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Mutated Cadherin Alleles from a Field Population of *Helicoverpa armigera* Confer Resistance to *Bacillus thuringiensis* Toxin Cry1Ac▽

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The cotton bollworm *Helicoverpa armigera* is the major insect pest targeted by cotton genetically engineered to produce the *Bacillus thuringiensis* toxin (transgenic Bt cotton) in the Old World. The evolution of this pest's resistance to *B. thuringiensis* toxins is the main threat to the long-term effectiveness of transgenic Bt cotton. A deletion mutation allele (r_1) of a cadherin gene (*Ha_BtR*) was previously identified as genetically linked with Cry1Ac resistance in a laboratory-selected strain of *H. armigera*. Using a biphasic screen strategy, we successfully trapped two new cadherin alleles (r_2 and r_3) associated with Cry1Ac resistance from a field population of *H. armigera* collected from the Yellow River cotton area of China in 2005. The r_2 and r_3 alleles, respectively, were created by inserting the long terminal repeat of a retrotransposon (designated *HaRT1*) and the intact *HaRT1* retrotransposon at the same position in exon 8 of *Ha_BtR*, which results in a truncated cadherin containing only two ectodomain repeats in the N terminus of *Ha_BtR*. This is the first time that the *B. thuringiensis* resistance alleles of a target insect of Bt crops have been successfully detected in the open field. This study also demonstrated that bollworm larvae carrying two resistance alleles can complete development on Bt cotton. The cadherin locus should be an important target for intensive DNA-based screening of field populations of *H. armigera*.

The global area of cotton (*Gossypium hirsutum* L.) genetically engineered to produce the *Bacillus thuringiensis* toxin (transgenic Bt cotton) planted in 2006 was 12.1 million hectares (12). Transgenic Bt cotton has been adopted extensively to reduce reliance on insecticides and save labor and costs in many countries. The rapid evolution of resistance by target pests could completely undermine the advantages of this new technology. Despite the expectations of many scientists and the predictions from models, resistance to Bt crops in the field has not yet been reported after a decade of adoption of Bt crops (2, 10, 19). However, we should not underestimate the ability of target insects to adapt to Bt crops, because resistance to Bt crops by target insects remains a question not of "if" but of "when."

Invertebrates have demonstrated a range of resistance mechanisms to *B. thuringiensis* toxins (11). The diamondback moth, *Plutella xylostella*, has already evolved resistance to *Bacillus thuringiensis* subspecies in the field (16, 18). The cotton bollworm *Helicoverpa armigera* (Hübner) is the most important target pest of transgenic Bt cotton in China, India, Australia, and South Africa. Laboratory selection experiments from several countries demonstrate that this pest has the capacity to evolve resistance to the *B. thuringiensis* toxin Cry1Ac protein contained in Bt cotton (1, 14, 24).

Understanding the molecular mechanisms of *B. thuringiensis* resistance is critical for developing efficient DNA-based monitoring of the resistance frequency for early resistance warning and is key to informed and proactive resistance management. Mutations in a cadherin gene were identified as the causes for the recessive resistance to Cry1Ac in three major lepidopteran cotton pests, *Heliothis virescens*, *Pectinophora gossypiella*, and *H. armigera* (7, 15, 24). However, the mutant alleles found in laboratory-selected strains may not be identical to those present in field populations (3). In fact, two recent publications reported that no resistance alleles of cadherin of *P. gossypiella* and *H. virescens* were detected from field populations after large-scale screening (8, 20). Because the cadherin genes are not essential to the survival of *P. gossypiella*, *H. virescens*, and *H. armigera* (at least under laboratory conditions) (7, 15, 24), any mutation that nullifies the cadherin protein as a key functional receptor could confer Cry1Ac resistance. It is therefore very important to establish whether any such mutant cadherin alleles are present in the field, leading to the development of an appropriate DNA-based resistance surveillance program.

As suggested by Yang et al. (26), it is both feasible and necessary to screen for resistance-associated cadherin alleles from field populations of *H. armigera* by using the single-pair mating technique (or F_1 screen). Field-derived moths of *H. armigera* can be crossed individually with moths from the laboratory-selected GYBT strain (r_1r_1), which shows recessive resistance to Cry1Ac (24). The mutated cadherin genotype status of field-derived moths will be revealed if their F_1 offspring show significant Cry1Ac resistance. Here, we report the isolation of two new cadherin alleles conferring Cry1Ac resistance from a field population of *H. armigera* by using this strategy. Our results provide an important foundation for a DNA-based detection method for monitoring the frequency of mutant cadherin alleles in field populations of *H. armigera*.

