The owls remained calm for the duration of the experiment. We conducted the experiments in the rostrolateral ICX, where neurons are tuned to frontal space in normal owls (ITDs range from 0 to 20 μs, contralateral ear leading). In prism-reared owls, the ITD tuning of neurons in this region has been shown to shift reliably in response to juvenile prism experience (*3, 16*). We recorded unit activity with a five-barrel glass microelectrode, the central barrel of which contained a carbon fiber 7 μm in diameter. The remaining four barrels had 2- to 4-μm tips and were used to apply bicuculline methiodide (Sigma) (10 mM in 0.9% saline, adjusted to pH 3.0 with HCl). Units were isolated with a waveform detector. We consistently attempted to isolate the largest unit waveforms. We do not know, however, the degree to which these waveforms corresponded to the discharges of single neurons. We used unit response properties (tuning for frequency, ITD, and interaural level difference) and the progression of these properties with electrode advance to indicate that the recording sites were in the ICX (*3, 8*). The experimental protocol was approved by the Animal Care and Use Committee at Stanford University School of Medicine.

10. Auditory stimulation: ITD tuning properties were characterized as described in (*3*). Briefly, broadband (4 to 12 kHz) noise stimuli were generated digitally and presented dichotically at 10 dB above unit threshold. We determined unit tuning for ITD by presenting a series of 50-ms noise bursts in which ITD was varied in a random, interleaved pattern. We measured ITD tuning by using the optimal interaural level difference for the site. Responses were defined as the number of spikes in the 100 ms after stimulus onset minus the number of spikes in the 100 ms before stimulus onset (baseline).
11. Iontophoresis protocol: At each site, we first assessed ITD tuning three to seven times without drug ejection by using a 20-repetition stimulus series. We then applied bicuculline iontophoretically at a current that resulted in a clear increase in responses; this current ranged from 30 to 60 nA. Once responses were stable, we reassessed ITD tuning three to seven times by using the same 20-repetition stimulus series. Finally, we halted drug ejection, allowed responses to recover to the predrug level, and assessed ITD tuning again.
12. Rearing conditions: This study is based on neurophysiological recordings from eight barn owls (*Tyto alba*), all of which were raised together in our colony. Two of the owls were raised normally and six were raised wearing Fresnel prismatic lenses (VisionCare/3M), mounted in spectacle frames, that displaced the visual field horizontally 23° to the left or right. The spectacle frames were secured with a bolt that was cemented to the skull when the owls were 60 to 70 days old, the age at which they are full grown and leave the nest. The flight room in which they lived provided them with a rich visual and auditory environment. Neurophysiological recordings began when the owls were 130 days old, after they had worn spectacles continuously for at least 60 days.
13. Predicting normal best ITD: The value of ITD to which a site in the ICX will be tuned in a normal owl can be predicted (±10 μs) by recording units along a transect that passes through the representation of a given value of ITD both in the central nucleus of the inferior colliculus (ICC) and in the optic tectum (*3*). In normal owls, sites in the ICX that lie along this transect are also tuned to this same value of ITD. In prism-reared owls, the representation of ITD in the ICC is not altered from normal, and normal ITD tuning in the optic tectum can be inferred from the location of a site's visual receptive field, which is also unaltered by prism experience (*3*). In this study, the predicted normal ITD tuning for a transect was determined at the beginning of each experiment from the best ITD measured in the ICC and from the best ITD inferred from the visual receptive field measured in the optic tectum. All ICX recordings were made along this transect.
14. We calculated effectiveness of inhibition for each ITD that evoked at least 15% of the maximum response at each site. To compare the effectiveness of inhibition across different groups of owls (Fig. 3A), we averaged the values of this metric across all sites from each group for a given ITD value [C. Koch, T. Poggio, V. Torre, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2799 (1983); N. Qian and T. J. Sejnowski, *ibid.* **87**, 8145 (1990)].
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17. To evaluate whether an ITD tuning curve was fully returned back to normal after prism removal, we compared the responses on the two flanks of the tuning curve. A tuning curve was deemed fully returned back to normal when the sum of the responses to ITDs 30, 40, and 50 μs on each side away from the predicted normal best ITD were not significantly different (two-tailed t test, $P > 0.05$).
18. Unpaired two-tailed t tests were used throughout this study.
19. At 12 of 28 ICX sites tested in prism-reared owls, responses to normal ITDs were significantly weaker than responses to learned ITDs when inhibition was blocked (two-tailed t tests, $P < 0.05$; Fig. 3B, filled circles) (at the remaining 16 sites, responses to normal and learned ITDs were not significantly different when inhibition was blocked; $P > 0.05$). In addition, with inhibition blocked, responses to normal ITDs were weaker in prism-reared owls than in normal owls ($P = 0.009$; Fig. 3B, open circles versus filled circles) even though there was no difference between the strengths of responses to normal ITDs in normal owls and responses to learned ITDs in prism-reared owls ($P = 0.19$).
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Inheritance of Resistance to *Bacillus thuringiensis* Toxin (Dipel ES) in the European Corn Borer

