Inheritance, Stability, and Dominance of Cry Resistance in *Culex quinquefasciatus* (Diptera: Culicidae) Selected With the Three Cry Toxins of *Bacillus thuringiensis* subsp. *israelensis*

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ABSTRACT Mendelian crosses were used to study the mode of inheritance of Cry toxin resistance in a *Culex quinquefasciatus* Say (Diptera: Culicidae) colony (CqAB11A) that evolved insecticide resistance under laboratory selection with a deletion mutant of *Bacillus thuringiensis* subsp. *israelensis* de Barjac lacking the Cyt1Aa toxin component but containing its three major Cry toxins, Cry4Aa, Cry4Ba, and Cry11Aa. High levels of resistance were observed to Cry toxins. F1 offspring of reciprocal crosses to a sensitive colony showed intermediate levels of resistance with no maternal effect, indicating autosomal inheritance. Dose–response data for backcross offspring deviated significantly from the monofactorial model when tested with Cry4Aa + Cry4Ba + Cry11Aa, Cry4Aa + Cry4Ba, or Cry11Aa. However, tests with Cry11Ba from *B. thuringiensis* subsp. *jegathesan* (Seleena, Lee, Lecadet) fit the monofactorial model. Dominance of F1 offspring was calculated at different concentrations of Cry-toxin suspensions and, as reported for other Cry-resistant *Culex*, generally decreased as concentration increased. A subset of colony CqAB11A was reared without selection pressure for 18 generations with little change in susceptibility, indicating a highly homozygous population. Consistent with reports for other Cry-resistant *Culex*, the data show these mosquitoes evolved resistance to *B. thuringiensis* Cry toxins at multiple loci in response to selection pressure and that cross-resistance to Cry11Ba was conferred by one of those loci.

KEY WORDS genetics, resistance, Cry toxin, dominance, mosquito

The bacterium *Bacillus thuringiensis* (Bt) is distinguished by the production of crystalline proteins at sporulation, many of which are toxic to insects (Lacey 2007). Depending on the strain of *B. thuringiensis* and the specific endotoxins it produces, different insecticidal effects are observed. For example, Cry4Aa, Cry4Ba, and Cry11Aa from *B. thuringiensis* subsp. *israelensis* de Barjac are toxic to nematoceran dipterans, including many mosquito and blackfly vectors of important human diseases, whereas Cry1 and Cry3 endotoxins are active against lepidopterans and coleopterans, respectively (Federici et al. 2010). When B. *thuringiensis* cells lyse at sporulation, a crystallized endotoxin is released into the environment where it can be ingested by susceptible insect larvae in their natural feeding process. After ingestion, the crystal endotoxin dissolves inside the insect midgut environment and releases the insecticidal proteins, which are activated by natural proteases. The activated toxins bind to receptors on the brush-border membrane of the insect midgut epithelium, destroying the epithelium and killing the larvae by destroying this tissue through the formation of cationic pores that result in cell death (Bravo et al. 2007).

Although widespread use against lepidopteran pests of the closely related bacterium *Bacillus thuringiensis* subsp. *kurstaki* (de Barjac and Lemille) has resulted in the evolution of insecticide resistance in several field populations of *Plutella xylostella* (L.) (Tabashnik et al. 1990, Shelton et al. 1993) and a few greenhouse populations of *Trichoplusia ni* (Hübner) (Janmaat and Meyers 2003), larvicides based on *B. t.* subsp. *israelensis* have been successfully used for >20 yr with no evidence of field control failure in mosquito populations (Becker and Ludwig 1993, Becker 1997). This failure to evolve insecticide resistance is in sharp contrast to the widespread resistance that evolved in many mosquito populations in response to conventional insecticide treatments. The lack of resistance to *B. t.* subsp. *israelensis* is attributed to the diversity and mechanisms of action of the toxins produced by this species, which includes the three major Cry toxins noted above, and Cyt1A, a cytolytic toxin, all of which are packaged in a single, large parasporal body (Ibarra and Federici 1986). A unique characteristic of *B. t.* subsp. *israelensis* is its relatively high toxicity, on the order of 10 ng/ml against fourth instars of various mosquito species; activity that is higher than would be

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expected from the activities of the individual toxic components. Numerous studies have shown that the various toxins interact with one another synergistically (Crickmore et al. 1995, Poncet et al. 1995) and that synergy is responsible for masking the evolution of resistance in *B. t.* subsp. *israelensis*–selected mosquitoes (Wirth et al. 1997). The Cyt1Aa component plays a critical role in both synergy and in resistance suppression (Wirth et al. 1997). For example, studies have demonstrated that mosquitoes selected with *B. t.* subsp. *israelensis* Cry toxins in the absence of the Cyt1Aa toxin can evolve high levels of resistance (Georghiou and Wirth 1997, Wirth et al. 2004), but those high levels of resistance can be overcome by combining Cyt1Aa with the Cry toxins (Wirth et al. 1997). More importantly, resistance evolves more slowly and to much lower levels in mosquitoes exposed to a single Cry toxin presented in combination with Cyt1A (Wirth et al. 2005).

