Cq4AB were consistently negative, but less than −0.01, suggesting a very minor decline in resistance. Resistance was stable toward Cry4a + Cry4Ba in both colonies.

Offspring of the reciprocal crosses between Cq11A and Cq4AB were tested with the various toxin powders, and resistance levels were determined (Table 5). The offspring of the hybrid crosses were not significantly different from each other in their susceptibility, indicating autosomal inheritance with no maternal effects. Resistance and cross-resistance alleles were strongly complementary because the offspring of both crosses were as resistant, or more resistant, than their homozygous resistant parents. This was particularly notable at the LC95, in which resistance ratios were an order of magnitude higher than those of either resistant parent. In the absence of selection for 13 gener-

![Fig. 1. Results of dose-response testing with B. thuringiensis israelensis toxins (A) Cry4Aa + Cry4Ba and (B) Cry11Aa against the parental (Cq4AB and CqSyn), F1, and backcross generations of colony Cq4AB. The dashed line is the line expected under a monofactorial model.](image-url)

The bioassay data with the various Cry proteins against offspring from the backcross experiments showed interesting similarities and differences between the phenotypic expression of resistance in the two colonies. Inheritance of Cry11Aa resistance was polyfacial in Cq11A, whereas inheritance better fit a monofacial model for colony Cq4AB. Cry4Aa + Cry4Ba resistance was a polyfacial trait in both colonies. Cry11Ba cross-resistance inheritance patterns were consistent with the monofacial model for Cq4AB, but better fit a polyfacial model for Cq4AB. These differences could result from experimental error as a result of the inherent problems with the classical Mendelian approach. However, the robustness of the data sets for all backcross offspring suggests these data reflect the distinct evolutionary path of each colony in response to selection pressure with different Cry toxins.

The phenotypic expression of dominance of resistance and cross-resistance also differed between the two selected colonies and was dependent on the treatment concentration and the specific protein(s) tested. Despite these differences, dominance generally decreased as the toxin concentration increased. Dominance of *B. thuringiensis* resistance has been reported to vary with treatment concentration, decreasing in response to increasing treatment concentration, in a number of insect species, including the following: *L. decemlineata* (Rahardja and Whalon 1995), *P. xylostella* (Liu and Tabashnik 1997, Sayyed et al. 2000, Liu et al. 2001), *P. gossypiella* (Tabashnik et al. 2002), and *O. nubilalis* (Alves et al. 2006). However, other expressions of dominance, such as incomplete dominance (Sims and Stone 1991, Huang et al. 1999), incomplete recessive (Hama et al. 1992, Kaín et al. 2004), and fully recessive inheritance (Augustin et al. 2004, Tabashnik et al. 1992, Mahon et al. 2007) have been reported.

Although both resistant colonies followed the same general relationship between dominance and treatment concentration, distinctly different levels of dominance and overall resistance levels were characteristic. For example, Cry11Aa resistance in Cq11A F1 offspring was codominant to partly dominant. Cross-resistance to Cry11Aa in colony Cq4AB F1 offspring was partly recessive to partly dominant, and resistance ratios were an order of magnitude lower than observed in Cq11A. Differences were also observed in dominance for the F1 offspring using Cry4Aa + Cry4Ba; dominance was generally lower in Cq11A F1 offspring than Cq4AB F1 offspring, although the levels of resistance attained after selection were very similar.

### Table 3. Estimation of dominance based on treatment concentration of *B. thuringiensis* toxins for F1 offspring from reciprocal crosses between Cq11A, Cq4AB, and SynP

<table>
<thead>
<tr>
<th>Toxin(s)</th>
<th>Concentration µg/ml</th>
<th>h value (crosses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cry11Aa</td>
<td>0.2</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.28</td>
</tr>
<tr>
<td>Cry4Aa +</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>0.2</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.29</td>
</tr>
<tr>
<td>Cry11Ba</td>
<td>0.02</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The h value indicates the degree of dominance of a phenotypic trait. An h value of 0 indicates fully recessive inheritance; an h value of 1 indicates a fully dominant trait; and an h value of 0.5 indicates a codominant trait. When 0 < h < 0.5, the trait is partially recessive, whereas when 0.5 < h < 1, the trait is partially dominant.

