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# Inheritance of Resistance to *Bacillus thuringiensis* subsp. *kurstaki* in *Trichoplusia ni*

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**The genetic inheritance of resistance to a commercial formulation of *Bacillus thuringiensis* subsp. *kurstaki* was examined in a *Trichoplusia ni* colony initiated from a resistant population present in a commercial vegetable greenhouse in British Columbia, Canada. Progeny of $F_1$ reciprocal crosses and backcrosses between $F_1$ larvae and resistant ($P_R$) and susceptible ($P_S$) populations were assayed at different *B. thuringiensis* subsp. *kurstaki* concentrations. The responses of progeny of reciprocal $F_1$ crosses were identical, indicating that the resistant trait was autosomal. The 50% lethal concentration for the $F_1$ larvae was slightly higher than that for $P_S$, suggesting that resistance is partially recessive. The responses of both backcross progeny ($F_1 \times P_R$, $F_1 \times P_S$) did not correspond to predictions from a single-locus model. The inclusion of a nonhomozygous resistant parental line in the monogenic model significantly increased the correspondence between the expected and observed results for the $F_1 \times P_R$ backcross but decreased the correspondence with the $F_1 \times P_S$ backcross results. This finding suggests that resistance to *B. thuringiensis* subsp. *kurstaki* in this *T. ni* population is due to more than one gene.**

Insecticides based on *Bacillus thuringiensis* are now the most widely used natural insecticides against lepidopteran pests in the world. However, the potential for the development of resistance in targeted insect populations is a continual threat to the long-term use of *B. thuringiensis*-based products. The first report of resistance to *B. thuringiensis* subsp. *kurstaki* outside the laboratory involved the resistance of populations of diamondback moth, *Plutella xylostella*, in Hawaii (39). Within a decade resistance to *B. thuringiensis* subsp. *kurstaki* in *P. xylostella* has been observed worldwide (9). Recently, resistance to *B. thuringiensis* subsp. *kurstaki* was found in commercial greenhouse populations of the cabbage looper, *Trichoplusia ni* (15). This finding provides a unique opportunity to compare the inheritance of a newly evolved resistant trait in *T. ni* to the inheritance of resistance in *P. xylostella*.

In order to devise strategies to delay the evolution of resistance to *B. thuringiensis* subsp. *kurstaki*, knowledge of the genetic inheritance of *B. thuringiensis* subsp. *kurstaki* resistance is required. The most widely publicized resistance management strategy is the high-dose–refuge strategy that has been employed in conjunction with the planting of transgenic crops expressing *B. thuringiensis* toxins (31). The success of this strategy depends on a variety of assumptions, one of which is that the resistance trait in the insect population is recessive at the dose expressed by the transgenic plant (19, 38). Furthermore, resistance management strategies such as the use of toxin mixtures or rotations with different toxins are more likely to succeed if the inheritance of resistance to each toxin is recessive (26, 36).

Dominance relationships are measured in a variety of ways, the most common of which is comparison of dose-mortality curves for susceptible homozygous, resistant homozygous, and heterozygous individuals (4). As determined by this method, the inheritance of resistance to *B. thuringiensis* subsp. *kurstaki* products or toxins in the diamondback moth varied from almost completely recessive to partially recessive (9). However, resistance to *B. thuringiensis* subsp. *kurstaki* in a laboratory population of *Ostrinia nubilalis* was incompletely dominant (13), and resistance to the Cry1Ab toxin of *B. thuringiensis* subsp. *kurstaki* in *Heliothis virescens* was found to be codominant (33). These exceptions demonstrate that species-specific knowledge of the inheritance of *B. thuringiensis* subsp. *kurstaki* resistance is required to devise appropriate resistance management strategies.

Much debate has centered on the role of monogenic or polygenic traits in the evolution of resistance to insecticides in the field (22). The majority of the examples of field-evolved resistance to synthetic insecticides involve monogenic traits (18, 27). It is, therefore, commonly assumed in resistance management strategies that resistance is due to one gene with a susceptible allele and a resistant allele (35). Unlike synthetic insecticides, foliar insecticides based on *B. thuringiensis* are composed of a suite of bacterial toxins (9, 12). Therefore, it is possible that resistance to *B. thuringiensis* subsp. *kurstaki* may arise due to a suite of genes, as opposed to a single monogenic response.

In previous studies, monogenic models of *B. thuringiensis* subsp. *kurstaki* resistance corresponded fairly well to backcross data. For example, studies of the inheritance of *B. thuringiensis* subsp. *kurstaki* resistance in *P. xylostella* (11, 29, 42, 43) and in *O. nubilalis* (13) were consistent with monogenic models of resistance. However, exceptions have been noted for resistance

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to the individual *B. thuringiensis* subsp. *kurstaki* toxin Cry1Ab in a laboratory colony of *H. virescens* (33) and in a field-derived strain of *P. xylostella* (30). Similar exceptions have been noted for resistance to the *B. thuringiensis* subsp. *aizawai* toxin Cry1C in *P. xylostella* (32) and for resistance to Cry1Ca in *Spodoptera littoralis* (7). These exceptions further emphasize the need for knowledge of the genetic inheritance of *B. thuringiensis* subsp. *kurstaki* resistance in *T. ni* in order to develop a species-specific or even population-specific resistance management strategy.

In the present study, the inheritance of *B. thuringiensis* subsp. *kurstaki* resistance in a *T. ni* colony derived from a commercial vegetable greenhouse population was examined. Reciprocal $F_1$ crosses between susceptible laboratory populations and a resistant strain were performed to examine the dominance of *B. thuringiensis* subsp. *kurstaki* resistance. $F_1$ larvae and parental populations were backcrossed to determine if *B. thuringiensis* subsp. *kurstaki* resistance corresponded to a monogenic trait.

## MATERIALS AND METHODS

**Insects.** To study the genetic inheritance of *B. thuringiensis* subsp. *kurstaki* resistance in *T. ni*, a susceptible colony obtained from a laboratory culture was crossed with a field-derived resistant colony. The crosses were conducted simultaneously at two separate locations (University of British Columbia [UBC] in Canada and Cornell University [Cor] in New York) to compare the inheritance results in the two laboratories. Two different susceptible laboratory colonies ($P_S$-UBC and $P_S$-Cor) were used at the two different sites, and both colonies were obtained from laboratory colonies that had been reared in the absence of *B. thuringiensis* subsp. *kurstaki* for over 10 years. The susceptible colonies were obtained from the Department of Plant Sciences, UBC, and the Department of Entomology, Cornell University, Geneva, N.Y.

