

Segregation of European Corn Borer, *Ostrinia nubilalis*, Aminopeptidase 1, Cadherin, and Bre5-Like Alleles, from a Colony Resistant to *Bacillus thuringiensis* Cry1Ab Toxins, are not Associated with F2 Larval Weights when Fed a Diet Containing Cry1Ab

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Segregation of European corn borer, *Ostrinia nubilalis*, aminopeptidase 1, cadherin, and bre5-like alleles, from a colony resistant to *Bacillus thuringiensis* Cry1Ab toxins, are not associated with F₂ larval weights when fed a diet containing Cry1Ab

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Abstract

Protein receptors may be required for activated *Bacillus thuringiensis* Cry toxins (Cry1Ab) to bind midgut epithelium prior to pore formation. Single nucleotide polymorphism markers from two *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) midgut peptide receptors, cadherin (OnCad), aminopeptidase N 1 (OnAPN1), and OnBre5 (Onb3GalT5; a β -1,3-galactosyltransferase family 5 member) were used to examine segregation in F₂ families derived from paired matings of Cry1Ab-resistant females and Cry1Ab-susceptible males. Genotypic frequencies for these markers did not deviate from Mendelian expectations. Analysis of F₂ larvae indicate the segregation of single nucleotide pores in OnAPN1, OnBre5 (Onb3GalT5), and OnCad marker loci were independent of the segregation of log₁₀ weights of larvae feeding on Cry1Ab diet.

Keywords: *Ostrinia nubilalis*, single nucleotide polymorphism

Abbreviations: Cry1Ab - toxins from *Bacillus thuringiensis*, OnAPN1 - *Ostrinia nubilalis* aminopeptidase N isoform 1, OnCad - *Ostrinia nubilalis* cadherin, OnBre5 - *Ostrinia nubilalis* homolog to *Caenorhabditis elegans* bre5 gene

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Introduction

Bacillus thuringiensis (Bt) Berliner is a gram-positive soil bacterium originally described as an insect pathogen against Lepidoptera, Diptera, and Coleoptera. Insoluble crystalline inclusion bodies of Bt spores contain three domain toxin proteins called Cry toxins. Toxicity occurs by insertion of oligomerized toxin into midgut epithelial membranes, resulting in formation of pore channels causing osmotic imbalance and sepsis (Schnepf et al. 1998). Susceptibility of Lepidoptera to transgenic *B. thuringiensis* crystalline (Cry) toxins has been shown to occur via interaction with midgut receptors. Cry toxins may bind extracellular domains of cadherin (Vadlamudi et al. 1993; Francis & Bulla, 1995), aminopeptidase N (APN; Knight et al. 1994), or alkaline phosphatase receptors (Jurat-Fuentes et al. 2002). Furthermore, carbohydrate modifications to peptide receptors were shown to enhance toxin-receptor interactions (Knowles et al. 1991; Masson et al. 1995) suggesting that glycosylation may be common among midgut receptors (Griffitts et al. 2001). *Caenorhabditis elegans* Bt resistant (*bre*) mutants evaded membrane pore formation when exposed to Cry5B and Cry14A toxins (Maroquin et al. 2000; Griffitts et al. 2001), and a putative β -1,3-galactosyltransferase family 5 member (*b3GalT5*) gene was correlated with resistance for the mutant *bre5* (Griffitts et al. 2001). *Bre5* established the theory that glycosylation pathways that modify midgut peptide receptors can be a mechanism of resistance to Cry toxins.

Feeding by larval stage European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), causes economic loss via yield decrease to cultivated corn (Mason et al. 1996). Crop injury caused by *O. nubilalis* has been reduced by transgenic maize hybrids expressing Cry1Ab toxins (Kozziel et al. 1993). In 2005, 35% of United States corn acreage was planted with commercial hybrids expressing Cry1Ab toxins (USDA-ERS, 2005). If genetic variance for resistance to Cry1Ab were present in wild populations of *O. nubilalis*, high adoption rates of Bt corn may not only result in a high selection pressure for resistance, but also increase potential for *O. nubilalis* populations to respond to the selection. Failure of transgenic crops due to insect resistance has not been observed in the field, but varying levels of resistance levels of resistance were selected for in laboratory colonies (Bolin et al. 1999; Chaufaux et al. 2001; Alves et al., 2006).

