functions in the two tissues, as occurs with receptor kinases in other systems (*16*). The findings that *cr4* encodes a receptor kinase and that the mutant affects the nonclonal progression of aleurone differentiation imply that cell interactions are involved in the differentiation of aleurone and epidermis and suggest *cr4* may function in a differentiation signal. The mutant phenotype and the similarity to a known ligand binding domain will provide advantages for identifying other components of the *cr4* signal transduction chain.

REFERENCES AND NOTES

1. P. S. Stinard and D. S. Robertson, *Maize Genet. Coop. Newsl.* **61**, 7 (1987); P. S. Stinard, *ibid.* **65**, 17 (1991); *ibid.* **66**, 3 (1992).
2. M. Freeling and B. Lane, in *The Maize Handbook*, M. Freeling and V. Walbot, Eds. (Springer-Verlag, New York, 1994), pp. 17–28.
3. R. N. Stewart and H. Dermen, *Am. J. Bot.* **66**, 47 (1979); S. Satina, A. F. Blakeslee, A. G. Avery, *ibid.* **44**, 311 (1940); R. A. Tilney-Bassett, *Plant Chimeras* (Arnold, Baltimore, MD, 1986).
4. D. B. Walker and D. K. Bruck, *Can. J. Bot.* **63**, 2129 (1985); R. Moore, *ibid.* **62**, 2476 (1984); D. K. Bruck, R. J. Alvarez, D. B. Walker, *ibid.* **67**, 303 (1988); D. K. Bruck and D. B. Walker, *Am. J. Bot.* **72**, 1602 (1985).
5. S. J. Lolle, A. Y. Cheung, I. M. Sussex, *Dev. Biol.* **152**, 383 (1992).
6. B. A. Siegel and J. A. Verbeke, *Science* **244**, 580 (1989).
7. L. F. Randolph, *J. Agric. Res.* **53**, 881 (1936).
8. B. McClintock, in *The Clonal Basis of Development*, S. Subtelny and I. M. Sussex, Eds. (Academic Press, New York, 1978), pp. 217–237.
9. P. W. Becraft and D. R. McCarty, unpublished data.
10. H. Loetscher *et al.*, *Cell* **61**, 351 (1990); C. A. Smith *et al.*, *Biochem. Biophys. Res. Commun.* **176**, 335 (1991); C. Upton, A. M. DeLange, G. McFadden, *Virology* **160**, 20 (1987).
11. D. W. Banner *et al.*, *Cell* **73**, 431 (1993).
12. G. Pearce, D. Strydom, S. Johnson, C. A. Ryan, *Science* **253**, 895 (1991).
13. D. M. Braun and J. C. Walker, *Trends Biochem. Sci.* **21**, 70 (1996).
14. C. Chang, S. F. Kwok, A. B. Bleeker, E. M. Meyerowitz, *Science* **262**, 539 (1993).
15. B. D. Kohorn, S. Lane, T. A. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10989 (1992).
16. P. van der Geer, T. Hunter, R. A. Lindberg, *Annu. Rev. Cell Biol.* **10**, 251 (1994).
17. K. U. Torii *et al.*, *Plant Cell* **8**, 735 (1996); H.-S. Lee, B. Karunanandaa, A. McCubbin, S. Gilroy, T.-h. Kao, *Plant J.* **9**, 613 (1996); J. B. Nasrallah, S. J. Rundle, M. E. Nasrallah, *ibid.* **5**, 373 (1994).
18. R. F. Barker, D. V. Thompson, D. R. Talbot, J. Swanson, J. L. Bennetzen, *Nucleic Acids Res.* **12**, 5955 (1984).

19. We thank J. Cicero for technical assistance. Supported by an NSF postdoctoral fellowship to P.W.B. awarded in 1992 and the Florida Agriculture Experiment Station. Journal series number R-05317.

2 May 1996; accepted 8 August 1996

Similarity of a Chromatic Adaptation Sensor to Phytochrome and Ethylene Receptors

David M. Kehoe* and Arthur R. Grossman

Complementary chromatic adaptation in cyanobacteria acts through photoreceptors to control the biosynthesis of light-harvesting complexes. The mutant FdBk, which appears black, cannot chromatically adapt and may contain a lesion in the apparatus that senses light quality. The complementing gene identified here, *rcaE*, encodes a deduced protein in which the amino-terminal region resembles the chromophore attachment domain of phytochrome photoreceptors and regions of plant ethylene receptors; the carboxyl-terminal half is similar to the histidine kinase domain of two-component sensor kinases.