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Resistance in the European corn borer, *Ostrinia nubilalis* (Hübner), to a commercial formulation of *Bacillus thuringiensis* (*Bt*) Berliner toxin, Dipel ES, appears to be inherited as an incompletely dominant autosomal gene. This contrasts with the inheritance of resistance to *Bt* in other insects, where it has usually been characterized as a recessive trait. The proposed high-dose/refuge strategy for resistance management in *Bt* maize depends on resistance being recessive or partially recessive. If field resistance turns out to be similar to this laboratory resistance, the usefulness of the high-dose/refuge strategy for resistance management in *Bt* maize may be diminished.

Maize and several other crops have been bioengineered to express, in plant tissues, endotoxins derived from *Bacillus thuringiensis* (*Bt*) Berliner (*1, 2*). Such transgenic maize hybrids are known as *Bt* maize (*Bt* corn) hybrids. *Bt* maize hybrids have been developed to protect the crop against corn borers such as the European corn borer, *Ostrinia nubilalis* (Hübner) (order Lepidoptera, family Crambidae). *Ostrinia nubilalis* ranks among the most important pests of maize in North America, causing losses in excess of $1 billion annually (*1*). The efficacy of *Bt* crops against this insect has been impressive and is resulting in widespread adoption of this bioengineered technology. Selection for pest resistance to *Bt* is expected to be intense and is likely to result in the evolution of resistance to *Bt* endotoxins. An effective resistance management program will be needed to preserve the long-term utility of this technology. The U. S. Environmental Protection Agency (EPA) has approved conditional registrations for several *Bt* maize transformations and is requiring the development of a scientifically sound resistance management strategy by the year 2001 (*1, 2*). The currently favored resistance management strategy for *Bt* maize is the "high-dose/refuge strategy" (*1*). Implicit in this strategy is the assumption that genes promoting resistance in the insect will be recessive or partially recessive (*1, 2*).

We analyzed resistance to Dipel ES in a laboratory colony of *O. nubilalis* (*3*). Dipel ES is a commercial formulation of *Bt* endotoxins (*4*). Our results suggest that resistance to Dipel ES in *O. nubilalis* is inherited as an incompletely dominant autosomal gene. In

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contrast, the inheritance of resistance to *Bt* in most other insects is controlled by recessive genes (*5, 6*). If field resistance to *Bt* turns out to be similar to this laboratory resistance, the usefulness of the high-dose/refuge strategy will be greatly diminished, and its application in the proposed resistance management plans required by EPA for the re-registration of *Bt* maize hybrids will need to be reevaluated.