Although we currently have a basic understanding of how the *B. t.* subsp. *israelensis* toxin characteristics influence the evolution of Cry resistance in mosquitoes, little is known of the genetics underlying this resistance. Data on the genetic basis of insecticide resistance is important to our general understanding of an insect population's response to selection pressure, and this information also can lead to the development of tactics for avoiding or managing insecticide resistance. For example, the data generated from laboratory studies on the genetics of *B. thuringiensis* subsp. *kurstaki*–resistant crop pests were used in the development of the Environmental Protection Agency–mandated refuge strategies implemented for genetically engineered Bt crops (Glaser and Matten 2003). Furthermore, this information is invaluable for designing recombinant bacterial insecticides that are refractory to driving resistance evolution in treated populations. Although field resistance to *B. t.* subsp. *israelensis* is not yet a problem, it would be shortsighted to assume that it cannot occur. In fact, significant levels of resistance have evolved in several field populations of *Culex pipiens* L. complex treated with *Bacillus sphaericus* Neide, an unrelated microbial control agent (Sinègre et al. 1994, Rao et al. 1995, Silva-Filha et al. 1995, Yuan et al. 2000, Mulla et al. 2001) and a suspected case of *B. t.* subsp. *israelensis* resistance was recently reported from New York (Paul et al. 2005). Here, we examined the Mendelian patterns of inheritance of Cry-toxin resistance and cross-resistance in a colony of *Culex quinquefasciatus* Say with high levels of resistance that evolved under laboratory selection pressure by using a deletion mutant of *B. t.* subsp. *israelensis.* This mutant strain synthesizes the three major Cry toxins that naturally occur in *B. t.* subsp. *israelensis*, but lacks Cyt1Aa. Traditional Mendelian backcross experiments were used to determine whether phenotypic expression of resistance was monogenic or polygenic. Dominance values were calculated at various treatment concentrations and the stability of resistance and cross-resistance in the absence of selection pressure was examined. The patterns of inheritance are discussed in relation to patterns observed for other *C. quinquefasciatus* laboratory colonies with Cry resistance.

Materials and Methods

Mosquito Colonies. Two *C. quinquefasciatus* colonies were used in these experiments. Colony CqSyn is a synthetic susceptible colony that originated in 1995; it was formed by pooling multiple field collections and used for baseline bioassays to estimate resistance levels and as the susceptible colony for the genetic crosses (Wirth et al. 2004). The second colony, CqAB11A, originated in 1990 from a different synthetic population and has been maintained under selection pressure since that time with a *B. t.* subsp. *israelensis* deletion mutant expressing the natural Cry toxins (Cry4Aa + Cry4Ba + Cry11Aa) but not the Cyt1Aa toxin component (Georghiou and Wirth 1997).

Recombinant Bacterial Strains. Acrystalliferous *B. t.* subsp. *israelensis* that synthesizes Cry4Aa + Cry4Ba (Delécluse et al. 1993b), Cry11Aa (Wu et al. 1994), or Cry11Ba from *B. thuringiensis* subsp. *jegathesan* (Seleena, Lee, Lecadet) (Delécluse et al. 1995) were used in bioassays. The deletion mutant of *B. t.* subsp. *israelensis* that synthesizes Cry4Aa + Cry4Ba + Cry11Aa but lacks Cyt1Aa was used in both selection and bioassays (Delécluse et al. 1993a). All recombinant materials were in the form of lyophilized crystal/spore powders. The bacterial strains were grown on liquid media as described previously (Park et al. 1998). Sporulated cells were washed in distilled water and sedimented, and the resulting pellet was lyophilized and stored at 4°C.

