Sequence variation in the cadherin gene of Ostrinia nubilalis: a tool for field monitoring

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Keywords
Cadherin, Bacillus thuringiensis receptor, Midgut cDNA

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Sequence variation in the cadherin gene of *Ostrinia nubilalis*: a tool for field monitoring

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Abstract

Toxin-binding proteins of insect midgut epithelial cells are associated with insect resistance to *Bacillus thuringiensis* (Bt) Cry toxins. A 5378 nt cDNA encoding a 1717 amino acid putative midgut cadherin-like glycoprotein and candidate Cry1Ab toxin-binding protein was characterized from *Ostrinia nubilalis*. Intraspecific alignment of partial *O. nubilalis* cadherin gene sequences identified variance within proposed Cry1A toxin binding region 2 (TBR2), 1328IPLQTSILVVT\[I/V\] N1340, and flanking Cry1A toxin binding region 1 (TBR1), 861DIEIEIIDTNN871. DNA sequence and PCR-RFLP detected single nucleotide polymorphism between cadherin alleles, and pedigree analysis demonstrated Mendelian inheritance. A population sample from Mead, Nebraska showed allelic polymorphism. These assays may be useful for linkage mapping and field surveillance of wild populations and of *O. nubilalis*.

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Keywords: Cadherin; *Bacillus thuringiensis* receptor; Midgut cDNA

1. Introduction

Resistance to insecticides commonly evolves in agricultural fields when insecticides are frequently applied at high doses to control insect pests (Georgiou and Lagunes-Tejeda, 1991). The western corn rootworm (*Diabrotica* sp.; Miota et al., 1998; Zhu et al., 2001) evolved field resistance to carbaryl insecticides. Indian mealmoth (*Plodia interpunctella*; McGaughey, 1985) and diamondback moth (*Plutella xylostella*; Tabashnik et al., 1990) evolved resistance to insecticides with toxins of the bacterium *Bacillus thuringiensis* var. kurstaki. European corn borer, *Ostrinia nubilalis* (Lepidoptera Crambidae), a lepidopteran pest of maize, has been controlled with foliar applications of the bacterium *B. thuringiensis* (Bt) var. kurstaki. Incorporation of the *B. thuringiensis* Cry1Ab toxin gene into maize germplasm (Koziel et al., 1993) results in expression that controls *O. nubilalis* larvae (Walker et al., 2000). High selection pressure and adaptation to transgenic toxins by *O. nubilalis* larvae raises concerns regarding sustainability of Bt technology. Commercial hybrid maize expressing Cry1Ab toxins were grown on 29% of 2003 United States corn acres (22.9 million acres; USDA-NASS, 2003). Resistance has not been detected in the field; but
moderate resistance was found in *O. nubilalis* laboratory colonies fed artificial Bt containing diets (Bolin et al., 1999; Huang et al., 2001; Chaufaux et al., 2001).

Modification of midgut receptor proteins that bind Cry toxins may reduce susceptibility of insects. Ligand blots demonstrate that Cry1Ab binds to a 220-kDa cadherin-like glycoprotein, and 145 and 154-kDa aminopeptidase (APN) isoforms in *O. nubilalis* (Hua et al., 2001). A *Manduca sexta* 210-kDa E-cadherin-like peptide bound activated (trypsinized) Cry1A toxin (Francis and Bulla, 1997). Midgut expressed cadherin-like cDNAs were isolated from *M. sexta* (Vadlamudi et al., 1995), *Bombyx mori* (Nagamatsu et al., 1998a), *Heliothis virescens* (Gahan et al., 2001), and *Pectinophora gossypiella* (Morin et al., 2003). Furthermore, a cadherin knockout from a transposon insertion resulted in 40,000-fold resistance in the *H. virescens* YHD2 colony (Gahan et al., 2001). Segregation of alleles at a susceptible locus was associated with resistance in *P. gossypiella* strain AZP-R (Morin et al., 2003). CRY1A domain II binds lepidopteran midgut epithelial receptors (de Maagd et al., 2001) by interacting with two cadherin motifs (Gomez et al., 2003). Cadherin Cry1A toxin binding region (TBR1), 865NITIHITDTNN875 was identified on *M. sexta* gene Br-R1, and implicated in binding loop 2 of CRY1A domain II (Gomez et al., 2001, 2002a, b). A second binding region (TBR2) was identified in *B. mori* cadherin repeat 9 (CR9; Nagamatsu et al., 1999) and *M. sexta* cadherin CR11 (Dorsch et al., 2002). Cadherin TBR2 was narrowed to 12 amino acids of Br-R1, 1331IPLPASILTVTV1342, and predicted to interact with loop z-8 of CRY1A domain II (Gomez et al., 2003). Effects of cadherin toxin binding region variation on resistance traits are unknown.