The *O. nubilalis* midgut expresses a 220-kDa cadherin-like protein, and 145- and 154-kDa aminopeptidase (APN) isoforms that bind Cry1Ab (Hua et al. 2001). A full-length cadherin cDNA from *O. nubilalis* was shown to have putative N-glycosylation sites (Coates et al. 2005), and was identified as a major midgut receptor (Flanagan et al. 2005). Reduced trypsin transcript T23 levels were associated with *O. nubilalis* KS-SC colony resistance to native toxins present in Dipel® Bt formulations, but did not show decreased susceptibility to truncated Cry1Ab toxins expressed by transgenic maize (Li et al. 2005). Cry toxin resistance has occurred due to mutations in aminopeptidase N 1 in *Spodoptera exigua* (Herrero et al. 2005) and in cadherin in *Heliothis virescens* (Gahan et al. 2001) and *Pectinophora gossypiella* (Morin et al. 2003). Molecular tools for monitoring *O. nubilalis* cadherin (Coates et al. 2005) and serine protease genes (Coates et al. 2006) were developed, and assessed in pedigrees. Herein we report the use of molecular markers for *O. nubilalis* aminopeptidase N 1 (*OnAPN1*), *OnBre5* (*Onb3GalT5*), and cadherin (*OnCad*) genes to assess the relationship between segregation of the candidate-gene markers and Cry1Ab-resistance phenotypes within F₂ progeny originating from resistant female by susceptible male crosses (*Cry1Ab*^R ♀ × *Cry1Ab*^S ♂).

Materials and Methods

Pedigrees and measurement of Cry1Ab resistance traits

A field-collected colony of *O. nubilalis* was exposed to laboratory selection for resistance to Cry1Ab since 2003 (> 25 generations) at USDA-ARS, Corn Insects and Crop Genetics Research Unit (CICGRU), Ames, IA. Resistance ratios were measured by comparing dose response of the Cry1Ab-resistant colony (*Cry1Ab*^R) and its parental control colony (*Cry1Ab*^S) at their respective LD₅₀ values. Dose-response studies and Cry1Ab bioassays employed in the current study use the surface overlay method developed by Marçon et al. (1999). In this method, solutions of trypsinized Cry1Ab toxins are applied to surfaces of an artificial diet that absorbs the toxin. Doses of Cry1Ab used in this study are reported in units of surface area (ng cm⁻²), as difficulties in assessing equivalent doses result when direct comparisons are made to volumetric units (cm⁻³) used by Gahan et al. (2005). During the current study, the LD₅₀ value of the *Cry1Ab*^S colony was 8.9 ng