Selection and Bioassay Procedures. Stock suspensions of crystal/spore powder were prepared by weight in deionized water and homogenized by incorporating glass beads into the suspension and agitating on a vortex mixer to obtain a fine particle suspension. Stocks were prepared monthly, and 10-fold serial dilutions were prepared weekly. All stocks and dilutions were stored at −20°C when not in use. Early fourth instars were used for bioassay tests and selections. Bioassays exposed groups of 20 larvae to different concentrations of crystal/spore suspension in 100 ml of deionized water in 237-ml (8-ounce) plastic cups. At least seven different concentrations, plus a water control, were replicated a minimum of five times on five different days. Mortality was determined 24 h after treatment, and data were analyzed using a Probit program for the PC (Raymond et al. 1993). Resistance ratios were determined by comparing concurrently obtained dose–response values for CqSyn and CqAB11A and calculated by dividing the LC_{50} (or LC_{95}) of the selected colony by the corresponding value for CqSyn. Dose–response values with overlapping fiducial limits were not considered significantly different.

Selection consisted of feeding the recombinant bacterial suspension to groups of 1,000 early fourth instars in 1 liter of deionized water in enamel metal pans for 24 h. The treatment concentration was adjusted to

Table 1. Dose–response values and resistance ratios for the colony CqAB11A (R), CqSyn (S), and F1 offspring of reciprocal crosses to CqSyn by using suspensions of recombinant powders expressing toxins Cry11Aa, Cry11Ba, Cry4Aa + Cry4Ba, or Cry4Aa + Cry4Ba + Cry11Aa

Toxin(s)	Colony	LC_{50} (FL), μg/ml	LC_{95} (FL), μg/ml	Resistance ratio	
				LC_{50}	LC_{95}
Cry4Aa, 4Ba, 11Aa	CqSyn	0.0249 (0.0175–0.0345)	0.345 (0.178–0.699)	1.0	1.0
	CqAB11A	1.21 (0.796–1.83)	64.4 (26.7–163)	49.1	187
	R X S	0.210 (0.171–0.255)	5.01 (3.54–7.67)	8.5	14.5
	S X R	0.122 (0.0805–0.186)	4.39 (2.09–9.46)	4.9	12.7
Cry4Aa, 4Ba	CqSyn	0.122 (0.02313–0.646)	1.35 (0.0705–25.5)	1.0	1.0
	CqAB11A	126.3 (37.5–444)	41974 (1176–1.8×10^6)	634	15,011
	R × S	2.62 (1.64–4.18)	173 (65.9–470)	21.5	128.1
	S × R	2.98 (1.75–5.07)	171.7 (56.0–539)	24.4	127.2
Cry11Aa	CqSyn	1.64 (0.664–3.99)	130 (25.1–695)	1.0	1.0
	CqAB11A	13,004 (1,646–12×10^6)	428,483 (∞)	11,708	506,310
	R × S	108.5 (36.9–340.5)	38,052 (1,150–1.7×10^6)	66.6	292
	S × R	83.4 (18.2–390)	28,820 (170–5.5×10^6)	52.2	222
Cry11Ba	CqSyn	0.0486 (0.0369–0.0638)	0.294 (0.181–0.496)	1.0	1.0
	CqaB11A	0.957 (0.583–1.56)	92.1 (35.4–248)	24.6	427
	R × S	0.222 (0.130–0.379)	6.07 (2.11–17.8)	4.6	20.6
	S × R	0.186 (0.148–0.228)	5.54 (3.88–8.59)	3.8	18.8

ensure 50–80% mortality. Survivors were removed to clean water, fed, and used to continue the colony. Throughout their selection history, 500–1,000 adults were used to establish any single generation, to avoid population bottlenecks. After 28 generations of selection pressure, generations were allowed to overlap.

Genetic Crosses. Reciprocal mass crosses were prepared between susceptible (S) CqSyn and the resistant (R) CqAB11A colonies. Virgin male and female adults were obtained by isolating pupae in scintillation vials. A minimum of 300 males and 300 females were used for each mass cross. The following crosses and backcrosses were prepared, with the female parent listed first: 1) CqSyn × CqAB11A; 2) CqAB11A × CqSyn; 3) (CqAB11A × CqSyn)F1 × CqSyn.

We accepted the assumption that CqAB11A was homogeneous for resistance because the resistance levels, in combination with the stability of resistance in the absence of the insecticide, were consistent with a high proportion of homogeneity for *B. thuringiensis* resistance. Backcross offspring were tested with the various recombinant powders by using 12–14 different concentrations of crystal/spore suspension to provide robust dose–response data and tested for goodness-of-fit to a monofactorial modal as described previously (Wirth et al. 2010).