MATERIALS AND METHODS

Insect strains. A resistant strain (GYBT) of *H. armigera* was derived from the original GY strain (collected from the Gaoyang district of Hebei Province of

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China) by 28 generations of selection with activated Cry1Ac by surface contamination bioassay. The GYBT strain has 564-fold resistance to Cry1Ac compared with that of the GY strain (24) and is homozygous for the mutant cadherin r_1 allele (26). Detailed information for the GYBT strain is presented in the report by Xu et al. (24). More than 20,000 eggs were collected from transgenic Bt cotton plants in Anyang (36°06′N, 114°21′E) of Henan Province, China, in early July of 2005. Bt cotton has been planted in Anyang since 1997 and has exceeded 90% of the total cotton area since 2001. Field-collected insect eggs were moved from Bt cotton leaves to an artificial diet, hatched, and reared to the second-instar larval stage for screening bioassays.

Larvae of *H. armigera* were reared on an artificial diet based on wheat germ and soybean powder (17) at 27 ± 1°C with a 16:8 light/dark (L:D, respectively) photoperiod. Adults were held under the same temperature and light conditions at an rH of 60% and were supplied with a 10% sugar solution.

Bioassay. A surface contamination bioassay was used in this study. The toxin suspensions of activated Cry1Ac toxin were diluted with a 0.01 M, pH 7.4, phosphate buffer solution (PBS). PBS was used as a control. A disc of artificial diet (1.6-cm diameter) was put into a 24-well plate and made to fit into the inner wall and bottom of the plate by gentle pressure. The toxin solution (100 μl) was applied to the diet surface and allowed to air dry. One second-instar larva, starved for 4 h, was placed in each well of the plate, which was covered with two layers of nylon net to prevent escape. The mortality rates (for dead larvae or those with a body mass of less than 5 mg) and the body masses of survivors were measured after being maintained for 5 days at 26 ± 1°C, with a 16:8 L:D photoperiod and a 60% rH.

Biphasic screen. A schematic diagram of the experimental design for the biphasic screen of mutant cadherin alleles in field populations of *H. armigera* is shown in Fig. 1. In the first phase, second-instar larvae hatched from field-collected eggs were screened with a discriminating dose of activated Cry1Ac (1 μg/cm^2 of diet surface, the 95% lethal concentration [LC_{95}] of the susceptible homozygotes), which killed most of the susceptible larvae. In the second phase, survivors from the first-phase screen were individually crossed to homozygous-resistant moths (r_1r_1) from the GYBT strain, and then F_1 offspring (48 second-instar larvae) from each single-pair family were screened with a second discriminating dose of activated Cry1Ac (2.5 μg/cm^2 of diet surface, the LC_{95} of the r_1S heterozygotes). If a field-derived moth was a susceptible homozygote (*SS*), then all of the F_1 offspring would be heterozygous (r_1S) and would have only a few survivors following the second discriminating dose. If a field-derived moth was a heterozygote (r_xS) at the *Ha_BtR* locus, then 50% of the F_1 offspring would be resistant homozygotes (r_1r_x), which would survive the second discriminating dose. If a field-derived moth was homozygous (r_xr_x) at the *Ha_BtR* locus, then 100% of the F_1 offspring would be resistant homozygotes (r_1r_x).

cDNA cloning of *Ha_BtR* alleles of *H. armigera*. Total RNA from the midguts of final-instar larvae was extracted with an SV total RNA isolation system (Promega, Madison, WI), according to the manufacturer's instructions, and reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Promega). Two pairs of primers (1-F/7-R and 7-F/9-R) were used to amplify the full-length cDNA of the r_1 allele originating from the GYBT strain. Three primer pairs (1-F/4-R, 4-F/7-R, and 7-F/9-R) and 3′ rapid amplification of cDNA ends were used to amplify cDNA fragments of other *Ha_BtR* alleles derived from field moths. The sequences of primers can be found in the supplementary material of the article by Yang et al. (26).