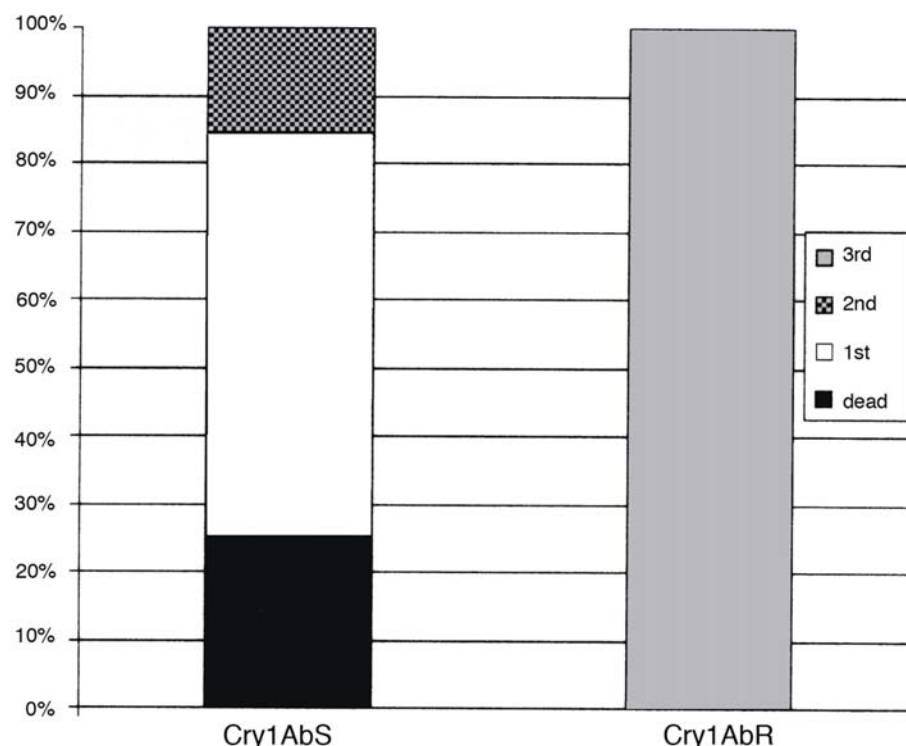


Figure 1. Developmental differences between larvae from Cry1Ab susceptible (Cry1Ab^S) and Cry1Ab resistant (Cry1Ab^R) colonies after 7 days feeding on 5.0 ng Cry1Ab toxin cm⁻².

cm⁻², compared to > 23,000 ng cm⁻² for the Cry1Ab^R colony, thus estimating a resistance ratio of > 2,500-fold. Other Cry1Ab resistant *O. nubilalis* colonies are reported to have resistance ratios of 2,000- to 1,300-fold (Alves et al., 2006). Alves et al., (2006) also assessed resistance via the surface overlay method and found LD₅₀ values of 640 to 1000 ng cm⁻². Cry1Ab^R larvae can complete development on freeze-dried, whorl-stage corn tissue containing Cry1Ab, and also survive on reproductive stage corn expressing Cry1Ab (Sumerford, personal observation). In comparison, *P. gossypiella* resistant strains AZP-R and APHIS-98R survive on Cry1Ac toxin concentrations > 10 µg g⁻¹ (Tabashnik et al., 2004), and *H. virescens* Cry1Ac resistant strains have an LC₅₀ of 506 µg ml⁻¹ of diet (Gould et al. 1992; Gould et al., 1995). Direct comparison between µg toxin cm⁻² and µg toxin ml⁻¹ of diet is difficult, but at 23 µg cm⁻², Cry1Ab^R may be considered to show high levels of resistance to Cry1Ab toxin.

In order to recover larvae with susceptible phenotypes for DNA extraction, a sublethal bioassay was used as described in other mapping studies involving Bt-resistant colonies (Heckel et al. 1997; Gahan et al. 2005). Larval development of Cry1Ab^R individuals is less delayed on sublethal

doses of Cry1Ab toxin compared to Cry1Ab^S. At a dose of 5 and 7.5 ng cm⁻², Cry1Ab^R individuals develop to 3rd instar whereas development of Cry1Ab^S larvae is delayed (Figure 1). Development differences, measured as log₁₀-transformed weights (mg), and 5 and 7.5 ng cm⁻² doses provide a quantitative measure of Cry1Ab resistance and recovery of susceptible individuals for subsequent genetic analysis. Two *O. nubilalis* F₁ families (Fam5 and Fam8) were established via paired matings of a female from the Cry1Ab^R colony with a Cry1Ab-susceptible male from the CICGRU colony (Cry1Ab^S). Two matings of full-sib F₁ pairs were made from each family for a total of four F₂ families (Fam 3-14, Fam 3-15, Fam 8-09 and Fam 8-19). F₂ neonates from each family were fed one of the Cry1Ab-overlay diets (5 and 7.5 ng cm⁻²; 100 – 200 larvae per family), or control overlay (24 larvae) for 7 days, after which larval weights were measured. All larvae were transferred to artificial control diet, reared to adults, and then frozen at -80°C.