The phycobilisomes (PBS), macromolecular complexes in cyanobacteria that are peripherally associated with thylakoid membranes, harvest light energy in the visible region of the electromagnetic spectrum between 540 and 660 nm and efficiently transfer that energy to the photosynthetic reaction centers within the thylakoid membranes (*1*). The PBS are composed of two types of proteins, pigmented phycobiliproteins and nonpigmented linker polypeptides. In the filamentous cyanobacterium *Fremyella diplosiphon*, the major species of phycobiliproteins are allophycocyanin (AP), phycocyanin (PC), and phycoerythrin (PE). Linker polypeptides associate with phycobiliproteins and are important for assembly, stability, and efficient energy transfer within the PBS and to the photosynthetic reaction centers (*2*).

As noted almost a century ago (*3*), the pigmentation of cyanobacteria can change in response to light quality; these changes reflect an altered pigment-protein composition of the PBS. In *F. diplosiphon*, red light causes the synthesis of large amounts of the blue chromoprotein PC and small amounts of the red chromoprotein PE, making the color of the organism blue-green. Conversely, in green light the organism has small amounts of PC and large amounts of PE, making it red in color. This change in PBS pigment-protein composition in response to different wavelengths of light is called complementary chromatic adaptation (CCA); it affords the cyanobacterium an adaptive advantage as the light quality of the environment changes because PC effectively absorbs red light and PE effectively absorbs green light. The differences in composition of the PBS in cells grown in red compared with green light are mostly a reflection of differential transcriptional activities of a specific set of red light–inducible PC genes, designated *cpcB2A2* (the protein designation is PCi, for inducible PC) and green light–inducible PE genes, designated *cpeBA* (*4*). The genes encoding linker polypeptides associated with PCi and PE are also regulated by light quality (*5*).

Classes of CCA mutants (*6–10*) that appear red (FdR), blue (FdB), green (FdG), or black (FdBk) have been characterized. FdR strains always express PE and cannot express PCi; they are always red. FdB strains appear bluer than wild-type cells and are less sensitive to light quantity; it takes a greater fluence rate to suppress PCi synthesis in FdB strains than in wild-type cells (*11*). In FdG strains PCi expression is normal, but PE genes are never activated. Finally, FdBk mutants have moderate amounts of both PE and PCi; these amounts are constant in red and green light (*10*).

The gene *rcaC* (regulator of chromatic adaptation) (*9*), which complements some FdR mutants, encodes a polypeptide with similarity to a bacterial response regulator. Response regulators are elements of two-component regulatory systems, which control a wide range of responses to environmental cues in both prokaryotes and eukaryotes (*12, 13*). The RcaC polypeptide (73 kD) is larger than most response regulators and has two conserved receiver domains, one at the COOH-terminus and the other at the NH_2-terminus, each with a putative aspartate phosphorylation site.

Here we identify the DNA fragment (on plasmid pDK1) capable of complementation of one of the FdBk mutants (Fig. 1). It includes open reading frames (ORFs) I, II, and III. ORFIII is truncated in the region encoding the NH_2-terminus of the putative protein. Subclones were modified (Fig. 1) from pDK1 and used for further complementation tests.

Results of complementation studies with each of the modified clones are shown in Fig. 2. The FdBk strain has the same pigmentation in red and green light. When transformed with pDK2 or pDK4, normal CCA is restored. pDK3 cannot complement the mutant phenotype, although it rescues another class of CCA mutants and therefore must produce functional ORFII protein (*14*). Spectral measurements demonstrate

Department of Plant Biology, Carnegie Institution of Washington, 290 Panama Street, Stanford, CA 94305, USA.

*To whom correspondence should be addressed. E-mail: davidk@andrew.stanford.edu

that PC and PE amounts in red and green light in FdBk transformed with pDK2 and pDK4 are quantitatively identical to those of wild-type cells (*14*). These results demonstrate that ORFI alone complements the FdBk mutant.

The putative protein encoded by ORFI (Fig. 3A), designated RcaE, has a molecular mass of 74 kD. Within the NH_2-terminal half of the protein, a region of ~140 amino acids is similar to the chromophore attachment domain of phytochromes, photoreceptors that control a wide range of responses in plants (*15*). This similarity exists primarily in regions of this domain designated region I and region III (Fig. 3A and Table 1). The RcaE sequence is significantly diverged from plant phytochrome sequences in region II. In particular, it lacks conserved amino acids that are associated with the cysteine that covalently binds the chromophore in phytochromes. The COOH-terminal region of RcaE has similarity to the histidine kinase domain of proteins that act as sensors in two-component regulatory systems. Four motifs required for histidine kinase activity, N, G1, F, and G2, and the motif containing the histidine that undergoes phosphorylation, designated H, are also shown (Fig. 3A).