PCR amplification of the genomic DNA fragments of the r_2 and r_3 alleles of *Ha_BtR*. Genomic DNA of individual adults was prepared according to the method detailed by Yang et al. (26). The genomic sequences flanking the mutation sites of the r_2 and r_3 alleles (between exon 1 and exon 11) were determined by sequencing overlapping PCR products amplified from genomic DNA of the field-derived moths of the single-pair families 15 and 17.

PCR products of the expected size were excised and purified using a Wizard DNA purification system (Promega) and cloned using pGEM-T easy vector (Promega). All clones were sequenced by Invitrogen (Shanghai, China).

DNA-based detection of the r_1, r_2, and r_3 alleles of *Ha_BtR*. Allele-specific PCR for the r_1 allele was performed as described by Yang et al. (26). A pair of specific primers (the forward cadherin primer for family 15 [15Cad-F], 5′-AGACAGGGACACTCTTGAGAAG-3′; and 15Cad-R, 5′-GGCTCGTTCGTTACACTCAGTA-3′) was used to detect both the r_2 and the r_3 alleles of *Ha_BtR*, and the expected length of the target genomic DNA fragment was 180 bp.

The amplification-reactive mixture (25 μl) contained 100 ng of genomic DNA, 1 μM of each primer, 150 μM of each deoxynucleoside triphosphate, 2 mM of $MgCl_2$, 1 U of r*Taq* DNA polymerase, and 2.5 μl of 10× PCR buffer. PCR was performed for 30 cycles of 30 s at 94°C, 1 min at 55°C, and 2 min at 72°C. PCR products were analyzed by 1.5% agarose gel electrophoresis and ethidium bromide staining.

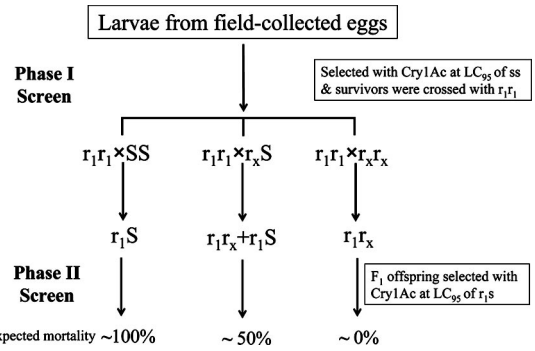

FIG. 1. Schematic diagram of the experimental design for a biphasic screening for cadherin mutations in field populations of *Helicoverpa armigera*. The laboratory-selected GYBT strain is homozygous for the cadherin allele r_1. The cadherin allele r_x is putatively carried by field-collected insects.

Survival test on a Cry1Ac-coated diet and on Bt cotton for larvae carrying cadherin mutations. The 15Sel strain was established from survivors of the family 15 after the second screen. In the seventh generation of the 15Sel strain, 48 second-instar larvae were exposed to an activated Cry1Ac-coated diet (2.5 μg/cm^2 of diet surface), which killed all of the susceptible and most of the heterozygous larvae. Five days later, survivors (those with a body mass of more than 5 mg) were collected, and their genomic DNA was extracted for cadherin genotyping.

Transgenic Bt cotton plants (Bollgard DP410B, Monsanto, China) were grown in a plant growth chamber (25°C to 30°C). Five plants were planted in each pot (20-cm diameter). Ten first-instar larvae (1 day old) from the seventh generation of the 15Sel strain were placed on each of 50 transgenic Bt cotton plants with five to six fully expanded leaves.

Nucleotide sequence accession numbers. Genomic DNA sequences of the r_2 and r_3 alleles of *Ha_BtR* have been deposited in the GenBank database under the accession numbers EU016078 and EU016079, respectively. cDNA sequences of the r_2 and r_3 alleles of *Ha_BtR* are available in the GenBank database under accession numbers DQ973282 and EU016080, respectively.