The extracellular domain of cadherin peptide also has been implicated as a Cry1Ab binding receptor in the *O. nubilalis* midgut (Hua et al., 2001). Candidate Cry1Ab binding receptor genes have not been reported in *O. nubilalis*. In this paper, we report research that isolated a full-length midgut cadherin-like cDNA from 5th instar *O. nubilalis* larvae, determined allelic variation (single nucleotide polymorphism; SNPs) in and near two putative Cry1Ab toxin-binding regions (TBR1 and TBR2), and characterized Mendelian segregation of SNPs among pedigrees by PCR-RFLP. Additionally, in a preliminary field screening experiment we estimated cadherin allele variation in a susceptible population of *O. nubilalis* near Mead, Nebraska.

### 2. Materials and methods

#### 2.1. Cadherin alignment and phylogeny

A 3048 residue consensus alignment was constructed among 11 lepidopteran cadherins, excluding *O. nubilalis*, using Align X software (Informax, San Francisco, CA; gap penalty = 5). Regions of peptide similarity were identified and corresponding cDNA sequence used to design degenerate 3′ RACE oligonucleotides with Primer3 (Rozen and Skaletsky, 1998; see primer Cad3pR in Section 2.3). Twelve lepidopteran cadherin amino acid sequences, including *O. nubilalis*, were placed into a single phylogeny using programs from the PHYLIP package (Felsenstein, 1989). One thousand bootstrap resampling steps were produced by the SeqBoot program, parsimony trees were generated using AA pars, a strict consensus tree was estimated from all possible phylogenies with CONSENSE, and was viewed using TreeView (Page, 1996).

#### 2.2. Complementary DNA (cDNA) synthesis and cloning

Ten dissected larval *O. nubilalis* midguts were bulked, ground to powder in liquid nitrogen, and RNA extracted with RNeasy extraction kits (Qiagen, Valencia, CA) according to manufacturer instructions. First strand cDNA synthesis used 0.5 μM of poly(T) adapter (PT-AD; GGT GTA ATA CGA CGG CCT GGA ATT), 10 μM of poly(T) adapter primer Cad3pR and poly(T) adapter primer Cad5pR (CTG CAT CGA CKC T) was designed using Primer3 (Rozen and Skaletsky, 1998) to anneal transcript sequence corresponding to a NNTPPLT amino acid sequence, which was conserved among lepidopteran cadherins. Hot start PCR of first strand cDNA used 5 pmol each of degenerate primer Cad3pR and poly(T) adapter primer 1 PT-Adc1 (GTG TAA TAC GAC GGC CTG G), 1.0 μM *Tli* proofreading polymerase (Promega), 100 μM dNTPs, 1.5 mM MgCl₂, and 2.5 μl of 10 × thermal polymerase buffer (Promega) in a 25 μl reaction volume. A PTC-100 thermocycler (MJ Research, Watertown, MA) cycled 40 times at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 4 min. Amplification was confirmed and fragment size estimated by electrophoresis of 10 μl PCR product on a 10 cm 1.5% agarose EDTA gel containing 0.5 μg/ml ethidium bromide. PCR product was ligated using the pGEM-T easy cloning system (Promega) by overnight incubation at 4 °C, and was used to transform 80 μl *E. coli* SURE (Stratagene). DNA sequencing of plasmid inserts used dye-terminator cycle sequencing (DTCS) quick-start kits (Beckman-Coulter, Fullerton, CA) and fragments separated on a CEQ8000 capillary electrophoresis system (Beckman-Coulter). Identity of insert sequence was checked by BLAST search, and transcript-specific 5′ RACE primers designed (Primers3; Rozen and Skaletsky, 1998).