Genotyping assays

Genomic DNA was isolated from parental, F₁, and F₂ adult *O. nubilalis* thorax tissue using DNAeasy isolation kit (Qiagen, www.qiagen.com) according to manufacturer directions. OnAPN1, OnBre5

Table 1. APN1, bre5 (Onb3GalT5), cadherin primers used in genotyping of *Ostrinia nubilalis* pedigrees.

Name	Sequenced	PCR annealing temperature	GenBank number
OnAPN1-F	5'-CGT GTG GCC AAT ATC GTG TC-3'	60 °C	EF100608
OnAPN1-R	5'-AAG ATG CAC GCT CCT CTT GC-3'		
Onb3GalT5-F1	5'-CGT GAC AAT GAT GTC GTT CAA-3'		
Onb3GalT5-R1	5'-TGC TGC GGC ACT AAG CCC AC-3'	62 °C	AY821557
OnCad5-F EPIC	5'-CTG CCG CAA GCA GAA GAC CT-3'	56 °C	DQ225104
OnCad5-R EPIC	5'-ATG ACG TTG CTT GAC TGG TC-3'		
OnCad6-F EPIC	5'-ATC GAC TGG GAC TCC ACC TG-3'	56 °C	DQ225104
OnCad6-R EPIC	5'-AAG CAT CTC GAA TTC CTC GAA-3'		

(Onb3GalT5), and OnCad gene fragments were PCR amplified using 2.5 mM MgCl₂, 50 μM dNTPs, 2.5 pmol each of primer (Table 1) and, 0.45 U Taq DNA polymerase (Promega, www.promega.com), and 100 ng of DNA template in a 12.5 μl reaction. PTC-100 thermocycler conditions used 95 °C for 2.5 m, followed by 40 cycles of 95 °C for 30 s, 30 s annealing (Table 1), and 72 °C for 1 m. Individual digest reactions included 7.5 μl of *O. nubilalis* Bre5 (Onb3GalT5) or APN1 PCR product, 3.0 μl 10x buffer, 0.1 mg/μl BSA, and 0.25 U of MspI (Onb3GalT5) or RsaI (OnAPN1) in 30 μl, and were incubated at 37 °C for 10 to 16 h. OnCad PCR products (OnCad5 and OnCad6; Table 1) comprise fragments that amplify across introns, and both show length variations that were used for allele identification. Entire volumes of all reactions were loaded onto 10 cm 2% agarose gels containing 0.5 μg/ml ethidium bromide, and separated at 100 V for 1 h. Digital images were taken under UV illumination on a BioRad ChemiDoc System (BioRad, Hercules, CA).

Data analysis

F₂ families were used to determine if OnAPN1, OnBre5 (Onb3GalT5) or OnCad genotypes exhibited Mendelian inheritance, and also to determine if their segregation was related to larval

development on sublethal Cry1Ab diets. Replicated goodness-of-fit tests (Sokal and Rohlf 1995) were used to determine if genotype frequencies were inherited in 1:2:1 ratio (Mendelian expectation) at each locus. The replicated goodness-of fit tests produce several G statistics. G_H tests whether the frequencies of genotype are homogeneous across all F₂ families. The pooled-G statistic (G_{Pooled}) tests whether the genotypes pooled across all F₂ families fit a 1:2:1 Mendelain expectation. Finally, the total G (G_{Total}) statistic measures whether the data as a whole fit Mendelian expectations. Analyses were performed separately for genotypic data from control and Cry1Ab diets.