RcaE also has some features in common with plant putative ethylene receptors [(*13, 16, 17*) GenBank numbers Z54099 and U41103] (Fig. 3B), which are similar to sensors of two-component regulatory sys-

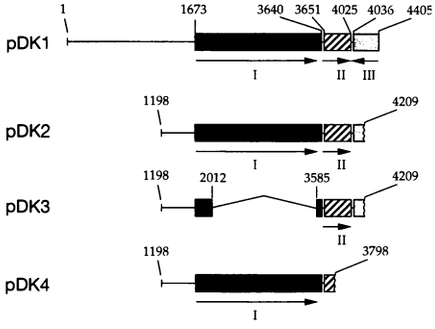

Fig. 1. Clones used in complementation of the FdBk mutant (*26*). Roman numerals refer to ORF numbers, arabic numerals to the nucleotide position relative to the start of pDK1, and arrows indicate the direction of transcription. Constructs pDK2, pDK3, and pDK4 were each tested three times for their ability to restore CCA to the FdBk mutant. Modifications: pDK2, 65 codons deleted from ORFIII; pDK3, in-frame deletion from ORFI; pDK4, deletion of ORFIII and truncation of ORFII.

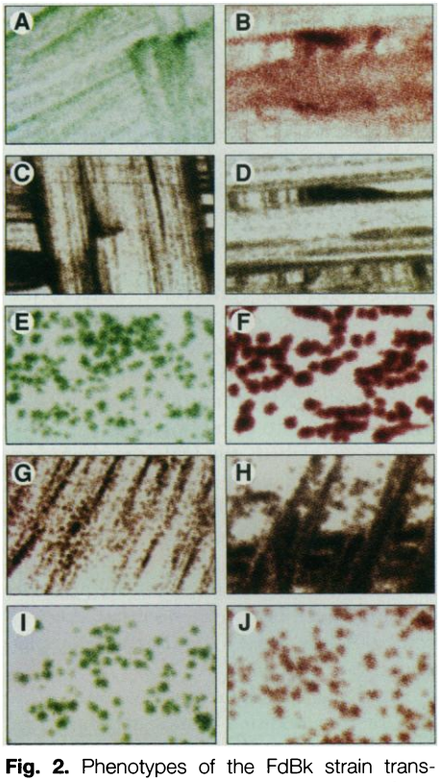

Fig. 2. Phenotypes of the FdBk strain transformed with plasmids containing ORFI and ORFII (*27*). Panels show the phenotypes of wild-type Fd33 (**A** and **B**), FdBk (**C** and **D**), and FdBk transformed with pDK2 (**E** and **F**), pDK3 (**G** and **H**), or pDK4 (**I** and **J**) grown in red light (left panels) and green light (right panels). FdBk cells transformed with unmodified pPL2.7 did not exhibit CCA.