Oligonucleotide primer Cad3pR (CTG CAT CGA TCG CCT CGT T) was designed from 3′ RACE cDNA...
sequence. First Choice RLM RACE kit (Ambion) was used to isolate 5′ cDNA end of the *O. nubilalis* cadherin transcript according to manufacturer instructions; RLM-RACE template was generated in 20 μl reactions with 1 μg of midgut total RNA. Subsequent hot start PCR used 1.0 U *Tli* proofreading polymerase (Promega), 200 μM dNTPs, 1.5 mM MgCl₂, and 2.5 μl of 10 × thermal polymerase buffer (Promega), 1 μl RLM mRNA template, 5 pmol each of degenerate primer Cad5pR, and 1 μl RLM-RACE 5′ adapter outer primer (Ambion; GCT GAT GGC GATG AAT GAA CAC) in a 25 μl reaction. A PTC-100 thermocycler (MJ Research) cycled 40 times at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 4 min. RT-PCR products were ligated, cloned, and sequenced as described previously.

2.3. Polymorphism with putative cadherin Cry1Ab toxin binding domains

Two putative Cry1A toxin-binding domains, 861DIEIEIHTNN871 (TBR1) and 1328IPLQT-SILVVTV1339 (TBR2), were identified from a single 1717 residue *O. nubilalis* midgut cDNA open reading frame (ORF). TBR1 and TBR2 were PCR amplified using primers OnCadTBR1-F (CAA ATT GAG GTG GAT GGC AA) with OnCadTBR1-R (TGT CAC GGT GGG AGT ACA), and OnCadTBR2-F (TYT CTT CAACAGACGACA A) with OnCadTBR2-R (ACC CGT GGT GTA AGC AAC TCC C). PCR products were referred to as *O. nubilalis* Cry1A toxin binding region 1 (OnTBR1) and 2 (OnTBR2), and amplified using 2.5 mM MgCl₂, 50 μM dNTPs, 7.5 pmol of each primer, 0.9 U *Tag* DNA polymerase (Promega), and 100 ng of DNA template (equal mix of 10 individual DNA samples) in a 12.5 μl reaction. PTC-100 thermocycler conditions used 95 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 20 s. OnTBR1 and OnTBR2 were ligated into pGEM-T vector (Promega), and used to transform *E. coli* SURE (Stratagene). Plasmid inserts were sequenced as described previously. Sequence data were imported into Vector NTI Suite 7.0 (Informax, San Francisco, CA) and multiple sequence alignments were made using AlignX software (Informax; gap penalty = 5).

A third PCR product (OnTBR2-s) used primer OnCadTBR2-F with OnCadTBR2-R2 (CTY AGC TTT TGC ASG AGA AAA G) to amplify an *O. nubilalis* cadherin gene region containing 1328IPLQT-SILVVTV1339. The OnTBR2-s PCR product is smaller and eliminates length polymorphism contained within an intron spanned by the original OnTBR2 product, thus facilitated ease of SNP genotyping. Polymorphic restriction endonuclease cleavage sites from OnTBR1, NdeII, RsaI, *Tag I*, and *Tsp*509I, and OnTBR2, *Cfo I*, *Hae*III, and *Hpy*CH4III, were identified from DNA sequence alignments. All endonucleases were purchased from Promega, except *Tsp*509I and *Hpy*CH4III (New England BioLabs). PCR-RFLP reactions of fragments OnTBR1 and OnTBR2-2 used PCR conditions described previously scaled to 25 μl. *Cfo I*, *Nde*II, *Rsa*I, *Tag*I, and *Tsp*509I digests included 6.0 μl of PCR product, 2.5 μl 10 × Buffer, 0.1 mg/μl BSA, and 0.25 U of enzyme in 25 μl OnTBR2-2 *Hae*III and *Hpy*CH4III double digestions used 0.5 U of each enzyme. Reactions were incubated at 37 or 60 °C (*Tsp*509I) for 8–14 h. Entire digest volumes were loaded onto 1.0 mm × 16 cm 6% polyacrylamide (19:1 acrylamide: bisacrylamide) 0.5 × TBE gels, and separated at 140 V for 6 h. A 25 bp step-ladder (Promega) was used for size comparison. Gels were stained with ethidium bromide, and digital images taken under UV illumination on a PC-FOTO/Eclipse Documentation System (Fotodyne, Hartland, WI). Allele frequencies for cadherin loci were estimated from 96 *O. nubilalis* adults.