Two *O. nubilalis* strains were used in this analysis: an unselected control strain (KS-SC-S) and a Dipel ES–resistant strain (KS-SC-R) (*3, 7*). The resistant strain has demonstrated 70-fold resistance to Dipel ES (*3*). These tests began when the strains were in the eighth and ninth generations in culture (seventh selected generation). Pupae were divided by sex before eclosion. Females from one population were mass-crossed with males from the other population. Four types of crosses were made: (i) reciprocal parental crosses between resistant (R) and susceptible (S) moths, (ii) $F_1 \times F_1$ crosses, (iii) backcrosses of F_1 with susceptible (S) moths (*8*), and (iv) three successive backcrosses between heterozygous (RS) and susceptible (S) moths (*9*). The susceptibility of corn borer neonates to Dipel ES was determined with a bioassay (*10*). The dose/mortality data were analyzed with probit regression.

Maternal effects on Dipel ES resistance were examined by comparing median lethal concentrations (LC_{50}'s) of progeny derived from the reciprocal parental crosses. The LC_{50}'s for the two reciprocal crosses did not differ significantly, based on overlapping 95% confidence intervals (CIs) (Table 1). This result confirms that Dipel ES resistance is inherited autosomally and that it is not sex-linked (*11*).

Examination of Dipel ES resistance inheritance indicates that the trait is incompletely dominant. The pooled LC_{50} for the F_1 crosses was significantly higher than that for the susceptible strain and significantly lower than that for the resistant strain, based on a nonoverlap of 95% CIs (Table 1). The dose/mortality regression line for the F_1 was closer to that of the resistant strain than to that of the susceptible strain (Fig. 1). Stone's method (*12*) indicates a dominance value D of 0.721 ± 0.02, consistent with incomplete dominance (*12*).

We tested the standard monogenic inheritance model by comparing observed and expected mortality at different Dipel ES concentrations (*6, 13*). There was no significant deviation between observed and expected mortality for six of seven concentrations for the pooled backcross data and for five of seven concentrations in the pooled F_2 generation. The minimum number of effective genes calculated with a modification of Lande's method (*14*) yielded a calculated number of genes n_E of 0.57. Both results suggest that one gene (or a few genes) influenced Dipel ES resistance in this European corn borer strain.

Repeated backcrosses between heterozygous (RS) and susceptible (S) populations (*9*) showed no significant increase in mortality over the three successive backcross generations, and the mortalities for the three backcrosses were not significantly different from expected values based on criteria for monofactorial inheritance (Table 2). This result also suggests the presence of a single gene governing Dipel ES resistance in this strain of *O. nubilalis* (*13*).

There was a somewhat higher genetic variation for the backcross and F_2 populations than for the resistant parent, susceptible parent, or F_1 progeny, based on the slopes of the probit regression lines (Table 1 and Fig. 1). This increased variance suggests that the number of loci that affected Dipel ES resistance in the strain is small (*6, 15*).

The inheritance of resistance to *Bt* has been assessed in several other insects. In *Plodia interpunctella* (Hübner), resistance was autosom-

Table 1. Dose/mortality results for the progeny of susceptible crosses (S), resistant crosses (R), reciprocal crosses R × S (F_1), F_1 crosses (F_2), and F_1 × susceptible backcrosses of *O. nubilalis* in bioassays with Dipel ES incorporated in the diet. The RS and SR are heterozygous strains of *O. nubilalis*. The first letters in crosses represent the female. Numbers in parentheses indicate the 95% CI.