Marker regressions were used to test the null hypothesis of no relationship among the segregation of resistance phenotypes (log₁₀ weight of larvae feeding on Cry1Ab overlay) with OnAPN1, OnBre5 (Onb3GalT5) and OnCad and genotypes (0, 1, or 2 copies of the allele originating from the Cry1Ab^R grandmother; Table 2) within F₂ families. Regressions were performed using the MIXED procedure of SAS (v. 9.1.3) via restricted-maximum-likelihood methods (Littell et al. 2006). Genotype for each marker was the only fixed effect entered into the model. F₂ family, block nested within an F₂ family, and larvae

Table 2. APN1, bre5 (Onb3GalT5), and cadherin alleles observed in pedigrees Fam3–14 and 3–15, and Fam8–09 and Fam8–19. *Ostrinia nubilalis* APN1 *RsaI* PCR-RFLP alleles A1 (310 bp) and A2 (160 and 150 bp); bre5 (Onb3GalT5) *MspI* PCR-RFLP alleles M1 (520, 240, and 127 bp) and M2 (428, 240, 127, and 92 bp). Initial parental cross were between Cry1Ab^R femalex Cry1Ab^S male.

Pedigree	Parental genotypes			
	OnAPN1	Onb3GalT5	OnCad5	OnCad6
Fam 3 F ₀ male	NP	M1/M2	490/490	485/485
F ₀ female	NP	M2/M2	510/510	485/485
Fam 3–14 F ₁ male	NP	M1/M2	490/510	NA
F ₁ female	NP	M1/M2	490/510	NA
Fam 3–15 F ₁ male	NP	M1/M2	490/510	NA
F ₁ female	NP	M1/M2	490/510	NA
Fam 8 F ₀ male	A2/A2	M1/M2	400/510	515/515
F ₀ female	A1/A1	M2/M2	510/510	485/485
Fam 8–06 F ₁ male	A1/A2	M1/M2	NA	485/515
F ₁ female	A1/A2	M1/M2	NA	485/515
Fam 8–19 F ₁ male	A1/A2	M1/M2	NA	485/515
F ₁ female	A1/A2	M1/M2	NA	485/515

NP = no polymorphism
NA = not analyzed

nested with a block ("Residual" error) were considered random sources of variance in their effects on larval $\log_{10}$ weight. The relationship between marker genotypes and $\log_{10}$ weight was considered significant if $P \leq 0.05$.

Results and Discussion

Mendelian inheritance and larval weights on Cry1Ab diet overlays

Four F_2 pedigrees (Fam3–14 and 3–15, and Fam8–09 and Fam8–19) were derived from two initial Cry1Ab^R ♀ × Cry1Ab^S ♂ parental crosses (Fam3 and Fam8). The F_0 parents were genotyped using OnAPN1, OnBre5 (Onb3GalT5), and OnCad assays (Table 2). The OnAPN1 RsaI PCR-RFLP marker differentiated parents from Fam8, but no polymorphism was shown between Fam3 parents. All Cry1Ab^R female parents were homozygous for the M2 allele at the OnBre5 (Onb3GalT5) locus, and since all Cry1Ab^S male parents were heterozygous M1/M2 (sharing the M2 MspI SNP allele observed in the Cry1Ab^R colony) all subsequent full-sib crosses were screened in order to select only M1/M2 by M1/M2 for F_1 matings. Heterozygous M1/M2 F_1 parents in Fam3–14, 3–15, 8–09 and 8–19 allowed determination that the M2 allele was inherited from the Cry1Ab^R F_0 female parent. Two cadherin gene markers, OnCad5 and OnCad6, differentiated parents from Fam3 and Fam8, respectively (Table 2).

Fidelity of allele inheritance is critical in pedigree analysis (Pemberton et al. 1995). Analysis showed that genotypic ratio of F_2 individuals from Fam3–14, 3–15, 8–09, and 8–19 fed control diet did not deviate from predicted 1:2:1 Mendelian expectations. F_2 families were homogeneous for observed frequencies of OnAPN1 ($G_H = 0.08$, $df = 2$, $P \geq 0.9608$), OnBre5 (Onb3GalT5; $G_H = 1.85$, $df = 6$, $P \geq 0.933$), and OnCad genotypes ($G_H = 1.91$, $df = 6$, $P \geq 0.9280$). Pooled genotype frequencies also fit 1:2:1 Mendelian expectations for OnAPN1 ($G_{Pool} = 1.09$, $df = 2$, $P \geq 0.5809$), OnBre5 ($G_{Pool} = 13.90$, $df = 8$, $P \geq 0.084$), and OnCad ($G_{Pool} = 0.67$, $df = 2$, $P \geq 0.716$). In addition, the total G-statistic indicated that all families were homogeneous for Mendelian expectation of genotypic frequencies for OnAPN1 ($G_{Total} = 1.017$, $df = 4$, $P \geq 0.8836$), OnBre5 ($G_{Total} = 5.43$, $df = 8$, $P \geq 0.711$), and OnCad ($G_{Total} = 2.58$, $df = 8$, $P \geq 0.9581$). Mendelian inheritance of OnAPN1 RsaI PCR-RFLP, OnBre5 (Onb3GalT5) MspI PCR-RFLP, and both cadherin markers among F_2 progeny fed on control diet