RESULTS

Biphasic screen for cadherin mutants from field populations of *H. armigera*. Second-instar larvae (9,984) of *H. armigera* from a population from Anyang district in Henan Province were individually treated with 1 μg of activated Cry1Ac/cm^2 of diet surface. Of the 123 surviving insects, 70 larvae completed development. The resulting 70 moths were single-pair crossed with adults from the laboratory-selected GYBT strain. Twenty-two single-pair crosses produced fertile offspring. F_1 offspring from these 22 single-pair families were subjected to a secondary screen (activated Cry1Ac, 2.5 μg/cm^2 of diet surface). The survival rates and average body weights of the survivors of each single-pair family are shown in Fig. 2. Of 22 single-pair families, 5 single-pair families had about 50% survival (family 5, 51%; family 13, 50%; family 15, 63%; family 17, 53%; and family 112, 52%); others were less than 30%. The average body weights of survivors from the second screen varied significantly among these five single-pair families. The average body weights of survivors from families 15 (14.0 ± 1.2 mg) and 17 (17.0 ± 1.4 mg) were most similar to that of survivors from the GYBT strain (18.9 ± 1.2 mg), with the dosage of 2.5 μg/cm^2 of diet surface, but all of them were significantly heavier than survivors from families 5 (7.2 ± 0.7 mg), 13 (6.7 ± 0.4 mg), and 112 (7.7 ± 0.8 mg) (analysis of variance, $\alpha = 0.05$). This suggested that the field-derived par-

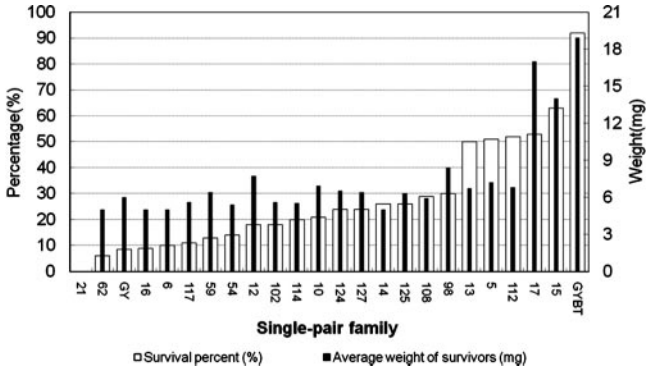

FIG. 2. Survival percentage and average body weight of survivors of the second screen with Cry1Ac (2.5 μg/cm^2 of diet surface) with F_1 offspring of single-pair matings between survivors from the primary screening and moths of the GYBT strain of *H. armigera*.

ents of families 15 and 17 were possibly heterozygous for cadherin mutations.

Cloning and sequencing of cDNA of cadherin alleles from field populations of *H. armigera*. cDNA of F_1 survivors (three final-instar larvae) from the second screen of the aforementioned five single-pair lines was prepared for cadherin genotyping. cDNA of *Ha_BtR* from the three surviving F_1 larvae from each of the five single-pair families (5, 13, 15, 17, and 112) was individually cloned and sequenced. Each F_1 survivor carried two alleles of *Ha_BtR*, one from the GYBT strain (r_1) and the other from the field-derived parent. As expected, the r_1 allele was recovered from all F_1 survivors (GenBank accession no. DQ973284, DQ973285, DQ523168, DQ973286, and DQ973289 for families 5, 13, 15, 17, and 112, respectively). In the F_1 survivors of family 15, a new transcript of *Ha_BtR* (GenBank accession no. DQ973282), putatively encoding a truncated cadherin protein (with 371 amino acids relative to 1,730 amino acids of the wild type), was cloned and sequenced. In the F_1 survivors of family 17, another new transcript of *Ha_BtR* (GenBank accession no. EU016080, with the difference of only one nucleotide in the coding region compared with that in DQ973282) was cloned and sequenced from each of the three larvae. In the F_1 survivors of families 5, 13, and 112, besides the r_1 allele from the GYBT strain, we found only the full-length wild-type transcript of *Ha_BtR*, which was passed from their field-derived parents.

Identification of mutations in the genomic DNA of the r_2 and r_3 alleles of *Ha_BtR*. To further confirm whether the two new transcripts originated from field-derived parents, genomic DNA sequences of *Ha_BtR* of the field-derived moths were checked. From the field-derived parent of family 15, a 5,813-bp genomic DNA fragment (GenBank accession no. EU016078, named the r_2 allele of *Ha_BtR*) encoding the new transcript of DQ973282 was successfully amplified and sequenced. The insertion of a 1,007-bp fragment was identified between bp 111 and 112 of exon 8 of *Ha_BtR*. From the field-derived parent of family 17, a 10,605-bp genomic DNA fragment (GenBank accession no. EU016079, designated the r_3 allele of *Ha_BtR*) encoding the new transcript of EU016080 was obtained through a set of PCR amplifications. The insertion of a 5,800-bp fragment was also identified between bp 111 and 112 of exon 8 of *Ha_BtR*. The wild-type allele of *Ha_BtR* could also be detected with allele-specific PCR in the field-derived parents of both family 15 and family 17. It confirmed that these two field-derived moths are heterozygous for the *Ha_BtR* mutations, which is consistent with the results from the F_1 progeny bioassay and cDNA sequencing.