Strain	LC_{50} (ml of Dipel/kg of diet)	Slope ± SE	Resistance factor
	Parents		
S	0.17 (0.16 to 0.18)	2.26 ± 0.08	
R	10.96 (9.75 to 12.20)	3.76 ± 0.28	65.2
	Reciprocal crosses (F_1)		
R × S	7.10 (6.26 to 7.99)	3.40 ± 0.23	42.2
S × R	5.88 (4.18 to 8.00)	5.03 ± 1.05	35.0
Pooled	6.13 (5.22 to 7.21)	4.20 ± 0.39	36.5
	Backcrosses		
S × SR	1.33 (0.75 to 2.15)	1.77 ± 0.23	7.9
RS × S	1.62 (1.28 to 2.02)	1.94 ± 0.12	9.6
Pooled	1.47 (1.02 to 2.03)	1.85 ± 0.16	8.7
	F_2 crosses		
SR × SR	1.71 (1.44 to 2.01)	1.82 ± 0.10	10.2
RS × RS	2.65 (1.50 to 4.38)	2.45 ± 0.42	15.8
Pooled	2.14 (1.59 to 2.81)	2.06 ± 0.17	12.7
	Repeated backcrosses		
First repeated pooled	1.82 (1.45 to 2.22)	1.64 ± 0.12	10.8
Second repeated pooled	1.58 (1.30 to 1.90)	1.51 ± 0.09	9.4
Third repeated polled	1.28 (0.84 to 1.85)	1.34 ± 0.12	7.6

Table 2. Dose/mortality results for the progeny of three successive backcrosses of the heterozygote to the susceptible strain of *O. nubilalis* in bioassays with Dipel ES incorporated in the diet. Expected mortality for monogenic inheritance at 2.43 ml of Dipel ES per kilogram of diet = 59.5%. Numbers in parentheses indicate percentage mortality.

Repeated backcross	Number of insects tested	Number that died		χ^2	P value
		Observed	Expected		
1	125	70 (56.0)	74.4	0.64	0.4237
2	123	68 (55.3)	73.2	0.91	0.3401
3	128	68 (53.1)	76.2	2.18	0.1398

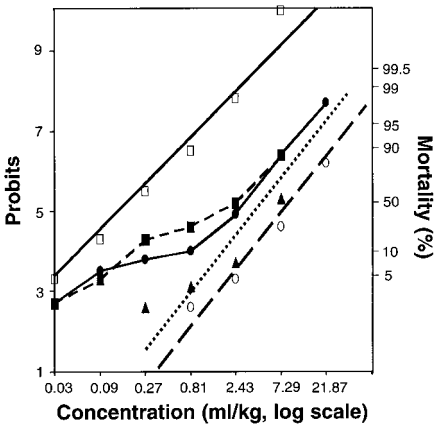

Fig. 1. Dose/morality regression lines for the progeny of susceptible strain (open squares, straight solid line), resistant strain (open circles, long-dashed line), reciprocal crosses R × S (F_1) (solid triangles, dotted line), F_1 crosses (F_2) (solid circles, angled solid line), and F_1 × susceptible backcrosses (solid squares, angled dashed line) of *O. nubilalis* in bioassays with Dipel ES incorporated in the diet.

al and recessive or partially recessive (*16*). In *Heliothis virescens* (Fabricius), resistance to a delta endotoxin of *Bt* subsp. *kurstaki* strain HD-1 was autosomal, incompletely dominant, but controlled by several genetic factors (*17*). The high level of resistance in *H. virescens* to CryIAc and to CryIAb was partially recessive and controlled by one or a few loci, but the low level of resistance to CryIIA was more dominant (*18*). In four colonies of *Plutella xylostella* (L.) (*6, 19*), inheritance of resistance to *Bt* subsp. *kurstaki* was recessive or incompletely recessive. Resistance was most likely autosomal and controlled by one or a few major loci. The genetic bases for resistance to *Bt* toxins appear to be different in different insects (*20*) and may even differ for different toxins. Our results agree with other studies in that Dipel ES resistance in *O. nubilalis* appears to be caused by a single autosomal gene (or by a few genes). However, our results differ in that resistance in this strain appears to be incompletely dominant rather than recessive.