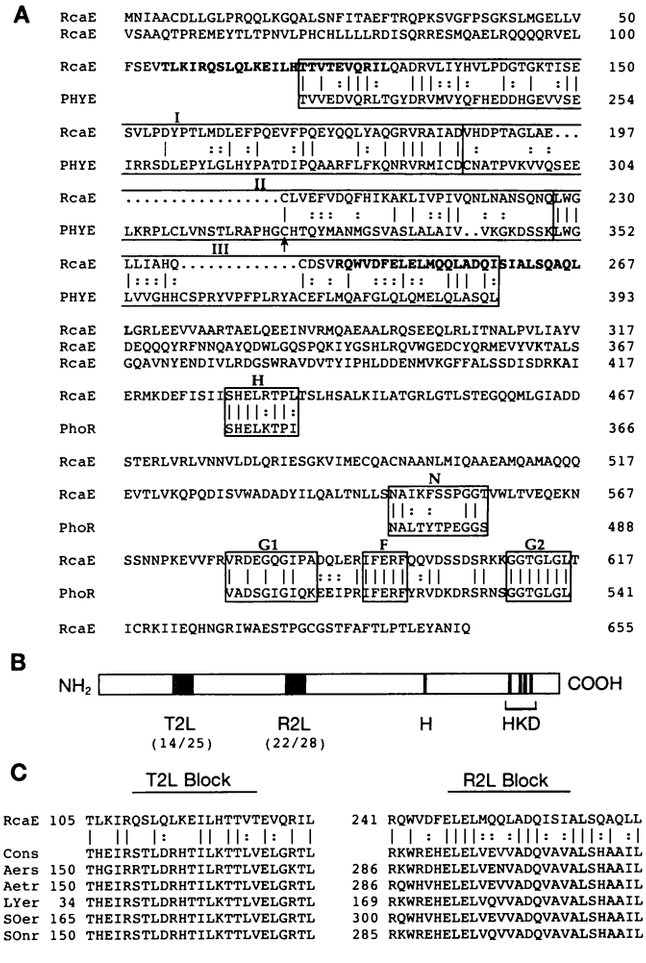

Fig. 3. Amino acid sequence of the predicted RcaE protein showing alignments to a phytochrome from *Arabidopsis*, a bacterial sensor kinase from *Bacillus subtilis*, and several higher plant putative ethylene response sensors (*28*). (**A**) RcaE amino acid sequence, deduced from nucleotide sequence of ORFI (by using the first methionine), is aligned with the chromophore attachment domain of *Arabidopsis* PHYE (*29*) and conserved motifs from the histidine kinase domain of PhoR [the sensor protein involved in the acclimation of *B. subtilis* to phosphorus deprivation (*30*)]. Sequence identity and similarity are represented by vertical lines and dots, respectively. Regions designated I and III are significantly similar between RcaE and eukaryotic phytochromes (Table 1). The region designated II is less similar and contains a cysteine that may correspond to the cysteine that binds the chromophore of phytochromes of plants. Gaps were introduced into the sequences to optimize the alignment (*28*). Alignments of the bacterial sensor kinase motifs H, N, G1, F, and G2 and the PhoR sequence are boxed. Amino acids in boldface type indicate positions of the T2L and R2L motifs. (**B**) Schematic representation of structures common to the sequenced plant putative ethylene receptors and RcaE, including the T2L and R2L motifs, H motif, and histidine kinase domain (HKD). Numbers in parentheses are the fraction of residues for each motif in RcaE that are similar to putative ethylene receptor consensus sequences shown in (C). (**C**) Conservation between plant putative ethylene receptors and RcaE in the sequence and spacing of the T2L and R2L motifs. Comparison of RcaE T2L and R2L sequences to the consensus (Cons) sequences derived from comparisons of ERS (Aers) and ETR1 (Aetr) from *Arabidopsis* (*13, 16*) and putative ethylene receptors from tomato [LYer, GenBank number Z54099; SOer, GenBank number U41103; and SOnr (*17*)]. In all six sequences, these blocks are separated by either 110 or 111 amino acid residues. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

tems. RcaE and the ethylene receptors have similar H, N, G1, F, and G2 domains (*18*). The NH_2-terminal sequences of the putative ethylene receptors contain two additional motifs, T2L and R2L, that are also present in the NH_2-terminal half of RcaE. These motifs are similar with respect to their sequences and their positions relative to each other (Fig. 3C); they also overlap regions of RcaE that are similar to phytochromes (Fig. 3A). Additionally, the T2L region partially overlaps the region of ETR1 that has been shown to bind ethylene (*19*).

The similarity of the COOH-terminus of RcaE to histidine kinases and the identification of RcaC as a response regulator controlling CCA suggests that RcaE operates by means of a histidine-aspartate phosphorelay. The relation of phytochromes to histidine kinases is still unclear. Many eukaryotic phytochromes have COOH-termini with limited similarity to histidine kinases, raising the possibility of an evolutionary relationship between sensor kinases and phytochromes (*20*). However, mutational approaches have uncovered several genes involved in higher plant photomorphogenesis, which is in part controlled by phytochrome. None of these appears to encode two-component regulatory proteins (*21*).

The phenotype of the FdBk mutant and the structural characteristics of RcaE are consistent with RcaE being positioned upstream of RcaC in the signal transduction chain for CCA, and possibly being involved in perceiving light quality. Mutants null for RcaC synthesize large amounts of PE and small amounts of PC in red or green light (*9*). Elevating PC synthesis and blocking PE synthesis in red light appears to require phosphorylation of RcaC (*9*) at an aspartate in the NH_2-terminal receiver domain (*22*). In FdBk mutants, the intermediate amounts of PE and PC may reflect partial, constitutive phosphorylation of the RcaC regulator (and perhaps other CCA regulatory proteins). Partial activity of a regulator protein in the absence of its cognate sensor has been observed and can result from regulator phosphorylation by other sensor proteins in the cell (*23*) or by small molecules such as acetylphosphate (*24*). However, if RcaE is a sensor-photoreceptor, the chromophore would have to be noncovalently associated with the apoprotein, bound to a cysteine lacking the adjacent, conserved residues found in all eukaryotic phytochromes, or bound to a second protein that associates with RcaE.