2.4. Cadherin allele frequencies and Mendelian inheritance

Over 300 adult *O. nubilalis* were collected in a single light trap at a non-Bt corn field near Mead, Nebraska in August 2002 (contributed by John Seymour, University of Nebraska), and were samples stored separately in 1.5 ml microcentrifuge tubes at −20 °C. A subsample of 92 individuals was screened with previously described PCR-RFLP protocols, and used to provide baseline estimates cadherin allele frequencies in a given population.

Two paired matings of *O. nubilalis* from the USDA-ARS, Corn Insects and Crop Genetics Research Unit laboratory colony were used to establish F₁ families. The F₁ progeny were sib-mated to produce F₂ larvae. F₂ larvae were reared to adults and used to estimate genotype frequencies for cadherin loci. Larvae were reared on artificial diet (Guthrie, 1987) and adult samples were stored separately in 1.5 ml microcentrifuge tubes at −20 °C prior to DNA isolation. DNA from field and pedigree samples was extracted from adult thorax tissue using methods described by Coates and Hellmich (2003). Genotyping by PCR-RFLP was performed as described previously. The goodness-of-fit of observed genotypic distributions during the F₂ generation were compared to expectations based on Mendelian inheritance.

3. Results and discussion

3.1. O. nubilalis cadherin identification

A 220-kDa cadherin-like peptide from *O. nubilalis* was identified as a midgut Cry1Ab receptor (Hua et al., 2001). We isolated a 5378 nt *O. nubilalis*
midgut-expressed mRNA (AY612336) similar to lepidopteran cadherin transcripts (*Helicoverpa armigera* AF519180, BLAST score = 92, E-value $10^{-14}$; *B. mori* AB041509, BLAST score = 54, E-value 0.003). A single 5154 nt open reading frame (ORF) encoded a putative 1717 amino acid polypeptide (Fig. 1). Protein Database (PDB) search with PredictProtein (Rost, 1996) demonstrated high similarity (S) and identity (IDE) of the putative *O. nubilalis* peptide to *B. mori* BtR175 ($S = 73\%$, IDE = 61%; Nagamatsu et al., 1998b), *L. dispar* BTR-CAD ($S = 72\%$, IDE = 60%; AF317621), *M. sexta* Bt-R1 ($S = 58\%$, IDE = 72%; Vadlamudi et al., 1995) and *H. virescens* BtR-4 ($S = 58\%$, IDE = 72%; Gahan et al., 2001). A parsimony-based phylogeny used a consensus 3048 amino acid alignment of 12 lepidopteran cadherins including *O. nubilalis* and indicated three main divisions: cadherins from superfamilies Noctuoidae, Bombycoidae, Sphingoidea, and Pyraloidea and Gelchioidea (Fig. 2). Internal branch support at internal nodes is greater than 85% based on 1000 bootstraps, but terminal nodes among *B. mori* alleles may show homoplasy due to homologous recombination. Placement of the *O. nubilalis* ORF within the cadherin peptide phylogeny and high peptide similarity to other lepidopteran cadherin, suggests the *O. nubilalis* midgut cDNA (AY612336) encodes a cadherin-like peptide.