suggests their appropriate use in population genetic and mapping experiments.

Similar conclusions were drawn from genotypic data analyzed from F_2 larvae exposed to sublethal doses of Cry1Ab. The *O. nubilalis* F_2 families were homogeneous for observed frequencies of OnAPN1 ($G_H = 2.41$, $df = 4$, $P \geq 0.662$), OnBre5 (Onb3GalT5; $G_H = 7.32$, $df = 6$, $P \geq 0.293$), and OnCad genotypes ($G_H = 1.85$, $df = 6$, $P \geq 0.933$). Pooled genotype frequencies also fit 1:2:1 Mendelian expectations for OnAPN1 ($G_{Pool} = 3.77$, $df = 6$, $P \geq 0.152$), OnBre5 ($G_{Pool} = 3.69$, $df = 2$, $P \geq 0.158$), and OnCad ($G_{Pool} = 0.62$, $df = 2$, $P \geq 0.734$). In addition, the total G-statistic indicates that all families were homogeneous for Mendelian expectation of genotypic frequencies for OnAPN1 ($G_{Total} = 6.17$, $df = 6$, $P \geq 0.404$), OnBre5 ($G_{Total} = 11.01$, $df = 8$, $P \geq 0.201$), and OnCad ($G_{Total} = 5.43$, $df = 8$, $P \geq 0.711$). Mendelian ratio of F_2 progeny after Cry1Ab bioassays indicated that genotypes were randomly present among larvae after bioassay (no genotypes were preferentially culled from survivors), and that subsequent regression analysis would be valid.

Segregation of marker and resistance phenotype analysis

Regression analysis was used to assess if segregation of OnAPN1 RsaI PCR-RFLP, OnBre5 (Onb3GalT5) MspI PCR-RFLP, or OnCad alleles derived from the Cry1Ab^R female parent explain the segregation of $\log_{10}$ weights for F_2 larvae feeding on Cry1Ab diets. Because two F_2 families were exposed to two Cry1Ab doses (5.0 and 7.5 ng/cm²), we examined variance attributed to F_2 family in the model to determine if $\log_{10}$ weights could be combined from both diets into one analysis. Examination of variance components for the random effects in the model supported the pooling of data across doses (percentages of the total variance for family, and replicates within a family were 6.1% and 9.6%, respectively).

Two-way marker regressions were performed to examine the relationship between the segregation of OnBre5 (Onb3GalT5) MspI PCR-RFLP and OnCad5 alleles for Fam 3–14 and 3–15). The OnBre5 × OnCad5 results suggested that no significant gene interactions were present ($F = 0.740$, $P \geq 0.3908$, $df = 1$, 190), and no single gene effects were present for OnBre5 ($F = 0.42$, $P \geq 0.5188$, $df = 1$, 190) or OnCad5 ($F = 0.42$, $P \geq 0.5172$, $df = 1$, 190). Similar results were found

Table 3. Regression analysis testing for significant 2- and 3-way interaction of genes, OnAPN1, OnBre5 (Onb3GalT5), and OnCad, (0, 1, or 2 copies of the allele derived within the Cry1Ab^R colony), with F₂ larval log₁₀ weights from Fam8–09 and 8–19 when fed on diet containing (5.0 and 7.5 ng Cry1Ab/cm⁻²).