Dominance Calculations. The single concentration method of Hartl (1992), as described by Liu and Tabashnik (1997), was used to estimate the degree of dominance of the resistance or cross-resistance trait, $h = (w_{12} - w_{22})/(w_{11} - w_{22})$, where h is the degree of dominance, w_{11} is the fitness of the homozygous resistant parent, w_{12} is the fitness of the heterozygous offspring, and w_{22} is the fitness of the homozygous susceptible parent. The fitness of the homozygous resistant parent at any treatment concentration was assumed to be 1. The fitness of the susceptible parent and the heterozygous F1 were estimated from the survival rate of the larvae at a specific treatment concentration divided by the survival rate of the resistant parent at the same concentration. Using this formula, 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 represents a codominant trait. When $0 < h < 0.5$, the trait is partly recessive, whereas when $0.5 < h < 1$, the trait is partly dominant.

Stability of Resistance. Three pans of larvae from CqAB11A were allowed to develop without exposure to insecticide and used to establish generation one of the unselected line. The unselected line was reared for 18 generations without exposure to insecticide with generations maintained separately. Susceptibility to insecticides was evaluated at generations 3, 7, 10, and 18.

The proportion of survivors of exposure to a 10-fold series of concentrations up to 200 μg/ml for each insecticide were used to calculate the change in frequency of resistant genotypes in the populations in the absence of insecticide exposure as described by Tabashnik et al. (1994). The rate of change in the absence of insecticide exposure (R) can be calculated from the following formula: R = (log [final proportion surviving treatment] – log [initial proportion surviving])/n, where n is the number of generations not exposed to insecticide. A negative R value indicates a decline in the proportion surviving exposure to insecticide. Values close to 0 or 0 indicate stable resistance.

Results

When assayed with Cry4Aa + Cry4Ba + Cry11Aa, colony CqAB11A had LC_{50} and LC_{95} values of 1.21 and 64.4 μg/ml and resistance ratios of 49.1 and 187, respectively (Table 1). F1 offspring of reciprocal crosses with CqSyn were intermediate in resistance to their respective susceptible and resistant parents, with LC_{50} values of 0.210 and 0.122 μg/ml. These values were not significantly different from each other based on overlap of their fiducial limits. Higher resistance was observed using recombinant powders expressing Cry4Aa + Cry4Ba. Lethal concentration values at 50 and 95% were 126.3 and 41,974 μg/ml, resulting in

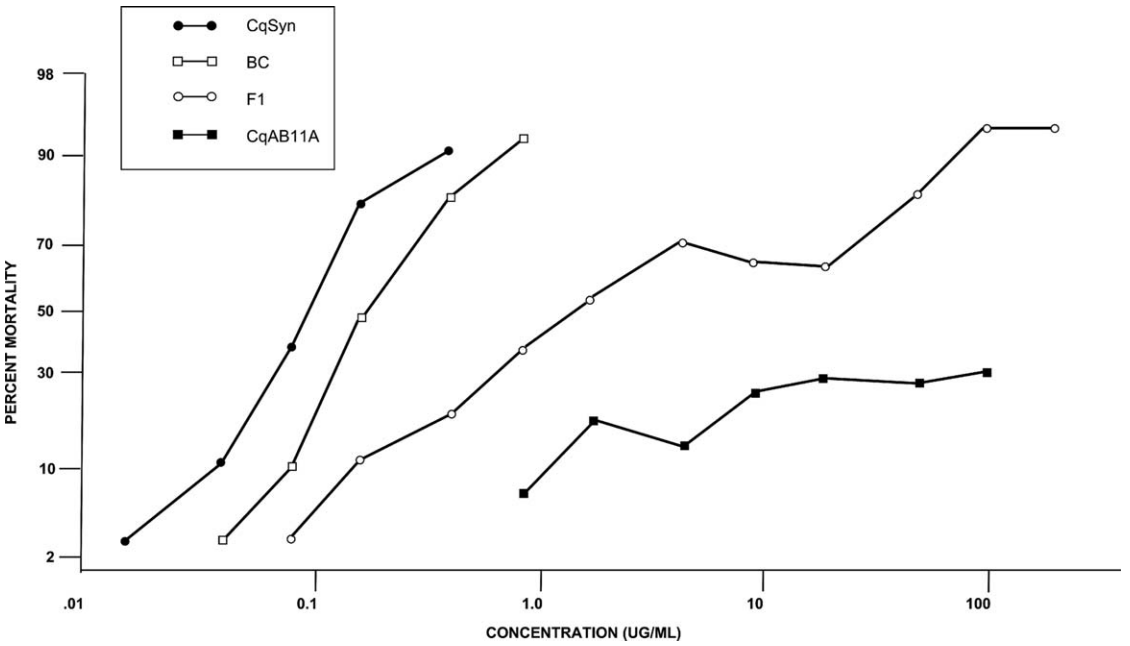

Fig. 1. Results of dose–response testing with *B. t.* subsp.. *israelensis* toxins Cry4Aa + Cry4Ba against two parental colonies (CqSyn, CqAB11A), the F1 (CqAB11A × CqSyn), and offspring of the backcross (BC) [(CqAB11A × CqSyn) F1 × CqSyn].