Cadherin-like motifs were identified within the derived *O. nubilalis* polypeptide. A ProSite database search identified a 1402 amino acid long extracellular domain, a 21 residue signal peptide (SIG), transmembrane region (TMR), and cytoplasmic domain (CPD; Fig. 1). Eleven consensus cadherin repeat motifs ([LIV]-x-[LIV]-x-D-x-N-D-[NH]-x-P) were identified by PredictProtein (Rost, 1996). The 1717 amino acid *O. nubilalis* cadherin-like peptide has predicted molecular weight (192 kDa) similar to *B. mori* and *M. sexta* homologs (Compute pI/Mw program; Bjellqvist et al., 1993). Cadherins are glycoproteins involved in calcium-dependent cell–cell adhesion (Takeichi, 1990). There are 13 predicted *O. nubilalis* cadherin peptide N-glycosylation sites (NP[S or T]; Gavel and von Heijne, 1990; Fig. 1). Difference between polyacrylamide mobility (220 kDa; Hua et al., 2001) and estimated 192 kDa may be due to posttranslational modifications. Predicted isoelectric point (pI) of the *O. nubilalis* cadherin peptide (4.23; Compute pI/Mw program; Bjellqvist et al., 1993) indicates solubility in alkaline midgut environments.

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Fig. 1. *O. nubilalis* 1717 residue long cadherin-like peptide sequence (AY612336) with a predicted 21 amino acid signal peptide (SIG), 11 cadherin repeats (CR1 to CR11), 13 extracellular glycosylation sites, membrane proximal region (MPR), transmembrane region (TMR), and cytoplasmic domain (CPD). Putative glycosylation sites are underscored by triangles in diagram and sequence. Two potential *O. nubilalis*, *B. thuringiensis* Cry1A toxin binding regions (TBR1 and TBR2) with homology to those proposed by Gomez et al. (2002a, b, 2003) are indicated.
which is congruent with required extracellular domain function.

3.2. Polymorphism within putative cadherin Cry1Ab toxin binding domains

Two putative *O. nubilalis* cadherin peptide Cry1A toxin-binding regions \(^{860}\text{DIEIEIDTN}^{871}\) and \(^{1328}\text{IPLQTSILVTV}^{1339}\) were identified (Fig. 1) that were identical to 7 of 11 homologous *M. sexta* Bt-R1 TBR1 residues \(^{860}\text{NITIHIDTN}^{875}\) (Gomez et al., 2001) and 9 of 12 homologous *M. sexta* Bt-R1 TBR2 residues, \(^{1331}\text{IPLASILTVTV}^{1342}\) (Gomez et al., 2003). A cadherin alignment of amino acid sequence (Fig. 3) indicates five TBR1 (I\(^{1608}\), I\(^{1870}\), D\(^{1872}\), N\(^{1874}\), and N\(^{1875}\)) and two TBR2 amino acids are fixed between all species (L\(^{1339}\) and T\(^{1341}\); with respect to *M. sexta* Bt-R1). Interspecies comparisons reveal little conservation of the cadherin toxin-binding region. Variability in cadherin sequences may allow flexibility of Cry1Ab toxin binding to midgut receptors or a generalized protein–protein interaction in Cry1A toxin mode of action.

Intraspecific DNA sequence alignment detected variation among cloned OnTBR1 (Fig. 4) and OnTBR2 (Fig. 5) cadherin gene PCR products, containing 214–232 and 222–232 nt introns, respectively. No insertions or deletions were observed in coding sequence (exons). In addition to intron length variation, 31 OnTBR1 SNPs were observed (\(\pi = 0.02707\) (Nei, 1987); \(\theta = 0.02809\) (Tajima, 1996)); 4 of 8 of these were synonymous changes in exons (Fig. 4). Thirty-one OnTBR2 SNPs were identified (\(\pi = 0.02410; \theta = 0.02490\)); 13 of 17 of these were synonymous.
changes in exons (Fig. 5). Eight SNPs were identified within restriction endonuclease recognition sites. Four polymorphic restriction sites (NdeII, Rsal, TaqI, and Tsp509I) were identified from OnTBR1 (Fig. 4); and four sites (CfoI, HaeIII, HpyCHVIII and MspI) were identified from OnTBR2 (Fig. 5). Subsequent
Fig. 5. Alignment of *O. nubilalis* cadherin Cry1A toxin binding region 2 (OnTBR2) genomic DNA sequence AY629584–AY629592. Primer annealing sites are indicated by arrows showing direction. Region bound by primers OnCadTBR2-F and OnCadTBR2-R encompasses the PCR product OnTBR2. Identical nucleotides (-) and deletions (*) are with respect to uppermost reference sequence. Introns are overlined (^) and amino acids encoded in exons are labeled using standard 21-symbol code. Polymorphic *CfoI*, *HaeIII*, *HpyCH4III*, and *MspI* restriction endonuclease sites are underlined and labeled appropriately within the region OnTBR2-s amplified with primers OnCadTBR2-F and OnCadTBR2-R.
PCR-RFLP assays used PCR product OnTBR2-s for \textsuperscript{1328}IPQTSILVTV\textsuperscript{1339} region SNP detection. One SNP within an HpyCHVIII recognition site represents a nonsynonymous peptide change \textsuperscript{1328}IPQTSILVVT[I/V] N\textsuperscript{1346}. Alternate amino acids (I or V) both have short chain aliphatic side groups and may not affect regional charge or peptide structure. Remaining PCR-RFLP indicate nonsynonymous changes of cadherin alleles outside 86\textsuperscript{6}DIEIELIDTNN\textsuperscript{871} and 1328\textsuperscript{IPQTSILVVT[I/V]} N\textsuperscript{1340} (Figs. 4 and 5).