Fixed effects and gene interaction(s)				
Interaction	Num d.f.	Den d.f.	F value	P-value
OnAPN1	1	95.4	0.34	0.5601
OnBre5	1	97.0	0.35	0.5557
OnCad	1	96.9	0.61	0.4377
OnAPN1 x OnBre5	1	94.8	0.73	0.3953
OnAPN1 x OnCad	1	95.3	0.50	0.4809
OnBre5 x OnCad	1	95.5	1.57	0.2130
OnAPN1 x OnBre5 x OnCad	1	96.7	1.04	0.3114

from a 3-way regression analysis of Fam8–09 and Fam8–19 for OnAPN1 RsaIII PCR-RFLP, OnBre5 (Onb3GalT5) MspI PCR-RFLP, OnCad6 allele for co-segregation with F₂ Cry1Ab resistance traits (Table 3).

Mutation of a single gene product has given rise to Cry1 toxin resistance traits (Gahan et al, 2001, Rajagopal et al. 2002, Herrero et al. 2005). Additionally, it was shown that independent mechanisms might evolve for resistance to different Cry toxins (Jurat-Fuentes et al. 2003), suggesting involvement of multiple midgut receptors (aminopeptidase isoforms, cadherin, and alkaline phosphatase), peptide and lipid modifiers (bre5 homologs), or serine proteases in the spectrum of resistance traits. Bt resistance may have developed by more-than-one independent mechanism in *O. nubilalis*. Resistance to native Bt toxins in Dipel[®] formulations was shown to result from decreased expression of an *O. nubilalis* trypsin transcript (T23; Li et al. 2005). In contrast, Siqueira et al. (2004) showed Cry1Ab resistance in the *O. nubilalis* was not associated with decreased serine protease activity, but did show decreased levels of binding at midgut receptors in a Europe-R strain (Siqueira et al., 2006). This evidence suggested midgut receptor binding was a potential point of resistance development in *O. nubilalis*.

The 3-way analysis of OnAPN1, OnBre5, and OnCad in Fam8–09 and 9–19 tested for epistasis, or gene interaction (Table 3). The F₂ family was included in the model as a random effect, but showed negligible variance between families ($v^2 = 0.0275$) and suggested most variance was contained within families. These analyses also suggest no significant gene interactions were present or that no particular genotype showed a correlation with higher larval log₁₀ weights when reared on sublethal doses of Cry1Ab toxin ($P \geq 0.213$). Analysis suggests individually, or in any combination, that OnAPN1, OnBre5, or OnCad

might not show significant effect on resistance trait shown by the Cry1Ab^R colony.

Conclusions

This research describes experiments to test correlations between *O. nubilalis* Cry1Ab resistance traits with segregation of alleles at candidate resistance gene loci; aminopeptidase N 1 (APN1), bre5 (Onb3GalT5), and cadherin. Studies with pink bollworm (*P. gossypiella*) indicated three cadherin alleles (r1, r2, and r3) were correlated with Cry1Ac resistance traits (Morin et al. 2003), and the function of cadherins as candidate midgut Bt receptors was shown by a transposon insertion-mediated knockout in *H. virescens* (Gahan et al. 2001). Two *O. nubilalis* Cry1Ab^R ♀ × Cry1Ab^S ♂ F₂ pedigrees independently showed a lack relationship between segregation of OnAPN1, Onb3GalT5, or cadherin alleles and factors that affect F₂ development (larval weight) in Cry1Ab bioassays. Additionally, 2- and 3-way regressions indicated that epistasis (gene interaction) was not involved in resistance traits, suggesting the traits shown by the Cry1Ab^R colony might not be polygenic for the genes tested in these experiments.

Additional experiments using other candidate resistance genes such as other aminopeptidases, and alkaline phosphatase, colonies resistant to other Cry toxins, or genomic scans using several genetic markers followed by detection of contributing quantitative trait loci (QTL) will be required to dissect the genetic components of *O. nubilalis* toxin resistance phenotypes.

Disclaimer

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