The resistant *T. ni* colony ($P_R$) was initiated from 74 individuals collected from a commercial greenhouse in British Columbia, Canada (labeled P5 in a previous paper [15]), in 2001, and this colony showed 24-fold-higher resistance to a product of *B. thuringiensis* subsp. *kurstaki* than a reference susceptible laboratory colony. In the fourth generation of laboratory culture in the absence of *B. thuringiensis* subsp. *kurstaki*, two lines were initiated at UBC. One line was subjected to selection with *B. thuringiensis* subsp. *kurstaki* each generation ($P_R$-UBC), and the other was reared without any *B. thuringiensis* subsp. *kurstaki* exposure (unsel$_1$-UBC). Selected lines were exposed to the *B. thuringiensis* subsp. *kurstaki* formulation DiPel WP (Abbott Laboratories), a product used in commercial vegetable greenhouses, which contained 16,000 IU per mg. International units are a standardized method of indicating *B. thuringiensis* subsp. *kurstaki* activity. In the United States, the standard *B. thuringiensis* subsp. *kurstaki* serotype (HD-1-S-1971) is assigned the value 18,000 IU, as it is 18 times more effective against *T. ni* than the French standard (E-61), which was assigned a value of 1,000 IU (5). The unsel$_1$-UBC line died out after seven generations in the laboratory due to disease. At this point, another unselected line (unsel$_2$-UBC) was initiated from the $P_R$-UBC colony.

After six generations of selection at UBC, eggs of the $P_R$-UBC colony were shipped to Cornell, and an additional selected line ($P_R$-Cor) and an additional unselected line (unsel-Cor) were initiated. At Cornell, the lines were selected with a DiPel 0.86% WP formulation (Bonide) that contained 4,320 IU per mg. At UBC, unselected *T. ni* larvae were reared in groups of 15 larvae in 175-ml Styrofoam cups on a wheat germ-based diet at 26°C with a photoperiod consisting of 16 h of light and 8 h of darkness by using methods described previously (15). A minimum of 200 larvae were reared each generation. Selected larvae were reared under similar conditions for 5 days prior to selection. Similarly, at Cornell, *T. ni* larvae were reared in 480-ml Styrofoam cups with 80 ml of a wheat-germ-based diet in groups of 35 larvae per cup (3). A minimum of 150 unselected larvae were reared for each generation. The cups were kept in an environmental chamber at 27 to 29°C with 50% relative humidity and a photoperiod consisting of 16 h of light and 8 h of darkness.

**Selection and survival bioassays.** Resistance in $P_R$-UBC was selected by placing groups of 20 to 25 5-day-old larvae (second and third instars) onto 10 ml of diet mixed with a *B. thuringiensis* subsp. *kurstaki* (DiPel WP) dose in 175-ml Styrofoam cups. All live larvae were transferred to new diet without *B. thuringiensis* subsp. *kurstaki* after 2 days. Levels of survival were recorded at pupation, and pupae were collected and pooled in a mating cage to produce progeny for the next generation (Table 1). For each generation, 500 to 1,000 larvae were selected. At Cornell University, selection was performed with neonates by using a diet overlay assay. In each cup, 2 ml of a DiPel solution was distributed over the diet surface. The concentration of DiPel was 10 to 80 kIU/ml of diet in the second to ninth generations of rearing at Cornell.

TABLE 1. History of selection of the $P_R$-UBC strain

| Lab generation | No. selected | Selective dose (kIU/ml of diet)[a] | % Survival to day 3 | % Survival to pupation |
|---|---|---|---|---|
| 3 | 200 | 0 | 100 | 100 |
| 4 | 661 | 5.0 | 35.4 | 22.7 |
| 5 | 200 | 0 | 100 | 100 |
| 6 | 550 | 10 | 84 | 75 |
| 7 | 560 | 80 | 20 | 7.5[b] |
| 8 | 1,028 | 80 | 9.3 | 2.9 |
| 9 | 543 | 80 | 29 | 16.9 |
| 10[c] | 800 | 80 | 20.6 | 6.6 |

[a] One milligram of DiPel WP contained 16,000 IU.
[b] Surviving pupae were used to initiate inbred lines.
[c] Progeny of the 10th lab generation were sent to Cornell.

At UBC the susceptibility of 5-day-old larvae to *B. thuringiensis* subsp. *kurstaki* was assessed by incorporating *B. thuringiensis* subsp. *kurstaki* into the artificial diet by using methods described by Janmaat and Myers (15). All assays were performed with a minimum of five to seven doses ranging from 1.25 to 160 kIU/ml of diet depending on the expected resistance level and a control. Twenty to 40 larvae were assayed per dose for each bioassay. Larval mortality was observed 3 days following the experimental setup. At Cornell University, a modified diet overlay assay method (45) was used to test the susceptibility of neonates to *B. thuringiensis* subsp. *kurstaki*. Five or six concentrations plus a control and four cups for each concentration were included in each bioassay. Ten neonates were transferred into each cup. The cups were covered with lids and kept at 27 ± 1°C with 50% ± 2% relative humidity and a photoperiod consisting of 16 h of light and 8 h of darkness for 4 days to determine mortality or growth inhibition. Preliminary results at Cornell indicated that growth inhibition (neonates reaching the second instar after 4 days) was a better indicator of neonate susceptibility to *B. thuringiensis* subsp. *kurstaki* than mortality. Therefore, 50% inhibitory concentrations ($IC_{50}$) are reported below for Cornell assays and 50% lethal concentrations ($LC_{50}$) are reported for UBC assays.

**Inbred lines.** After three rounds of selection at UBC, 22 pairs were chosen at random from the $P_R$-UBC colony and were then sib-mated for three generations to create inbred lines. Inbred lines were established to increase homogeneity for genetic analysis as the assumption of homozygous parental lines is critical for determination of inheritance from $F_1$ and backcross generations. Pupae were sexed, and single pairs were placed into 16-oz paper cups supplied with a 10% sugar solution and lined with paper towels for oviposition. Of the 22 crosses, 17 produced sufficient viable offspring for bioassays. The $LC_{50}$ for each pair was assayed for three subsequent generations. Several inbred lines exhibited poor fecundity and could not be maintained.

To further ensure that few susceptible alleles remained in the inbred lines, any line that exhibited a decrease in the $LC_{50}$ between generations or had an evident plateau in the concentration-mortality line was assumed to contain susceptible genes and was terminated. The presence of a plateau at concentrations lower than the family $LC_{50}$ indicated that the inbred line was not genetically homogenous. Five inbred lines (lines 6, 12, 13, 16, and 17) exhibited stable resistance over three generations and adequate fecundity, and four of these five lines were used for the genetic analysis (lines 6, 12, 13, and 17). Inbred lines 6, 12, 13, and 16 were maintained without *B. thuringiensis* subsp. *kurstaki* exposure for 14 generations and were assayed after 6, 10, and 14 generations to examine the stability of resistance.