resistance ratios of 634 and 15,011, respectively. Offspring of reciprocal crosses to CqSyn tested with Cry4Aa + Cry4Ba were intermediate to their respective resistant and susceptible parents and were not significantly different from one another, with LC_{50} values of 2.62 and 2.98 μg/ml. The highest resistance levels were observed in tests with Cry11Aa. Lethal concentration values were 13,004 and 428,000 μg/ml at the LC_{50} and LC_{95} and resistance ratios were estimated at 11,708 and 506,310. F1 offspring showed intermediate levels of susceptibility, with LC_{50} values of 108.5 and 83.4 μg/ml, and these values were not significantly different from each other. Cry11Ba assays on CqAB11A showed LC_{50} and LC_{95} values of 0.957 and 92.1 μg/ml, respectively, and resistance ratios of 24.6 and 427. F1 offspring were again intermediate to their respective parents, with LC_{50} values of 0.222 and 0.186 μg/ml and resistance ratios of 4.6 and 3.8 at the LC_{50} and 20.6 and 18.8 at the LC_{95}. The lethal concentration values of the F1 offspring were not significantly different from each other.

The F1 offspring of the cross CqAB11A × CqSyn were backcrossed to CqSyn and their offspring were bioassayed with the four recombinant powders and tested for goodness-of-fit to a monofactorial model. Tests using Cry4Aa + Cry4Ba + Cry11Aa ($\chi^2 = 31$, df = 13, $P < 0.05$), Cry4Aa + Cry4Ba ($\chi^2 = 29.8$, df = 13, $P < 0.05$) (Fig. 1), and Cry11Aa ($\chi^2 = 18.3$, df = 10, $P < 0.05$) did not fit the monofactorial model. All three dose–response lines deviated significantly from the expected mortality for the monofactorial model at moderately low and moderately high treatment concentrations. In contrast, the dose–response line for Cry11Ba (Fig. 2) showed no significant deviations from the mortality expected under the monofactorial model ($\chi^2 = 22.4$, df = 13, $P > 0.05$).

F1 offspring were generally dominant at low treatment concentrations; however, dominance declined at higher treatment concentrations (Table 2). For example, tests with Cry4Aa + Cry4Ba + Cry11Aa and Cry4Aa + Cry4Ba showed complete dominance (1.0) at 0.002 μg/ml, but dominance declined to semirecessive by treatment concentration of 20.0 μg/ml. Cry11Aa was also dominant at 0.20 μg/ml but declined to codominant at 200 μg/ml. Cry11Ba was fully dominant at 0.02 μg/ml and declined to completely recessive ($h = 0$) by 200 μg/ml.

A subset of CqAB11A was reared without selection for 18 generations and susceptibility to all the recombinant materials was periodically tested (Table 3). R values were calculated at the various treatment concentrations on generation 18 to detect declines in resistance. Most values were very close to 0, indicating generally stable resistance. Negative R values, indicating declines in resistance, were detected toward Cry4Aa + Cry4Ba + Cry11Aa and Cry11Aa; however, these negative values were 0.06 or smaller. Cry4Aa + Cry4Ba and Cry11Ba each showed a single low negative value, and all other R values were positive.

Discussion

C. quinquefasciatus larvae under long-term selection pressure with the *B. t.* subsp. *israelensis* toxins Cry4Aa + Cry4Ba + Cry11Aa evolved moderate levels of resistance to those toxins when presented in combination, but they expressed much higher levels of resistance and cross-resistance to the individual toxins