### Discussion

Larvae of two colonies of *C. quinquefasciatus* reared in the laboratory under selection pressure since 1990 with the insecticidal toxins Cry11Aa or Cry4Aa + Cry4Ba evolved high levels of insecticide resistance and cross-resistance to a variety of mosquitocidal toxins, including Cry11Ba, to which they had not been exposed previously. F1 offspring of reciprocal crosses in both colonies showed dose-response lines consistent with autosomal inheritance of the resistance alleles, and no maternal effects. These results are in agreement with previous reports of autosomal inheritance of resistance to *B. thuringiensis* in other insect species, including *Plodia interpunctella* (Hübner) (McGaughey 1985), *Plutella xylostella* (L.) (Tabashnik et al. 1992), *Heliothis virescens* (F.) (Gould et al. 1995), *Leptinotarsa decemlineata* (Say) (Rahardja and Whalon 1995), *Pectinophora gossypiella* (Saunders) (Tabashnik et al. 2002), *Ostrinia nubilalis* (Hübner) (Alves et al. 2006), and *Helicoverpa armigera* (Hübner) (Mahon et al. 2007).

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### Table 4. Stability of resistance in the absence of selection pressure in colonies Cq11A and Cq4AB

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Generation</th>
<th>Concentration µg/ml</th>
<th>Colony (% mortality)</th>
<th>R value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cq11A × CqSyn</td>
<td>Cq11A × Cq11A</td>
<td>Cq4AB × Cq11A</td>
</tr>
<tr>
<td>Cry11Aa</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4</td>
<td>200</td>
<td>4</td>
</tr>
<tr>
<td>Cry4Aa +</td>
<td>2</td>
<td>18</td>
<td>6</td>
<td>0.0005</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>20</td>
<td>38</td>
<td>35</td>
<td>-0.0005</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>27</td>
<td>41</td>
<td>0.0000</td>
</tr>
<tr>
<td>Cry11Ba</td>
<td>0.2</td>
<td>3</td>
<td>9</td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10</td>
<td>21</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>54</td>
<td>41</td>
<td>0.0112</td>
</tr>
<tr>
<td>Cry4Aa +</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>0.0027</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>75</td>
<td>63</td>
<td>0.0100</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>72</td>
<td>64</td>
<td>0.0150</td>
</tr>
</tbody>
</table>

R value measures the rate of change in the absence of insecticide exposure. A negative R value indicates a decline in the proportion of individuals surviving exposure to insecticide. R values close to 0 indicate no change in the proportion of individuals surviving insecticide exposure.
in the two parental colonies. These differences could result from multiple causes, including the evolution of resistance at independent loci, allelic differences between the two colonies, or from differential expression of the same alleles (Strickberger 1976).

Resistance remained stable in both Cq11A and Cq4AB in the absence of exposure to insecticides. Overall, R values were very close to 0 and positive, indicating no decline in resistance toward Cry11Aa or Cry4Aa + Cry4Ba. Only extremely small negative R values were observed for Cq4AB using Cq11A, suggesting a very slight decline in resistance level. All the R values, including the latter values, are much lower than those reported for several lepidopteran species (Tabashnik 1994). Of those studied, P. interpunctella had the lowest reported rate of instability with an R value of −0.02. Our R values were an order of magnitude smaller. In most cases, instability of resistance was attributed to fitness costs linked to the resistance alleles (Tabashnik 1994). However, the more likely explanation for stability in our colonies is alleles for resistance came close to fixation at the various loci in response to prolonged selection pressure at the various loci in two parental colonies. In the absence of selection pressure toward Cry11Aa and to Cry11Ba, the backcross data indicated the two colonies differed in the number of loci involved in resistance to Cry11Aa and to Cry11Ba. In fact, in the absence of selection pressure, the backcross data suggested that those alleles may be of more importance to the phenotypic expression of resistance.