**Analysis of inheritance.** To examine maternal effects, sex linkage, and dominance, $F_1$ larvae from reciprocal crosses between susceptible and resistant strains were tested. At Cornell, $F_1$ larvae from reciprocal mass crosses (50 pupae per sex) between the $P_S$-Cor and selected $P_R$-Cor strains were assessed, whereas at UBC the inheritance of resistance was examined in reciprocal single-pair crosses between the four resistant inbred lines and the $P_S$-UBC strain. Pupae were obtained following three generations of sib-mating from each inbred line and were paired with $P_S$-UBC pupae to produce $F_1$ hybrids. To examine the number of factors involved in resistance, hybrid larvae were backcrossed to parental resistant lines ($F_1 \times P_R$) at both locations and to $P_S$ at UBC. Progeny of mass crosses between 75 $F_1$ females and 50 resistant males were tested for suscepti-

bility to *B. thuringiensis* subsp. *kurstaki* at Cornell, and progeny of single-pair crosses were assayed at UBC.

At UBC, two single pairs per inbred line were crossed for each of two reciprocal backcrosses. All pairs that produced sufficient numbers of viable offspring were assayed. For the $F_1 \times P_R$ backcross, three pairs each for lines 6 and 12 were assayed, two pairs for line 13 were assayed, and one pair for line 17 was assayed. For the $F_1 \times P_S$-UBC backcross, two pairs were assayed for lines 6, 12, and 17 and three pairs were assayed for line 13. In addition, five single-pair crosses within two of the inbred lines (resistant × resistant) were performed to examine any remaining variation in resistance in the inbred lines.

**Data analysis.** $LC_{50}$s and slopes of concentration-mortality lines were estimated by using the probit analysis procedure in Genstat 5, release 4.1 (25) at UBC. The POLO program (16) was used for probit analysis of dose-response data (28) at Cornell University. Mortality was corrected by using Abbott's formula (1) for each probit analysis. In the UBC assays, no mortality was recorded in the majority of the control treatment groups, and if mortality occurred, the level was less than 5%. The $LC_{50}$s for different crosses or genetic lines were considered significantly different if their 95% fiducial limits did not overlap. Resistance ratios were calculated by dividing the $LC_{50}$ of the strain by the $LC_{50}$ of the corresponding $P_S$ population. $LC_{50}$s were rounded to the nearest hundredth. Below all $LC_{50}$s or $IC_{50}$s are expressed in thousands of international units per milliliter of diet or water.

Deviance statistics were used to test for differences in mortality over the dose range between groups by using the accumulated display setting in Genstat 5. Deviance ratios (devratio) and approximate chi-square probabilities are shown below. To test for dominance, the responses of $F_1$ offspring were compared to the responses of the parental resistant family and the susceptible parent. Dominance was estimated as described previously (17), and estimation of dominance based on the $LC_{50}$ was used (34). Dominance values range from −1 (completely recessive) to 1 (completely dominant).

Indirect methods based on estimated mortalities from normal distributions with the mean and standard deviation corresponding to the $LC_{50}$ and reciprocal of the probit slope, respectively, of different genotypes were used to compare responses of backcross progeny to responses predicted from models with one or two loci (40, 42). The assumptions of the models were (i) each locus had one resistant allele and one susceptible allele and (ii) the parental susceptible and resistant strains ($P_S$ and $P_R$) were homozygous for susceptible and resistant alleles, respectively.

Additional monogenic models with nonhomozygous parental lines were examined, in which the frequency of the resistant allele varied from 0.5 to 1.0 in the $P_R$ population or from 0 to 0.3 in the $P_S$ population in increments of 0.05. The expected proportions of susceptible, hybrid, and resistant genotypes were estimated for the $F_1$ and subsequent backcross generations and utilized to adjust the expected backcross $LC_{50}$ in the following three scenarios.

**Case 1.** The expected genotypic frequencies from a monogenic model in the $F_1 \times P_R$ backcross generation with a nonhomozygous $P_R$ line and a homozygous susceptible $P_S$ line, where R is the resistant allele and S is the susceptible allele, were as follows: $P = 0.5p^2$, $H = 0.5(3 - 2p)p$, and $Q = 0.5q(1 + q)$, where P is the frequency of the RR genotype, H is the frequency of the RS genotype, Q is the frequency of the SS genotype, $p$ is the frequency of R in $P_R$, and $q$ is the frequency of S in $P_R$.

**Case 2.** The expected genotypic frequencies from a monogenic model in the $F_1 \times P_S$ backcross generation with a nonhomozygous $P_R$ line and a homozygous susceptible $P_S$ line were as follows: $P = 0$, $H = 0.5p$, and $Q = 0.5(1 + pq)$.

**Case 3.** The expected genotypic frequencies from a monogenic model in the $F_1 \times P_S$ backcross generation with a nonhomozygous $P_S$ line and a homozygous resistant $P_R$ line were as follows: $P = 0.5p(1 + p)$, $H = 0.5(3 - 2q)q$ and $Q = 0.5q^2$, where $p$ is the frequency of R in $P_S$ and $q$ is the frequency of S in $P_S$.

For the two-locus model, four models with epistasis (nonadditive interactions between loci) were also tested and were analogous to models A, B, C, and D described by Tabashnik et al. (42). In model A, individuals heterozygous at one locus and homozygous resistant at the other locus were fully resistant ($R_1S_1R_2R_2$ and $R_1R_1R_2S_2$), whereas in model B the same genotypes responded like $F_1$ progeny ($R_1S_1R_2S_2$). In model C, $R_1S_1R_2R_2$ responded like $F_1$ progeny, and the $LC_{50}$ for $R_1R_1R_2S_2$ was the geometric mean of the $LC_{50}$s for the $F_1$ progeny and the resistant parent (assumed to be $R_1R_1R_2R_2$). In model D, $R_1R_1R_2S_2$ was fully resistant and the $LC_{50}$ of $R_1S_1R_2R_2$ was the geometric mean of the values for the $F_1$ progeny and the resistant parent. For all model comparisons, expected and observed mortalities at each concentration were compared by using a $2 \times 2$ test for independence at each of the concentrations used in the bioassay (42). Overall model $\chi^2$ values were calculated by adding the $\chi^2$ values for all doses for each model. The model with the lowest $\chi^2$ value was determined to have the best fit to the observed data. Results for the four inbred lines from the UBC backcrosses were pooled in the analyses, since the 95% confidence intervals of the $LC_{50}$s of the four lines overlapped.

TABLE 2. History of selection of the $P_R$-Cornell strain

| Lab generation | No. selected | Selective dose (kIU of DiPcl/ml)[b] | % Survival to pupation |
|---|---|---|---|
| 1[a] | 100 | 0 | 100 |
| 2 | 900 | 10 | 13 |
| 3 | 1,500 | 10 | 17 |
| 4 | 510 | 20 | 36 |
| 5 | 560 | 20 | 36 |
| 6 | 675 | 40 | 26 |
| 7 | 500 | 40 | 17 |
| 8 | 625 | 40 | 17 |
| 9 | 1,500 | 80 | 7 |
| 10 | 150 | 0 | 100 |
| 11 | 1,500 | 40 | 14 |
| 12 | 1,000 | 40 | 9 |
| 13 | NA[c] | 40 | NA |
| 14 | 775 | 40 | 8 |
| 15 | 720 | 40 | 7 |

[a] The first lab generation began with the original eggs received from UBC.
[b] Two milliliters of a DiPel suspension was added to the surface (~50 $cm^2$) of the diet.
[c] NA, number not recorded.