The nucleotide substitutions in the cadherin gene and PCR-RFLP assays provide a means for genotyping \textit{O. nubilalis}, mapping of resistance traits, and characterizing populations. Three cadherin alleles (r1, r2, and r3) in Cry1Ac resistant \textit{P. gossypiella} strain AZP-R differed by block deletion or truncation (Morin et al., 2003), and shared no fixed peptide sequence differences compared to alleles from a susceptible line. Resistant allele r2 had a truncation deletion that eliminated homologous \textit{P. gossypiella} TBR1 \textsuperscript{862DNVINIDINNK\textsuperscript{875}} and TBR2 \textsuperscript{1341PLPGSLLVTVV\textsuperscript{1352}}; and allele r1 had a nonsynonymous change directly flanking TBR1 (R\textsuperscript{861}) compared to of susceptible and r3 alleles (G\textsuperscript{861}). Morin et al. (2003) also established a correlation between cadherin resistance alleles and increased insecticide resistance levels and are increasingly being incorporated into resistance diagnostics (Brogdon and McAllister, 1998). Molecular assays have been developed to detect resistance genes in insect populations (Steichen and Ffrench-Constant, 1994; Field et al., 1996), and screening known resistance alleles in \textit{P. gossypiella} may show promise for population surveillance (Morin et al., 2003; Tabashnik et al., 2004). In a preliminary surveillance experiment we used PCR-RFLP to estimate frequencies of \textit{O. nubilalis} cadherin alleles (SNP haplotypes) in a population of susceptible adults collected near Mead, Nebraska. Three to six alleles were observed per PCR-RFLP assay (OnTBR1 \textit{RsaI}, \textit{TagI}, \textit{TaqI}, \textit{TaqI}, and OnTBR2-s \textit{CfoI} and \textit{HaeIII} with \textit{HpyCH4III} I) and 21 unique allelic combinations (genotypes) were observed. Frequency of cadherin alleles were defined from individual restriction assays and ranged from 0.012 to 0.455 (Table 2).

One or more genes may mediate lepidopteran resistance to Cry1A toxins. Aminopeptidase was implicated as a major midgut receptor (Knight et al., 1994; Gill et al., 1995), followed by cadherin (Vadlamudi et al., 1995). Screening \textit{P. xylostella} for survival on Cry1A and Cry1F toxins suggested cross resistance was imparted by a single gene (Tabashnik et al., 1997). This was corroborated when a major autosomal locus inherited as a recessive trait was identified (Heckel et al., 1999). Cadherin was implicated as a resistance factor by phenotypic association in \textit{P. gossypiella} (Morin et al., 2003) and transposon knockout in \textit{H. virescens} (Gahan et al., 2001). For \textit{Spodoptera litura}, transcript knockdown by RNA interference (RNAi) showed the role of aminopeptidase in Bt resistance (Rajagopal et al., 2002). Inheritance of resistance in an \textit{O. nubilalis} strain selected for survival on Dipel B formulations was autosomal and incompletely dominant (Huang et al., 1999). Additive gene action was predicted to mediate Bt resistance in \textit{H. zea} (Burd et al., 2003); and two copies of cadherin resistance alleles (r1, r2, or r3) were required for resistance in \textit{P. gossypiella} (Morin et al., 2003).