The 5,800-bp insertion in the r_3 allele was identified as a long terminal repeat retrotransposon (LTR-RT), which was named *HaRT1* (DNA sequences are included in GenBank accession no. EU016079). It revealed the common characteristics of an LTR-RT: LTRs of 1,007 bp, *gag* and protease domains (in open reading frame 1 [ORF1] of *HaRT1*), retrotranscriptase, and an RNase H domain (in ORF2) and an integrase domain (in ORF3). Interestingly, the sequence of the insertion in the r_2 allele was almost identical to that of the LTR of *HaRT1* (only 2 bases out of 1,007 bp were different). At a position 9 bp downstream of the 5′ end of the inserted LTR, there was a premature stop codon (TGA), which caused the truncation of the r_2 and r_3 alleles. The genomic structures of the wild-type allele and the r_1, r_2, and r_3 alleles of the *Ha_BtR* gene from *H. armigera* are shown in Fig. 3. The truncated transcripts of the r_2 and r_3 alleles putatively encode only 2 of the 11 ectodomain repeats in the N terminus of *Ha_BtR*, which lacks transmembrane and toxin-binding domains.

Survival of larvae carrying cadherin mutations on a Cry1Ac-coated diet and on Bt cotton. Survivors of family 15 following the second screen were maintained in the laboratory as the founders of the 15Sel strain for further investigation. In the seventh generation of the 15Sel strain, survival of larvae on a Cry1Ac-coated diet (2.5 μg/cm^2 of diet surface) and on Bt cotton was tested, and the results are detailed in Table 1.

For the untreated larvae from the 15Sel family, the ratio among homozygous-resistant individuals (carrying two *r* alleles, i.e., r_1r_1, r_2r_2, or r_1r_2), heterozygotes (r_1S or r_2S), and homozygous susceptible individuals (*SS*) is nearly 1:2:1 ($\sum\chi^2 = 0.36 < \chi^2$ [$P = 0.05$]; df = 2). Following treatment with 2.5 μg Cry1Ac/cm^2 of diet surface, 87% of survivors were homozygous individuals, and the remaining 13% were heterozygotes. This indicates that *H. armigera* carrying two mutated cadherin alleles can survive but that most heterozygotes carrying one mutated cadherin allele and all susceptible homozygotes cannot survive on this discriminating dose, exemplifying the recessive nature of the gene.

Treated with transgenic Bt cotton, only 2 out of 500 larvae (presumably containing about 25% larvae with two resistance alleles, i.e., around 125 larvae) completed their larval development. These two survivors (pupae) were determined to be r_1r_1 and r_1r_2. This demonstrates that *H. armigera* carrying two mutated cadherin alleles can survive on transgenic Bt cotton, and the survival is about 1 to 2%. Heterozygotes carrying one mutated cadherin allele cannot survive on transgenic Bt cotton. Under the conditions of selection by transgenic Bt cotton in this study, resistance conferred by the disruption of *Ha_BtR* is completely recessive.

DISCUSSION

The F_1 screen method was first developed to estimate the frequency of major *B. thuringiensis* resistance alleles in field populations of *H. virescens* (9). Field-trapped males and males