## RESULTS

**Response to selection and genetic variation within resistant colonies.** An increase in resistance of the $P_R$-UBC *T. ni* colony to *B. thuringiensis* subsp. *kurstaki* was observed in response to selection with increasing *B. thuringiensis* subsp. *kurstaki* doses (Table 1). Prior to selection, there was no survival to pupation at a dose of 80 kIU/ml of diet. Following five generations of selection, the level of survival to pupation increased to 17% at a dose of 80 kIU/ml of diet. Prior to selection, the $LC_{50}$ for the $P_R$-UBC colony after 3 days of larval feeding was 8 kIU/ml of diet (6.0 to 10.3 kIU/ml of diet), and the value increased to 44.8 kIU/ml of diet (36.5 to 54.7 kIU/ml of diet), for a resistance ratio of 44.8 compared to $P_S$-UBC (i.e., $LC_{50}$ for $P_R/LC_{50}$ for $P_S$). After eight or nine generations of selection at Cornell University, the $IC_{50}$ for the $P_R$-Cor neonates was 5.8 kIU/ml, and the resistance ratio relative to $P_S$-Cor was 37.7 (Tables 2 and 3).

Bioassays of offspring of 17 single-pair crosses between $P_R$-UBC resistant individuals demonstrated that genetic variation for *B. thuringiensis* subsp. *kurstaki* resistance remained in the population after selection at a dose of 80 kIU/ml of diet (pair devratio, 2.34; $p = 0.002$). The $LC_{50}$s of the pairs ranged from 16.7 ± 3.0 to 42 ± 6.0 kIU/ml of diet.

**Inbreeding results.** Five lines were sib-mated successfully for three generations and reared in the absence of selection with *B. thuringiensis*. In the first generation, six lines were discarded due to prominent plateaus corresponding to mid-range *B. thuringiensis* doses in the assays, which suggests that the lines were not genetically homogeneous for resistance. Two lines were discarded in generation 2, and four lines were discarded in generation 3, due to significant decreases in $LC_{50}$s between generations. Two-thirds of the lines displayed poor fecundity and adult survival after the third generation of inbreeding, presumably due to inbreeding effects. Four inbred

TABLE 3. Responses of susceptible ($P_S$-UBC), resistant ($P_R$-UBC, $P_R$-Cor), $F_1$, and backcross *T. ni* larvae to *B. thuringiensis* subsp. *kurstaki* (DiPel)[a]

| Inbred line | Cross | *n* | Slope (mean ± SE) | $LC_{50}$ (95% fiducial limits) (kIU/ml of DiPel inoculum) | Resistance ratio[b] | Dominance[c] |
|---|---|---|---|---|---|---|
| UBC | $P_S$-UBC | 180 | 0.761 ± 0.13 | 1.0 (0.6–1.4) | 1.0 | |
| | $P_R$-G1 | 396 | 1.32 ± 0.11 | 23.9 (21.2–26.4) | 23.9 | |
| | $P_R$-G14 | 800 | 0.77 ± 0.06 | 18.3 (15.7–21.7) | 18.3 | |
| | $F_1$[d] | 480 | 1.53 ± 0.23 | 2.4 (2.2–2.6) | 2.4 | −0.44 |
| | $F_1$[e] | 480 | 1.32 ± 0.20 | 2.3 (2.0–2.8) | 2.3 | −0.48 |
| | $F_1 \times P_R$ | 1,204 | 0.80 ± 0.04 | 5.1 (4.6–5.7) | 5.1 | |
| | $F_1 \times P_S$ | 1,325 | 1.03 ± 0.11 | 2.5 (2.2–2.7) | 2.5 | |
| Cornell | $P_S$-Cor | 160 | 2.26 ± 0.42 | 0.15 (0.11–0.20)[f] | 1.0 | |
| | $P_R$-Cor | 240 | 2.96 ± 0.49 | 5.8 (4.6–7.0)[f] | 37.7 | |
| | $F_1$[d] | 240 | 1.87 ± 0.20 | 0.35 (0.21–0.62)[f] | 2.3 | −0.55 |
| | $F_1$[e] | 240 | 1.40 ± 0.18 | 0.35 (0.26–0.48)[f] | 2.3 | −0.55 |
| | $F_1 \times P_R$ | 400 | 1.57 ± 0.18 | 0.90 (0.65–1.16)[f] | | |

[a] The responses of resistant inbred lines after 1 ($P_a$-G1) and 14 ($P_R$-G14) generations of rearing in the absence of selection are shown. The UBC results are the means for four inbred lines and their corresponding crosses.
[b] $LC_{50}$ of $P_R$ divided by $LC_{50}$ of the corresponding $P_S$. The mean $LC_{50}$s and resistance ratios of four inbred resistant lines are shown for the UBC $P_R$ cross.
[c] Dominance as defined by Stone (34). The dominance ranges from −1 (completely recessive) to 1 (completely dominant) at the $LC_{50}$.
[d] $F_1$ cross between $P_S$(f) and $P_R$ or $F_1$, where f is female.
[e] $F_1$ cross between $P_S$ and $P_R$(f), where f is female.
[f] $IC_{50}$, dose at which 50% of the larvae were growth inhibited.

lines were chosen and sib-mated for an additional 11 generations.

In the absence of selection pressure, the $LC_{50}$s of the four inbred lines remained relatively stable over 14 generations (Fig. 1). A regression of the natural logarithm of the $LC_{50}$s over time showed a negative change in $LC_{50}$ ($F = 4.15$; df = 19; $p = 0.057$; JMPIN (SAS Institute Inc. 2000), 4.03). An examination of the change over time in the individual lines revealed that line 6 displayed a significant decrease in $LC_{50}$. If line 6 was excluded from the analysis, there was no change in $LC_{50}$ over time in the remaining lines ($F = 1.09$; df = 14; $p = 0.32$).

In contrast, the resistance of $P_R$-UBC following field collection declined from an $LC_{50}$ of 52 kIU/ml of diet to $LC_{50}$ of 8.0 kIU/ml of diet in three generations (15). In the absence of selection, the $LC_{50}$ for $unsel_1$-UBC decreased to 4.3 kIU/ml of diet after seven generations. The $LC_{50}$ for $unsel_2$-UBC, which was initiated from the eighth generation of the selected $P_R$-UBC colony ($LC_{50}$, 44.8 kIU/ml of diet), also rapidly declined to 2.9 kIU/ml of diet after nine unselected generations (Fig. 2). Similarly, the nonselected strain at Cornell exhibited a decrease in resistance to an $IC_{50}$ of 0.9 kIU/ml (95% fiducial limits, 0.7 to 1.2 kIU/ml) after 11 generations of rearing in the absence of *B. thuringiensis* subsp. *kurstaki*, whereas the decrease in line 6 was much slower than that in the nonselected colonies, as the $LC_{50}$ decreased from 27 to 13 kIU/ml of diet over 14 generations. Therefore, susceptible alleles or costly resistant alleles were excluded from the inbred lines, which stabilized the $LC_{50}$s over time. However, bioassays for offspring of single-pair crosses within two of the inbred lines (line 13 × line 13; line 17 × line 17) after three generations of inbreeding revealed that significant variation remained in the two inbred lines (line 13 devratio = 4.5 [$p = 0.004$]; line 17 devratio = 17.4 [$p < 0.001$]). The $LC_{50}$s varied from 27.4 to 48.5 kIU/ml of diet and from 18.9 to 61.1 kIU/ml of diet for lines 13 and 17, respectively.