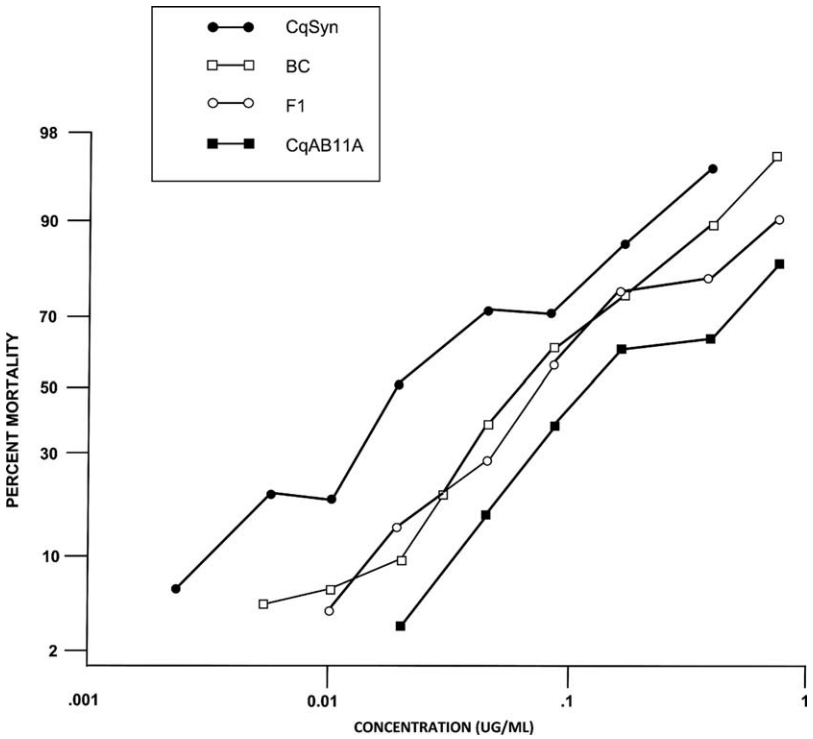

Fig. 2. Results of dose–response testing with *B. t.* subsp. *israelensis* toxins Cry11Ba against two parental colonies (CqSyn, CqAB11A), the F1 (CqAB11A × CqSyn), and offspring of the backcross (BC) [(CqAB11A × CqSyn) F1 × CqSyn].

such as Cry11Aa and Cry11BA, or the mixture Cry4Aa + Cry4Ba. This pattern of resistance evolution in response to selection with mixtures of Cry toxins was reported previously (Georghiou and Wirth 1997, Wirth et al. 1998), but these patterns have persisted under continuing selection pressure and the levels of resistance and cross-resistance have significantly increased. For example, resistance toward Cry4Aa + Cry4Ba + Cry11Aa was 91-fold at the LC_{95} after 28 generations of selection but rose to 187 in this study. Resistance rose more dramatically when the colony was tested with one, or a mixture of two, Cry toxins; from 13.5-fold (Wirth and Georghiou 1997) to 506,310 for Cry11Aa, and from 16.2-fold (Wirth and Georghiou 1997) to 15,011 at the LC_{95} for Cry4Aa + Cry4Ba. A moderate increase in cross-resistance to-

Table 2. Estimation of dominance based on the concentration of *B. thuringiensis* Cry toxin(s) for F1 offspring of reciprocal crosses between CqAB11A and CqSynP

Toxin(s)	Concn, μg/ml	Doninance value for cross	
		CqAB11A × CqSyn	CqSyn × CqAB11A
Cry4Aa + Cry4Ba + Cry11Aa	0.002	1	1
	0.02	0.92	0.48
	0.20	0.56	0.37
	2.0	0.30	0.16
	20.0	0	0.23
Cry4Aa + Cry4Ba	0.02	1.0	1.0
	0.20	0.86	0.95
	2.0	0.54	0.69
	20.0	0.47	0.33
	200.0	0.19	0.24
Cry11Aa	0.20	1.0	1.0
	2.0	0.88	0.91
	20.0	0.54	0.64
	200.0	0.52	0.52
Cry11Ba	0.02	0.94	1.05
	0.20	0.49	0.65
	2.0	0.31	0.26
	20.0	0.23	0.0
	200.0	0.0	0.0

Table 3. Stability of resistance and cross-resistance in the absence of insecticide selection pressure calculated from percent survival of larvae at different treatment concentrations

Toxin(s)	Concn, μg/ml	Generations without selection				Stability (R)
		1	4	13	18	
Cry4A + Cry4B + Cry11A	0.2	79	78	64	26	−0.027
	2.0	44	38	34	13	−0.028
	20.0	13	4	6	0	−0.06
	200	0	1	0	0	0
Cry4Aa + Cry4Ba	2.0	79	90	85	83	0.0012
	20	68	69	53	46	−0.0056
	200	31	50	41	44	0.0056
Cry11Aa	2	100	96	98	96	−0.0011
	20	92	90	85	86	−0.0017
	200	82	79	57	66	−0.0050
Cry11Ba	0.02	98.7	98	100	100	0.0006
	0.20	57	80	97	98.5	0.0133
	2.0	48	55	73	47	−0.0006
	20.0	11	22	47	29	0.0233
	200.0	1	38	20	26	0.0789

ward Cry11Ba was observed, from 347-fold (Wirth et al. 1998) to 427 at the LC_{95}.