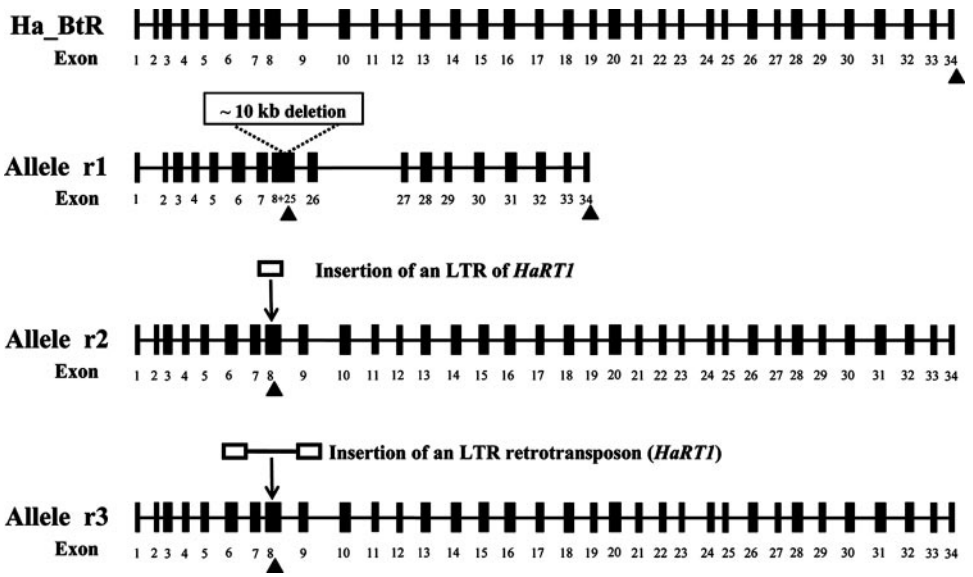

FIG. 3. Genomic structures of the wild-type allele and the r_1, r_2, and r_3 alleles of the *Ha_BtR* gene from *H. armigera*. Exons are shown as vertical bars and introns as thin horizontal lines. The stop codon and poly(A) sites are marked with ▲.

that developed from field-collected eggs of *H. virescens* were used in single-pair crosses to laboratory-selected homozygous-resistant YHD2 strain virgin females, and their progeny were screened with a discriminating dose of Cry1Ac. Three out of 1,025 field-derived males were tested heterozygous for a major resistance allele of *BtR-4*, and the field frequency of the *BtR-4* allele in field populations of *H. virescens* was estimated to be 0.0015 (9). *BtR-4* was identified later as a cadherin gene disrupted by the insertion of a retrotransposon (*Hel-1*) (7). However, the *Hel-1* insertion was not detected from the three field-caught males thought to carry *BtR-4* in a recent study (8). There are two possible explanations for this discrepancy: (i) the three field-caught males have other kinds of cadherin mutation than the *Hel-1* insertion or (ii) the *BtR-4* frequency could be overestimated because the F_1 screen procedure may detect dominant resistance alleles at other loci. In the present study, the progeny tests indicated that the field-derived parents of families 5, 13, and 112 may have been heterozygous for a *Ha_BtR* resistance allele, but no mutation was found other than that from the GYBT strain. This may be explained by the second-screen dosage of Cry1Ac (2.5 μg/cm^2 of diet surface, LC_{95} of the susceptible strain) not being high enough to kill 100% of the *Ha_BtR* heterozygotes. This is supported by results from the survival tests (Table 1). In future, the second-screen dosage should be increased to an LC_{99} (5 μg/cm^2 of diet surface) of the susceptible GY strain of *H. armigera*, which is expected to reduce ambiguity when choosing candidate families with resistance mutations from the field.

TABLE 1. Genotypes of survival of the 15Sel strain of *H. armigera* on Cry1Ac-coated diet and on Bt cotton

Treatment	No. of insects genotyped	No. of survivors of cadherin genotypes:					
		r_1r_1	r_2r_2	r_1r_2	r_1S	r_2S	SS
Artificial diet (without *B. thuringiensis* toxin)	50	4	2	7	11	12	14
Artificial diet with Cry1Ac (2.5 μg/cm^2)	15[a]	2	1	10	1	1	0
Bt cotton (DP410B)	2[b]	1	0	1	0	0	0

[a] Survivors of 48 larvae reared on a Cry1Ac-coated diet (2.5 μg/cm^2 of diet surface).
[b] Number of pupae that survived from Bt cotton plants infested with 500 1-day-old larvae of the 15Sel strain.

The biphasic F_1 screen employed in this study is very effective and promising for screening diverse resistance alleles in field populations. The primary screen was designed with the assumption of incomplete recessiveness of Cry1Ac resistance in *H. armigera*. This step is intended to keep most resistance alleles in the screen and to limit the scale of the secondary screen, providing huge labor and cost savings. It should be a standard practice before the initiation of any DNA-based detection program.