**Evaluation of dominance and maternal effects.** No difference was found between the $LC_{50}$s and slopes of the concentration-mortality lines for the hybrid progeny for the two reciprocal crosses (cross) between the inbred lines and the $P_S$-UBC strain (cross devratio = 0.005 [$p = 0.946$]; cross*dose (i.e., slope comparison) devratio = 0.62 [$p = 0.432$]) (Table 3). Little variation in the $LC_{50}$s was observed among the eight $F_1$ hybrid lines ($LC_{50}$ range, 1.5 ± 0.7 to 3.0 ± 0.4 kIU/ml of diet) ($p = 0.95$). Similarly, the $IC_{50}$s of the Cornell reciprocal $F_1$ crosses were identical (0.35 kIU/ml of DiPel suspension) (Table 3). Therefore, no maternal effects or sex linkage were evident, and inheritance of *B. thuringiensis* subsp. *kurstaki* resistance was assumed to be autosomal.

The mean resistance ratio for the UBC $F_1$ hybrids was 2.4 ± 0.2, compared to a mean resistance ratio for the resistant parents of 23.9 ± 1.4. The resistance ratio for the Cornell $F_1$ hybrids was 2.3, compared to the ratio of 37.7 for the resistant

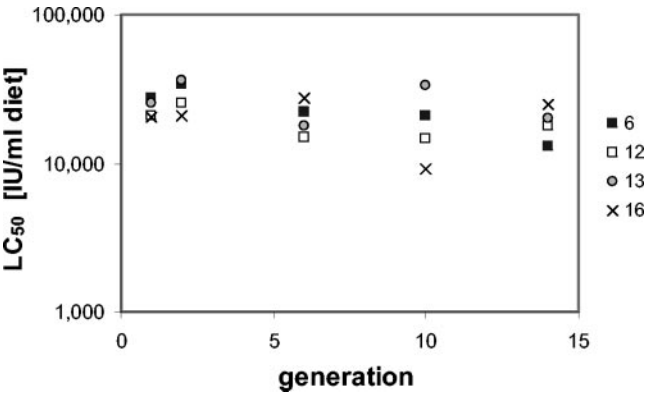


FIG. 1. Change in $LC_{50}$s over 14 unselected generations of four inbred $P_R$ lines (lines 6, 12, 13, and 16). A regression of the natural logarithm of the $LC_{50}$ on the generation yielded a slope of −0.0030 ± 0.0015 ($t = -2.04$; $p = 0.057$).

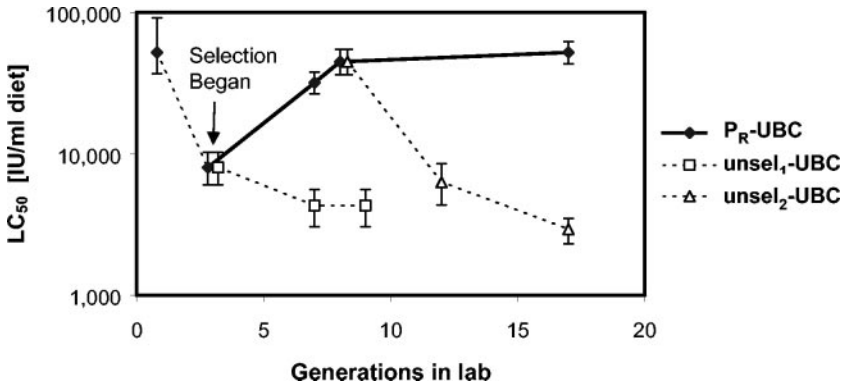


FIG. 2. Change in $LC_{50}$s over time for a selected field-derived strain of *T. ni* ($P_R$-UBC) and two unselected lines ($unsel_1$-UBC and $unsel_2$-UBC). Selection with *B. thuringiensis* subsp. *kurstaki* began after three generations of laboratory rearing.

strain. The mean dominance value calculated for the pooled inbred line assays was −0.46 for the two reciprocal $F_1$ crosses, compared to −0.55 for the Cornell crosses (Table 3). Therefore, resistance to *B. thuringiensis* subsp. *kurstaki* at the $LC_{50}$ or $IC_{50}$ was partially recessive, assuming that the parental lines were homozygous.

**Backcross results.** For the $F_1 \times P_R$-UBC backcross, the slope of the concentration-mortality line for the backcross progeny was lower than that for the $F_1$ hybrid, indicating that variation in the resistance levels increased, as expected when the inheritance is due to one or a few loci ($F_1$-UBC slope, 1.54 ± 0.12; $F_1$-UBC × $P_R$-UBC slope, 0.80 ± 0.04). However, a decrease in the slope was not observed for the concentration-mortality lines for the Cornell $F_1$ hybrids and the backcross progeny ($F_1$-Cor slope, 1.62 ± 0.13; $F_1$-Cor × $P_R$-Cor slope, 1.57 ± 0.18).

For all $F_1 \times P_R$ backcrosses, progeny exhibited higher mortality than expected under a model of monogenic inheritance (Fig. 3). There were significant deviations between observed and expected mortalities near the expected $LC_{50}$ (Table 4). Under the assumption that the parental lines were homozygous, this result suggests that a monogenic model did not adequately fit the observed data (37). However, relaxing the assumption of homozygosity in the parental lines increased the correspondence between the expected and observed results. This was shown by the reduction in model $\chi^2$ values from a monogenic model with a resistant allele frequency of 1.0 in $P_R$ to a frequency of 0.8 for both $F_1 \times P_R$ backcrosses (Table 4). Therefore, the discrepancy between predicted and observed results can be explained by either the presence of more than one resistance locus or the presence of susceptible alleles in the resistant parental lines.