### Table 5. Dose-response assay values for Cry4Aa + Cry4Ba, Cry11Aa, or Cry11Ba against offspring of reciprocal crosses between Cq11A and Cq4AB

<table>
<thead>
<tr>
<th>Toxin(s)</th>
<th>Cross</th>
<th>N</th>
<th>LC₅₀ (μg/ml)</th>
<th>LC₉₅ (μg/ml)</th>
<th>Resistance ratio</th>
<th>χ²</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cry4Ba</td>
<td>Cq11A</td>
<td>1,000</td>
<td>0.016 (0.106–0.297)</td>
<td>0.016 (0.106–0.297)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>Cq4AB</td>
<td>1,100</td>
<td>0.15 (0.12–0.18)</td>
<td>0.15 (0.12–0.18)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>Cq11A×Cq4AB</td>
<td>1,000</td>
<td>0.15 (0.12–0.18)</td>
<td>0.15 (0.12–0.18)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>Cq4AB×Cq11A</td>
<td>800</td>
<td>0.25 (0.20–0.30)</td>
<td>0.25 (0.20–0.30)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>Cq11A×Cq4AB</td>
<td>800</td>
<td>0.25 (0.20–0.30)</td>
<td>0.25 (0.20–0.30)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>Cq4AB×Cq11A</td>
<td>800</td>
<td>0.25 (0.20–0.30)</td>
<td>0.25 (0.20–0.30)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>Cq11A×Cq4AB</td>
<td>800</td>
<td>0.25 (0.20–0.30)</td>
<td>0.25 (0.20–0.30)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cry4Ba</td>
<td>Cq4AB×Cq11A</td>
<td>800</td>
<td>0.25 (0.20–0.30)</td>
<td>0.25 (0.20–0.30)</td>
<td>0.0001</td>
<td>1.0</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

R value measures the rate of change in the absence of insecticide exposure. A negative R value indicates a decline in the proportion of individuals surviving exposure to insecticide. R values close to 0 indicate no change in the proportion of individuals surviving insecticide exposure.
An alternative explanation for the high levels of resistance observed in the crosses between Cq11Aa and Cq4AB is overdominance or heterosis. Crossbreeding highly inbred strains can produce more vigorous hybrid offspring resulting from the increase in fitness associated with the dramatic reduction in homozygosity at multiple loci (Strickberger 1976). Because no increased fitness was noted in the hybrid offspring from crosses with the susceptible strain, this explanation seems less likely.

The cross-resistance that evolved between the colonies selected with Cry11Aa or Cry4Aa + Cry4Ba indicates that alleles selected by Cry4Aa + Cry4Ba confer resistance to Cry11Aa, whereas alleles selected by Cry11Aa confer resistance to Cry4Aa + Cry4Ba. Similarly, both colonies evolved cross-resistance to Cry11Ba in response to selection with Cry11Aa or Cry4Aa + Cry4Ba, despite the absence of prior exposure. Some similarity in evolutionary pathway for resistance would not be completely unexpected because the two selected colonies were derived from the same, albeit large, heterogeneous synthetic population (Georghiou and Wirth 1997). However, the extent of similarities suggests that the number of resistance alleles in the original source population was relatively constrained, because similar alleles at the same loci arose independently under two different selection regimes (Tabashnik et al. 1998). If these alleles represent variations in the binding affinities for these Cry proteins, as reported for many insects resistant to B. thuringiensis, then it is likely that Cry11Aa, Cry4Aa + Cry4Ba, and Cry11Ba share a binding site (or sites), or there is cross-recognition of the different binding sites by these toxins. Binding assays with B. thuringiensis subsp. israelensis Cry proteins using brush border membrane vesicles from Aedes aegypti L. suggested that Cry11Aa, Cry4Aa, and Cry4Ba toxins may share a common class of binding sites (de Barros Moreira Beltrão and Silva-Filha 2007). Shared receptors have also been reported for Cry1A-resistant P. xylostella, which has a single receptor that binds Cry1Aa, Cry1Ab, and Cry1Ac and Cry1 F toxins (Ballester et al. 1999, Granero et al. 1996). Binding studies are needed to determine whether this hypothesis is correct.

Cross-recognition of binding sites between Cry11Aa and Cry4Aa + Cry4Ba might also explain the synergy of these three Cry toxin components of B. thuringiensis subsp. israelensis. Earlier research clearly demonstrated that the activity of various combinations of Cry proteins from B. thuringiensis subsp. israelensis was much greater than expected than if presented singly (Crickmore et al. 1995, Ponce et al. 1995). Cooperative receptor binding and/or the formation of hybrid pores were proposed as a possible explanation for these results (Ponce et al. 1995). Although there are now several studies suggesting this possibility for B. thuringiensis subsp. israelensis, more research is needed before this possible mechanism can be accepted.

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