Pink bollworm (*P. gossypiella*) resistance to Cry1Ac was monitored for 8 years with laboratory bioassays of strains collected annually from 10 to 17 cotton fields in Arizona, and bioassay results showed no net increase from 1997 to 2004 in the mean frequency of pink bollworm resistance to *B. thuringiensis* toxin (19, 21, 22). A recent DNA-based screening of pink bollworm resistance to *B. thuringiensis* toxin across Arizona, California, and Texas from 2001 to 2005 detected no resistance alleles (20). Delayed resistance of target pests to Bt crops compared with the rapid evolution of resistance with laboratory selection could result from one or more of the following factors: refuges of non-Bt crops that enable survival of susceptible insects, recessive inheritance of resistance, incomplete resistance, and field fitness costs associated with resistance (5, 19).

Bt cotton has been commercialized in China since 1997. In 2006, China's Bt cotton area increased to 3.5 million ha (about two-thirds of the total cotton area) (12). In the Yellow River cotton area, Bt cotton has comprised nearly 100% of the crop for several years. The high-dose/refuge strategy is not man-

dated for resistance management in China. The susceptibilities of Chinese field populations of *H. armigera* to the Cry1Ac toxin were monitored using bioassays from 1997 to 2006, and no resistance was detected (23). In the current study, providing that the cadherin mutation frequency in the 70 survivors from the second screen is similar to that in the 22 survivors producing fertile progeny, the resistance mutation frequency in the Anyang field population in 2005 can be estimated to be around 3.5×10^{-4}. After nearly a decade of Bt cotton planting, the frequency of mutant cadherin alleles in the Anyang population of *H. armigera* is still low. Fitness cost, incomplete resistance, and alternative host crops as a natural refuge could be key factors for delayed resistance of *H. armigera* to Bt cotton in the small-scale cotton system of China. Ongoing work in this area will address these questions.

The mode of action of Cry1Ac toxins is a multistep process that involves interactions with several receptor molecules leading to membrane insertion and cell lysis (4). Cadherin appears to be one key functional receptor in the multistep intoxication process, at least in *H. virescens*, *P. gossypiella*, and *H. armigera* (25). It is possible that a large number of mutations in the cadherin gene can cause *B. thuringiensis* resistance, complicating DNA-based *B. thuringiensis* resistance-monitoring strategies. Together with previous results (24, 26), three resistance alleles (r_1, r_2, and r_3) of *Ha_BtR* of *H. armigera* have now been detected. All three alleles are associated with a mutation in exon 8 of *Ha_BtR*, which may be a regional hot spot for mutations. If other mutations are also uncovered in this region, it will simplify DNA-based monitoring methods.

As far as we know, this is the first time that *B. thuringiensis* resistance alleles of a target insect of a Bt crop have been successfully detected in the field. The biphasic F_1 screen method could be used to trap resistance alleles of other populations of *H. armigera* or other pest insects. Although the presence of additional resistance alleles at other loci cannot be excluded, the cadherin locus of *H. armigera* (*Ha_BtR*) should be a major resistance locus for molecular monitoring of resistance to Bt cotton, given that bollworm larvae with two mutant cadherin alleles can complete development on Bt cotton, albeit at a low frequency.

Transposable elements are mobile sequences that are abundant within eukaryotic genomes and that are endogenous mutators with the potential to produce a wide array of changes in the genomes of their hosts (13). Transposable elements can make insects become resistant to insecticides either by altering the metabolic enzymes that detoxify insecticides or by altering the targets of insecticides (6). In laboratory-selected *Heliothis virescens*, disruption of a cadherin (HevCLP) mediated by a defective LTR-RT (*Hel-1*) insertion is responsible for high levels of resistance to the *B. thuringiensis* toxin Cry1Ac (7). In the current study, an insertion of an LTR-RT (*HaRT1*) in the r_3 allele of *Ha_BtR* resulted in a truncated cadherin, thereby conferring resistance to the *B. thuringiensis* toxin Cry1Ac in *H. armigera*. The r_2 allele of *Ha_BtR* was disrupted by an insertion of a remnant LTR of *HaRT1*. Insertions in the r_2 and r_3 alleles occurred at exactly the same site in the *Ha_BtR* gene. This suggests that the r_2 allele originated from an earlier insertion event of *HaRT1* and that the r_3 allele of *Ha_BtR* came from a more recent insertion event of *HaRT1*.

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