To distinguish between these two plausible hypotheses, the results of the UBC $F_1 \times P_S$ backcross were examined. Unlike the $F_1 \times P_R$ progeny, the progeny of the $F_1 \times P_S$ backcross exhibited lower mortality than expected at all concentrations (Fig. 3). However, if $P_R$ lines contained susceptible alleles, it would be predicted that $F_1 \times P_S$ backcross progeny would have a higher mortality than expected. Some of the $F_1$ individuals would be homozygous susceptible, and therefore a higher proportion of the backcross progeny would also be homozygous susceptible. As expected, including a nonhomozygous $P_R$ line in the $F_1 \times P_S$ backcross model did not improve the fit to the observed data (Table 5). In contrast, including resistant alleles in the $P_S$ line (in an $F_1 \times P_S$ model with homozygous $P_R$) decreased the overall $\chi^2$ value from 22.3 to 10.6 with a change in the resistant allele frequency from 0 to 0.05 in the $P_S$ population (Table 5). However, the possibility of a nonhomozygous $P_S$ population would not account for the higher-than-expected mortality observed in the $F_1 \times P_R$ backcross. Therefore, the lack of correspondence between the backcross results and the results predicted from a monogenic model suggests that more loci are involved in resistance.

To further elucidate the inheritance of resistance, the $P_R \times F_1$ backcross results were compared to mortalities predicted from a two-locus model with additive or epistatic effects. The

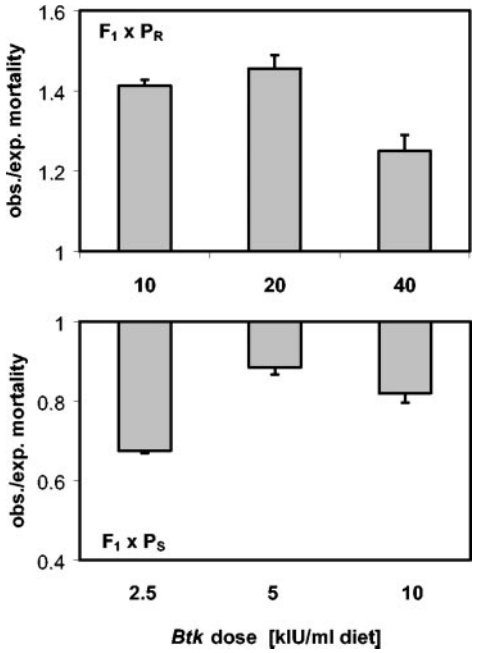


FIG. 3. Comparison of the mortality observed (obs.) in assays of backcross progeny ($F_1 \times P_R$; $F_1 \times P_S$) relative to the mortality expected (exp.) from a monogenic model with homozygous parental lines. The mean ratios of observed mortality to expected mortality for the four $P_R$ inbred lines are shown. The error bars indicate standard errors. *Btk*, *B. thuringiensis* subsp. *kurstaki*.

TABLE 4. Indirect tests of a monogenic model of *B. thuringiensis* subsp. *kurstaki* resistance inheritance by comparing observed and expected mortalities of backcrosses between $F_1$ and $P_R$, the resistant parent[a]

| Line | Dose[b] | *n* | Observed mortality | Monogenic model with $p = 1.0$ | | Monogenic model with $p = 0.9$ | | Monogenic model with $p = 0.8$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Expected mortality | $\chi^2$ | Expected mortality | $\chi^2$ | Expected mortality | $\chi^2$ |
| UBC | 1.25 | 172 | 9.3 | 9.9 | 0.04 | 11.7 | 0.52 | 14.0 | 1.86 |
| | 2.50 | 172 | 25.6 | 26.9 | 0.08 | 32.2 | 1.81 | 37.4 | 5.58 |
| | 5.00 | 174 | 56.3 | 42.8 | 6.37[c] | 51.4 | 0.83 | 58.4 | 0.15 |
| | 10.0 | 174 | 71.3 | 52.6 | 12.82[d] | 61.3 | 3.87[c] | 68.8 | 0.25 |
| | 20.0 | 172 | 88.4 | 64.9 | 26.39[d] | 88.4 | 14.58[e] | 77.8 | 6.85[d] |
| | 40.0 | 170 | 93.5 | 83.4 | 8.49[d] | 86.4 | 4.79[c] | 89.4 | 1.89 |
| $\Sigma\chi^2$ | | | | | 54.19 | | 26.40 | | 16.58 |
| Cornell | 0.20 | 40 | 15 | 5.1 | 2.11 | 7.7 | 1.05 | 12.8 | 0.46 |
| | 0.40 | 40 | 37.5 | 30.7 | 0.52 | 38.5 | 0.008 | 46.2 | 0.05 |
| | 0.80 | 40 | 52.5 | 48.7 | 0.11 | 56.4 | 1.22 | 66.7 | 0.30 |
| | 1.13 | 40 | 52.5 | 48.7 | 0.11 | 58.9 | 0.33 | 69.2 | 0.59 |
| | 1.60 | 40 | 65 | 51.2 | 1.53 | 58.9 | 0.30 | 69.2 | 0.14 |
| | 2.15 | 40 | 77.5 | 51.3 | 5.93[c] | 61.5 | 2.37 | 69.2 | 2.14 |
| | 2.88 | 40 | 85 | 53.8 | 9.06[d] | 61.5 | 5.57[c] | 71.8 | 4.91[d] |
| | 4.32 | 40 | 82.5 | 64.1 | 3.42 | 69.2 | 1.90 | 76.9 | 1.56 |
| | 6.48 | 40 | 87.5 | 79.5 | 0.92 | 84.6 | 0.13 | 87.2 | 0.11 |
| | 9.72 | 40 | 97.5 | 94.9 | 0.37 | 94.9 | 0.37 | 97.4 | 0.35 |
| $\Sigma\chi^2$ | | | | | 24.08 | | 13.25 | | 10.61 |

[a] Monogenic models were adjusted for the presence of a nonhomozygous resistant parental population ($P_R$) that had a resistant allele frequency of 0.9 or 0.85.
[b] The doses are expressed in thousands of international units per milliliter of diet for the UBC crosses and in thousands of international units per milliliter of DiPel inoculum for the Cornell crosses.
[c] Significant at a level of ≤0.05.
[d] Significant at a level of ≤0.01.
[e] Significant at a level of ≤0.001.

two-locus additive model yielded a $\chi^2$ value similar to that of the monogenic model with the nonhomozygous $P_R$ line (where $p = 0.8$) for the Cornell crosses, but a higher $\chi^2$ value was obtained for the UBC crosses. For both the Cornell and UBC backcrosses, the two-locus model with epistatic effects yielded the lowest overall model $\chi^2$ value (Table 6). In this model, the $R_1S_1R_2R_2$ genotype responded like $F_1$ progeny, and the $LC_{50}$ for $R_1R_1R_2S_2$ was midway between the values for the $F_1$ hybrid and the resistant parent. None of the two-locus models improved the correspondence between the observed and expected mortalities of the $F_1 \times P_S$ backcross. However, the limited difference in $LC_{50}$ between the $F_1$ and $P_S$ populations and the high dose range chosen may not have been adequate to effectively compare the different multilocus models.