When reciprocal crosses were undertaken between CqAB11A and the susceptible colony CqSyn, F1 offspring were not significantly different from each other in their phenotypic expression of resistance and cross-resistance to the different recombinant powders, indicating that resistance traits were not sex-linked and were not influenced by the maternal parent. These data are consistent with our earlier studies on other *Culex* colonies with *B. t.* subsp. *israelensis*-resistance (Wirth et al. 2010) and suggest that autosomal inheritance of Cry resistance may be common in this species. Autosomal inheritance also has been reported for other insect species with resistance to *B. thuringiensis* Cry toxins, including *Plodia interpunctella* (Hübner) (McGaughey 1985), *P. xylostella* (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).

The classical backcross method was used to determine whether resistance and cross-resistance were under monofactorial or polyfactorial control in CqAB11A. The F1 dose–response line for Cry4Aa + Cry4Ba + Cry11Aa failed to fit the monofactorial model. The deviations in observed mortality were near the LC_{50} range and could be indicative of nonadditive polygenic inheritance or experimental error (Tabashnik 1991). Although tests with Cry4Aa + Cry4Ba and Cry11Aa also failed to fit the monogenic model, the deviations showed higher than expected mortality at moderately low doses and lower than expected mortality at moderately high doses, including deviations near the LC_{50}, leading to a conclusion of nonadditive, polygenic inheritance (Tabashnik 1991). Assays with Cry11Ba were consistent with monofactorial inheritance of cross-resistance, suggesting that cross-resistance evolved at a locus shared with other Cry toxins or was closely linked with such a locus. Prior studies on resistant colonies of *C. quinquefasciatus*, selected with Cry11Aa or Cry4Aa + Cry4Ba, provide some interesting similarities and differences (Table 4) (Wirth et al. 2010). The Cry11Aa-selected colony evolved the same patterns of inheritance of resistance and cross-resistance observed in CqAB11A; polyfactorial inheritance of Cry11Aa and Cry4Aa + Cry4Ba-resistance and monofactorial inheritance of Cry11Ba cross-resistance. The Cry4Aa + Cry4BA-selected colony also expressed polyfactorial inheritance for Cry4Aa + Cry4Ba resistance, but differed from the other two colonies by evolving monofactorial inheritance of Cry11Aa resistance and polyfactorial inheritance of Cry11Ba cross-resistance. Together, these results indicate that multiple loci are involved in Cry toxin resistance in *C. quinquefasciatus* and that the number of loci involved in resistance to a particular toxin is influenced by the specific components of the Cry toxin mixture used in selection.

Table 4. Comparison of backcross experimental results from three *B. thuringiensis*–resistant colonies, CqAB11A, CqAB, and Cq11A, and tested with various Cry toxins or toxin mixtures

	Mosquito colony		
	CqAB11A	CqAB[a]	Cq11A[a]
Recombinant Bacterial Strain			
Cry11Aa	Polyfactorial	Monofactorial	Polyfactorial
Cry4Aa + Cry4Ba	Polyfactorial	Polyfactorial	Polyfactorial
Cry4Aa + Cry4Ba + Cry11Aa	Polyfactorial	NT[b]	NT
Cry11Ba	Monofactorial	Polyfactorial	Monofactorial

[a] Data from Wirth et al. (2010). Inheritance patterns, dominance, stability, and allelism for insecticide resistance and cross-resistance in two colonies of *Cx. quinquefasciatus* selected with *B. t.* subsp. *israelensis* Cry toxins.

[b] NT, not tested.

Complete or near complete dominance of resistance was observed at lower treatment concentrations in F1 larvae; however, dominance declined to codominant, or in one case, fully recessive inheritance, at the higher treatment concentration. Similar patterns of declining dominance also were observed in two other colonies of *C. quinquefasciatus* selected for resistance with Cry11Aa or to Cry4Aa + Cry4Ba (Wirth et al. 2010). Many other insect species resistant to *B. thuringiensis* Cry toxins have shown patterns of declining dominance, such as *P. xylostella* (Sayyed et al. 2000, Liu et al. 2001), *O. nubilalis* (Alves et al. 2006), *L. decemlineata* (Rahardja and Whalon 1995), and *P. gossypiella* (Tabashnik et al. 2002); however, dominance patterns can vary broadly among resistant insect species. For example, fully recessive inheritance (Tabashnik et al. 1992, Augustin et al. 2004, Mahon et al. 2007), incompletely recessive resistance (Hama et al. 1992, Kain et al. 2004), and incomplete dominance (Sims and Stone 1991, Huang et al. 1999) have been reported. Theoretically, the partly dominant, to dominant inheritance of Cry resistance in these mosquitoes could enhance survival of heterozygous larvae that are exposed to sublethal field treatment concentrations of *B. t.* subsp. *israelensis.* How relevant any such survival might be in nature is questionable, because the native *B. t.* subsp. *israelensis* used in the field expresses the Cyt1Aa protein and its presence overcomes resistance to the other Cry toxins (Wirth et al. 1997).