These results could have important implications for resistance management of *O. nubilalis* in *Bt* maize. The currently proposed resistance management strategy for *Bt* maize, the high-dose/refuge strategy (*1, 2, 21*), requires (i) that plant tissue be very toxic so that heterozygotes for resistance are killed, (ii) that the resistance alleles be very rare, and (iii) that susceptible insects are within an effective mating distance of resistant insects. The high-dose/refuge strategy would not be useful for resistance management if the trait is dominant. However, the genetic dominance we observed is expressed over a specific range of Dipel ES doses; at higher dosages, all individuals are expected to be susceptible. The practical importance of this genetic dominance will depend on whether these insects can survive on *Bt* maize. The high-dose/refuge strategy is subject to a number of stringent prerequisites that may be difficult to meet in practice. More robust resistance management options are needed.

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7. These *O. nubilalis* strains were initiated with 48 egg masses collected in July 1995 from maize fields near St. John, in south central Kansas. The exposure of the *O. nubilalis* population to *Bt* pesticides in the field is unknown but is believed to be low (*3*). However, this *O. nubilalis* population was probably exposed to other non-*Bt* foliar pesticide treatments on an annual basis over the past 20 to 30 years. After one generation, the progeny were divided into two strains: KS-SC-S (the unselected control strain) and KS-SC-R (the resistant strain). The corn borers were reared on a meridic agar-based diet. The selected strain was reared on the same diet, but for each selected generation, neonates were exposed to the diet laced with Dipel ES at concentrations that caused 80 to 99% mortality. Larvae from both strains continued to develop normally on greenhouse-grown maize plants. Neonates of the Dipel ES–resistant *O. nubilalis* strain were able to cause more damage than susceptible insects when placed on certain greenhouse-grown *Bt* maize hybrids (F. Huang, R. A. Higgins, L. L. Buschman, unpublished data).
8. The reciprocal crosses of resistant and susceptible strains demonstrated that resistance to Dipel ES in *O. nubilalis* was incompletely dominant. Therefore, we backcrossed the F_1 with the susceptible strain. This choice increased our ability to distinguish the modes of inheritance (*6*).
9. Because the resistance was incompletely dominant, according to the data obtained from the reciprocal crosses (F_1), we crossed the hybrid and susceptible strains. A discriminating dose of 2.43 ml of Dipel ES per kilogram of diet was employed in the selection of offspring. At this concentration, the susceptible strain exhibited 99% mortality, whereas the RS hybrids in these tests experienced 91% survival.
10. Dipel ES was incorporated in the diet at 0.03, 0.09, 0.27, 0.81, 2.43, 7.29, and 21.87 ml of Dipel ES per kilogram of diet. There was also a no–Dipel ES control. The dilutions were prepared with 0.05% Triton X-100. The diets were poured into cells in a 128-cell tray, and a single neonate larva was placed in each cell. For each concentration, there were four replicates of 32 larvae ($n = 128$ larvae). The bioassay trays were maintained at 27°C under continuous light. Mortality was assessed on the fifth day.
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An Essential Role for DNA Adenine Methylation in Bacterial Virulence

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Salmonella typhimurium **lacking DNA adenine methylase (Dam) were fully proficient in colonization of mucosal sites but showed severe defects in colonization of deeper tissue sites. These Dam$^-$ mutants were totally avirulent and were effective as live vaccines against murine typhoid fever. Dam regulated the expression of at least 20 genes known to be induced during infection; a subset of these genes are among those activated by the PhoP global virulence regulator. PhoP, in turn, affected Dam methylation at specific genomic sites, as evidenced by alterations in DNA methylation patterns. Dam inhibitors are likely to have broad antimicrobial action, and Dam$^-$ derivatives of these pathogens may serve as live attenuated vaccines.**

Methylation at adenine residues by Dam controls the timing and targeting of important biological processes such as DNA replication, methyl-directed mismatch repair, and transposition (*1*). In addition, Dam regulates the expression of operons such as pyelonephritis-associated pili (*pap*), which are an important virulence determinant in upper urinary tract infections (*2, 3*). The latter regulatory mechanism involves formation of heritable DNA methylation patterns, which con-

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