The presence of two plateaus would be expected in the concentration-mortality plot of the $F_1 \times P_R$ backcross progeny if the resistance trait were due to two loci with additive effects or nonadditive effects, such as the loci in model C (22). These two plateaus would correspond to the concentration at which all of the $R_1S_1R_2S_2$ (and $R_1S_1R_2R_2$ in model C) individuals had been killed and the concentration at which all of the $R_1R_1R_2S_2$ (and $R_1S_1R_2R_2$ in the two-locus additive model) had been killed. Interestingly, two plateaus are evident in the concentra-

TABLE 5. Indirect test of a monogenic model of *B. thuringiensis* subsp. *kurstaki* resistance inheritance by comparing observed and expected mortalities of backcrosses between $F_1$ and the susceptible parent ($P_S$)[a]

| Dose (μg of DiPel/ml of diet) | *n* | Observed mortality | Monogenic model with $p = 1.0$ in $P_R$ | | Monogenic model with $p = 0.8$ in $P_R$ | | Monogenic model with $p = 0.05$ in $P_S$ | |
|---|---|---|---|---|---|---|---|---|
| | | | Expected mortality | $\chi^2$ | Expected mortality | $\chi^2$ | Expected mortality | $\chi^2$ |
| 1.25 | 255 | 29.4 | 22.0 | 3.6 | 22.7 | 3.2 | 21.5 | 4.5[b] |
| 2.5 | 255 | 44.7 | 57.1 | 7.8[c] | 57.5 | 8.3[c] | 55.2 | 5.5[b] |
| 5.0 | 255 | 83.1 | 87.0 | 1.5 | 87.1 | 1.8 | 84.4 | 0.2 |
| 10.0 | 255 | 94.5 | 98.0 | 4.4[b] | 98.1 | 5.7[b] | 95.6 | 0.4 |
| 20.0 | 255 | 98.0 | 100.0 | 5.0[b] | 99.9 | 5.0[b] | 98.0 | 0 |
| $\Sigma\chi^2$ | | | | 22.3 | | 24.0 | | 10.6 |

[a] The model was adjusted for the presence of a nonhomozygous resistant ($P_R$) parental population with a resistant allele frequency of 0.85 or a nonhomozygous susceptible ($P_S$) parental population with a resistant allele frequency of 0.05.
[b] Significant at a level of ≤0.05.
[c] Significant at a level of ≤0.01.

TABLE 6. Indirect tests of a two-locus model of *B. thuringiensis* subsp. *kurstaki* resistance inheritance performed by comparing observed mortalities of backcrosses between $F_1$ and the resistant parental population ($P_R$) with mortalities predicted from a two-locus model with additive or nonadditive effects (model C of Tabashnik et al.)[a]

| Line | Dose[b] | *n* | Observed mortility | Two-locus model (additive) | | Two-locus model C (with epistasis) | |
|---|---|---|---|---|---|---|---|
| | | | | Expected mortality | $\chi^2$ | Expected mortality | $\chi^2$ |
| UBC | 1.25 | 172 | 9.3 | 5.3 | 2.07 | 9.9 | 0.04 |
| | 2.50 | 172 | 25.6 | 16.4 | 4.38[c] | 28.7 | 0.41 |
| | 5.00 | 174 | 56.3 | 35.8 | 14.65[d] | 49.7 | 1.52 |
| | 10.0 | 174 | 71.3 | 58.9 | 5.78[c] | 67.1 | 0.72 |
| | 20.0 | 172 | 88.4 | 77.8 | 6.85[e] | 80.1 | 4.41[c] |
| | 40.0 | 170 | 93.5 | 91.1 | 0.69 | 91.7 | 0.41 |
| $\Sigma\chi^2$ | | | | | 34.42 | | 7.51 |
| Cornell | 0.20 | 40 | 15 | 25.6 | 1.38 | 5.1 | 2.11 |
| | 0.40 | 40 | 37.5 | 25.6 | 1.28 | 30.8 | 0.39 |
| | 0.80 | 40 | 52.5 | 33.3 | 2.96 | 51.3 | 0.01 |
| | 1.13 | 40 | 52.5 | 46.2 | 0.32 | 61.5 | 0.66 |
| | 1.60 | 40 | 65 | 61.5 | 0.10 | 69.2 | 0.16 |
| | 2.15 | 40 | 77.5 | 69.2 | 0.41 | 71.8 | 0.34 |
| | 2.88 | 40 | 85 | 74.4 | 1.38 | 76.9 | 0.84 |
| | 4.32 | 40 | 82.5 | 82.1 | 0 | 82.1 | 0.003 |
| | 6.48 | 40 | 87.5 | 89.7 | 0.1 | 89.7 | 0.098 |
| | 9.72 | 40 | 97.5 | 97.4 | 0 | 97.4 | 0 |
| $\Sigma\chi^2$ | | | | | 7.93 | | 4.61 |

[a] See reference 42.
[b] The doses are expressed in thousands of international units per milliliter of diet for the UBC crosses and in thousands of international units per milliliter of DiPel inoculum for the Cornell crosses.
[c] Significant at a level of ≤0.05.
[d] Significant at a level of ≤0.001.
[e] Significant at a level of ≤0.01.

tion-mortality plot for the Cornell backcross progeny, providing further support for the two-locus models (Fig. 4).

## DISCUSSION

Resistance to *B. thuringiensis* subsp. *kurstaki* in a moderately resistant *T. ni* population appears to be due to an autosomal partially recessive trait, as determined by comparisons of dose-mortality curves for resistant, susceptible, hybrid, and backcross progeny. The similarity of the results obtained in the two separate laboratories was striking considering the differences in methodology. For example, the dominance value varied from −0.46 to −0.55 between the two locations, and therefore *B. thuringiensis* subsp. *kurstaki* resistance in this *T. ni* population conformed to a partially recessive trait. In other studies, resistance has varied from partial to complete recessivity in laboratory-selected strains of *Plodia interpunctella* (20, 21) and field-derived strains of *P. xylostella* (29, 42, 43), whereas in an *O. nubilalis* laboratory population resistance to *B. thuringiensis* subsp. *kurstaki* was found to be codominant (13).

One general assumption of studies of the inheritance of resistance is that the parent populations are homozygous. In previous studies, variation in the resistance of progeny of $F_1$ hybrid crosses showed that resistant alleles were present in susceptible laboratory populations (10, 41) and that susceptible alleles were present in selected resistant populations (17). In the present study, to increase homogeneity, four inbred lines derived from single-pair crosses were maintained by sib-mating for three generations and were used to produce $F_1$ and backcross progeny. Inbred lines which exhibited a decrease in resistance over time were discarded. A possible effect of this procedure was the exclusion of major resistance alleles that were heterozygous in either parent of the initial cross or the exclusion of minor alleles. However, the results of the single-pair crosses in this study agreed with the mass cross results, strengthening the overall conclusions.