Resistance was highly stable in the absence of selection pressure and any negative R values were very close to 0, with a single exception. Cry4A + Cry4B + Cry11A showed generally negative R values ranging from −0.027 to −0.06. Otherwise, R values were very close to 0 and were generally positive. Cry11Aa-selected and Cry4Aa+Cry4Ba-selected *Culex* colonies were previously reported to have generally stable resistance and cross-resistance in the absence of selection pressure (Wirth et al. 2010). Given the largely polygenic nature of its Cry resistance, it would not be unexpected for resistance to be unstable toward the most complex, three-toxin mixture. This slight instability may indicate that the colony CqAB11A was not fully homozygous at all resistance loci. In contrast to the high

stability of Cry resistance in *C. quinquefasciatus*, some *B. thuringiensis*–resistant *P. xylostella* colonies showed rapidly declining resistance and R values averaged −0.28 (Tabashnik et al. 1994). Other *B. thuringiensis*–resistant species exhibited slower declines in resistance in the absence of selection. For example, *P. interpunctella* lost resistance slowly (R = −0.02) as did *H. virescens* (R = −0.04) (McGaughey and Beeman 1988, Sims and Stone 1991). Instability of resistance in the absence of selection is generally attributed to reductions in fitness (Tabashnik et al. 1994). For CqAB11A, it is likely that prolonged selection has lead to fixation or near fixation of resistance alleles at most resistant loci. Fixation of resistance alleles is unlikely to occur in the field because mosquito populations are highly mobile, dispersed, and most populations are not genetically isolated. In view of the prolonged selection of this particular *Culex* colony, fitness studies might prove useful in understanding the basis of resistance stability.

The results of this study, and our prior genetic studies with *Culex* (Wirth et al. 2010), show interesting and complex patterns of inheritance of Cry resistance and cross-resistance in *C. quinquefasciatus.* To date, Cry resistance has been consistently autosomal with no maternal effect in the three colonies studied. However, differences in the composition of the toxins used for selection seemed to influence the number of loci involved in resistance and cross-resistance inheritance. It is well known that the different component toxins of *B. t.* subsp. *israelensis* interact to enhance activity (Crickmore et al. 1995, Poncet et al. 1995). Consequently, it is not surprising that those interactions, or the lack thereof because of the absence of a particular toxin, might exert influence during selection. This effect was apparent for Cry11Ba, a toxin to which the three Cry-resistant colonies had never been exposed. *Culex* selected Cry11Aa or a mixture including Cry11Aa showed monofactorial patterns of inheritance of Cry11Ba cross-resistance. The colony selected without Cry11Aa, Cry4Aa + Cry4Ba, evolved polyfactorial inheritance of Cry11Ba cross-resistance.

The cross-resistance data clearly demonstrated that Cry11Ba cross-resistance evolves in response to selection with *B. t.* subsp. *israelensis* Cry toxins. Furthermore, it is known that resistance to Cry11Aa confers cross-resistance to Cry4Aa + Cry4Ba and vice versa (Wirth et al. 1997, 2010). If resistance in these three colonies involves alterations in receptor binding, as is common in many Cry-resistant insects, then Cry11Ba, Cry11Aa, Cry4Aa, and Cry4Ba are likely to share binding sites. Cross-recognition of binding sites has been reported for *P. xylostella*, which has a single receptor that binds Cry1Aa, Cry1Ab, Cry1Ac, and Cry1 F toxins (Granero et al. 1996, Ballester et al. 1999). Furthermore, brush-border membrane vesicle binding studies in *Aedes aegypti* (L.), another mosquito species, suggested that Cry11Aa, Cry4Aa, and Cry4Ba might share a common class of binding sites (de Barros Moreira Beltrão and Silva-Filha 2007). To prove this hypothesis, receptor binding studies and identification of the Cry receptors in *C. quinquefasciatus* is needed.

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