### Table 1

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Parental genotype</th>
<th>Observed cadherin PCR-RFLP genotype ratios among F\textsubscript{2} progeny</th>
<th>Expected F\textsubscript{2} ratio</th>
<th>(\chi^2)</th>
<th>p-Value (df)</th>
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<tr>
<td>Ped31</td>
<td>(\delta R15\delta R16)</td>
<td>TBR1; R11(3/9); R16(12/39); R15(7/39); R56(6/39); R55(0/39); R66(1/39)</td>
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<td>(\delta C12\delta C11)</td>
<td>TBR2; C11(26/39); C12(14/39); C22(4/39)</td>
<td>9:6:1</td>
<td>1.07</td>
<td>0.586 (2)</td>
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<tr>
<td>Ped62</td>
<td>(\delta T22\delta T11)</td>
<td>TBR1; T11(10/44); T12(26/44); T22(8/44)</td>
<td>1:2:1</td>
<td>1.64</td>
<td>0.441 (2)</td>
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<td></td>
<td>(\delta H33\delta H34)</td>
<td>TBR2; H33(26/44); H34(14/44); H44(4/44)</td>
<td>9:6:1</td>
<td>1.01</td>
<td>0.604 (2)</td>
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</tbody>
</table>

Observed genotypic ration among F\textsubscript{2} offspring was compared to Mendelian expected F\textsubscript{2} ratio based on parental genotypes (R15 genotype is R1 and R5 heterozygote; see PCR-RFLP alleles on Table 1). Significance of observed allele frequency departures from expected tested by Chi-square (\(\chi^2\)).
To manage resistance effectively, it is necessary to understand the genetic mechanisms of resistance (Gould and Tabashnik, 1998; Caprio et al., 2000) and monitor insect pests of transgenic-insecticidal cultivars for changes in their tolerances of Bt toxins (Gould et al., 1997; Andow and Alstad, 1998). To do this, it is necessary to track the evolutionary changes of resistance alleles in pest populations. The full-length mRNA, partial-genome sequence, and molecular markers developed in this study have downstream applications that will aid in the determination of cadherin involvement in potential *O. nubilalis* resistance to Cry1Ab toxin. These markers are appropriate for incorporation into linkage mapping studies. Laboratory colonies of *O. nubilalis* that exhibit moderate resistance to Cry1Ab toxin in artificial diet (Bolin et al., 1999; Huang et al., 1999; Chaufaux et al., 2001), and diagnostic bioassays can discriminate phenotypes (Marcon et al., 2000). OnTBR1 and OnTBR2 PCR-RFLP markers show Mendelian inheritance (Table 1), which is required for genetic mapping procedures (Pemberton et al., 1995). F$_2$ pedigrees in conjunction with the bioassays can be used to determine if the segregation of the moderate *O. nubilalis* resistance phenotype is associated with cadherin allele segregation using OnTBR1 or OnTBR2 PCR-RFLP assays. A similar protocol was used to associate cadherin alleles r1, r2, and r3 with Cry1Ac resistance in the *P. gossypiella* AZP-R strain and field collections (Morin et al., 2003). Association of cadherin alleles with distinct phenotypic traits is a prerequisite for effective field surveillance, but marker polymorphism determines how effectively alleles are differentiated. Our cadherin loci will also be incorporated into linkage maps of other marker types (AFLP and microsatellites) to better examine cause and effect between resistant phenotypes and cadherin loci. This will help pinpoint the location of putative resistant loci and ask if their location is in the vicinity of our cadherin maker loci.

Polymorphisms among *O. nubilalis* cadherin alleles at Mead, Nebraska for OnTBR1 and OnTBR2 indicates that native genetic variation is present. The native genetic variation for cadherin loci can be incorporated into resistance monitoring or surveillance programs, pending allelic association with potential field resistance traits via bioassays. Studies are currently underway with *O. nubilalis* to map the genomic position of the cadherin locus. We will use the information obtained not only to better understand inheritance of Cry1Ab toxin resistance, but also examine the appropriateness of allelic association with phenotypic traits for potential incorporation into field monitoring or surveillance programs.

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