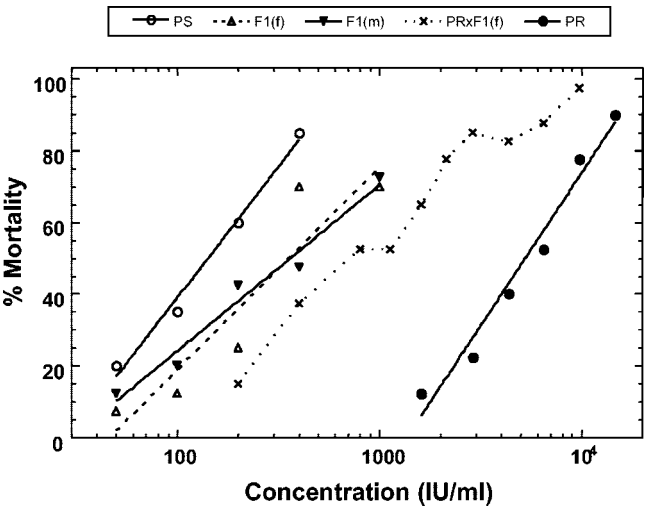


FIG. 4. Concentration-mortality curves for larvae of $P_S$-Cor, $P_R$-Cor, $F_1$ hybrids from the reciprocal crosses, and offspring of a backcross between $F_1$ and $P_R$-Cor.

The relative stability of resistance over 14 generations in the inbred lines suggests that the lines were homozygous for resistance; however, significant variation in the $LC_{50}$ did remain in at least two of the inbred lines. Therefore, it is unclear if susceptible alleles or multiple resistant loci were present in the inbred lines. However, the decrease in slope of the concentration-mortality lines from the $F_1$ progeny to the backcross progeny and the evident plateau in the backcross probit line suggested that resistance was due to a few major loci rather than a quantitative trait (22, 37, 42).

In the majority of studies on the inheritance of *B. thuringiensis* subsp. *kurstaki* resistance, resistance has corresponded to one or a few major loci (6, 13, 29, 43). The primary method currently used to determine the number of loci involved in resistance compares backcross results to mortalities predicted from a monogenic model. However, nonhomozygous parental lines could obscure the results of hybrid and backcross mortality assays and could lead to spurious rejection of monogenic models of resistance. Therefore, both the effect of nonhomozygous parental lines and multiple resistant loci were examined in this study.

No correspondence was found between the predictions of a monogenic model and backcross results in this study. Both the inclusion of a nonhomozygous $P_R$ population in a monogenic model and an additional resistance locus increased the correspondence between the observed and predicted results. A monogenic model with a resistance allele frequency of 0.80 resulted in one dose for which there was a significant deviation between the observed and predicted mortalities, as opposed to significant deviations for two to four doses when $p$ was equal to 1.0, whereas a two-locus model with epistatic effects (model C) produced a significant deviation at one dose in the UBC $F_1 \times P_R$ backcross and no deviations with the Cornell $F_1 \times P_R$ backcross. To distinguish between a model with a nonhomozygous resistant line and a model with multiple resistant loci, the results of the $F_1 \times P_S$ backcross were utilized. Given that the observed mortality of the $F_1 \times P_S$ progeny was lower than expected, a model with a proportion of susceptible alleles in the resistant population would not adequately describe the $F_1 \times P_S$ results. Therefore, the discrepancy between the monogenic model and the $F_1 \times P_R$ backcross was most likely due to the presence of more than one locus or more than two alleles in the resistant *T. ni* population.

Similar discrepancies between backcross results and models of monogenic inheritance have been found in other studies. For example, the resistance of *Leptinotarsa decemlineata* to the Cry3A toxin of *B. thuringiensis* subsp. *tenebrionis* (24) and the resistance of *S. littoralis* to Cry1C (7) did not correspond to monogenic inheritance. The resistance of *Pectinophora gossypiella* to Cry1Ac corresponded to a single resistance gene with three alleles or to more than one resistance locus (40). In field-derived populations of the diamondback moth, resistance to Cry1Ac did not correspond to monogenic inheritance in a population from Malaysia (30), and two different genes that confer resistance to Cry1Ab were present in a population originating from the Philippines (10). Direct tests of monogenic inheritance of Cry1C resistance in a *P. xylostella* population originating from New York suggested that significant deviations between observed and expected mortalities were the result of nonadditive polygenic inheritance or experimental error (44), and further tests indicated that there might be polygenic inheritance (45).

The presence of multiple resistance loci in *T. ni* is not surprising since the *B. thuringiensis* subsp. *kurstaki* toxin is comprised of five different toxic Cry proteins (Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, and Cry2B) (12). *T. ni* larvae have been shown to be most susceptible to Cry1Ac, followed by Cry1Ab and Cry2Aa, whereas Cry1Aa toxicity has varied from moderate to low (14, 23). In a previous study, a laboratory population of *T. ni* was selected for resistance to Cry1Ab, and no cross-resistance to Cry1Ac was found (8). Similarly, a strain of *P. xylostella* from the Philippines was shown to harbor multiple resistance genes that confer either resistance to only Cry1Ab (2) or combined resistance to Cry1Ab and Cry1Ac (10, 41). Therefore, it is possible that two separate loci that confer resistance to either Cry1Ac or Cry1Ab or both were present in the *T. ni* population due to selection with the multitoxin *B. thuringiensis* subsp. *kurstaki* formulation. In a selection of $P_R$-Cor with Cry1Ac alone, monogenic resistance to Cry1Ac was found, supporting the prediction that multiple resistance loci for the different toxins were present in this population (Wang, unpublished data).

A two-locus model with nonadditive effects (model C) provided the best fit to the observed backcross mortalities; however, a two-locus model with additive effects was adequate for the Cornell results. In model C, the $R_1S_1R_2R_2$ genotype responded like the $F_1$ hybrid progeny, thereby elevating the expected mortalities of the $F_1 \times P_R$ backcross from that of an additive two-locus model. In Tabashnik's (37) analysis of the determination of inheritance from backcross experiments, two-locus models with additive effects yielded equal and opposite expected differences on either side of the backcross $LC_{50}$. The differences observed with nonadditive two-locus models were consistently positive or negative over the dose range. In the present study, the observed mortalities were consistently higher for the $F_1 \times P_R$ progeny than expected from a two-locus model, suggesting the presence of epistatic effects.

A two-gene model, with nonadditive effects, of the inheritance of resistance in *T. ni* is undoubtedly more simplistic than the true nature of inheritance; however, it raises the possibility of epistatic interactions between loci. What is remarkable is that two genes for resistance to *B. thuringiensis* subsp. *kurstaki* with complex nonadditive interactions may have evolved in a *T. ni* population outside the laboratory. The probability of the evolution of resistance to two toxins is assumed to be extremely low, and this assumption provides the rationale for utilizing pesticide mixtures and rotations and the pyramiding of toxin genes in transgenic plants as resistance management strategies. It will, therefore, be pertinent to determine if epistatic interactions between loci that facilitate the evolution of resistance to multiple *B. thuringiensis* toxins in the field are a common occurrence. If epistatic interactions are common, then the prevalent models of resistance evolution may need to be reexamined.

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