Our data plus information in sequence databases suggest that *rcaE* is a member of a large, highly diverged gene family that is present in both prokaryotes and eukaryotes with varying degrees of similarity to phytochrome. Several deduced polypeptides encoded within the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803 show differing degrees of relatedness to both RcaE and phytochromes (Table 1). RcaE is as related to *Arabidopsis* PHYE in regions I and III as to the putative polypeptides from *Synechocystis*, but it is more related to the majority of these cyanobacterial sequences in region II. However, *Synechocystis* sequence 1001165 is more related to PHYE in regions I through III than is RcaE. The other *Synechocystis* sequences are no more related to PHYE than is RcaE. Because *Synechocystis* sp. strain PCC 6803 does not exhibit CCA, it is reasonable to speculate that such proteins are important for the regulation of other light-responsive processes in prokaryotes.

The similarity of RcaE to the phytochromes and bacterial sensor proteins, as well as its proposed position in the signal transduction chain of CCA, indicates that it may be the photoreceptor controlling CCA. The similarity of RcaE to plant ethylene receptors raises the possibility that ethylene receptors and the phytochromes may represent diverged proteins that have evolved from a common progenitor. Elucidation of signal transduction processes triggered by phytochrome-like photoreceptors in prokaryotes and an understanding of the evolutionary events that have shaped plant phytochromes and ethylene receptors will help establish the mechanisms for light- and ethylene-regulated gene expression in plants.

Table 1. Relatedness of regions I and III and region II of RcaE to *Arabidopsis* (*Arab.*) PHYE and several RcaE-related hypothetical proteins from *Synechocystis* (*Syn.*), and of regions I and III and region II of *Arabidopsis* PHYE to RcaE and RcaE-related hypothetical proteins from *Synechocystis*. Database numbers are used to identify the *Synechocystis* sequences, with GenBank accession numbers provided in parentheses. Numbers shown are percent identity; bracketed numbers are percent similarity. NA, not applicable.

Sequence	Relatedness to RcaE regions		Relatedness to *Arab.* PHYE regions	
	I and III	II	I and III	II
Arab. PHYE	37 [62]	13 [40]	NA	NA
Syn. 1001288 (D64003)	39 [61]	33 [60]	35 [52]	21 [42]
Syn. 1001300 (D64006)	37 [61]	28 [45]	22 [58]	26 [50]
Syn. 1001165 (D64001)	30 [53]	13 [40]	53 [75]	29 [48]
Syn. 1001174 (D64001)	26 [48]	23 [43]	23 [46]	19 [40]
RcaE	NA	NA	37 [62]	13 [40]

REFERENCES AND NOTES

1. G. Porter *et al.*, *Biochim. Biophys. Acta* **501**, 232 (1978); G. F. W. Searle *et al.*, *ibid.*, p. 246; A. N. Glazer, *J. Biol. Chem.* **264**, 1 (1989); N. Tandeau de Marsac and J. Houmard, *FEMS Microbiol. Rev.* **104**, 119 (1993).
2. A. N. Glazer, *Annu. Rev. Biophys. Biophys. Chem.* **14**, 47 (1985); in *The Cyanobacteria*, P. Fay and C. Van Baalen, Eds. (Elsevier Biomedical, Amsterdam, 1987), pp. 69–88.
3. T. W. Engelmann, *Arch. Anat. Physiol.* (physiological abstract) **1902**, 333 (1902); N. Gaidukov, *Ber. Dtsch. Bot. Ges.* **21**, 517 (1903); *ibid.* **22**, 23 (1904).
4. R. Oelmüller *et al.*, *Plant Physiol.* **88**, 1077 (1988); R. Oelmüller, A. R. Grossman, W. R. Briggs, *ibid.*, p. 1084.
5. T. L. Lomax, P. B. Conley, J. Schilling, A. R. Grossman, *J. Bacteriol.* **169**, 2675 (1987); N. A. Federspiel and A. R. Grossman, *ibid.* **172**, 4072 (1990).
6. N. Tandeau de Marsac, *Bull. Inst. Pasteur* **81**, 201 (1983).
7. J. G. Cobley and R. D. Miranda, *J. Bacteriol.* **153**, 1486 (1983).
8. B. Bruns *et al.*, *ibid.* **171**, 901 (1989).
9. G. G. Chiang, M. R. Schaefer, A. R. Grossman, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 9415 (1992).
10. D. M. Kehoe and A. R. Grossman, *Semin. Cell Biol.* **5**, 303 (1994).
11. E. Casey, thesis, Stanford University (1996).
12. J. S. Parkinson and E. C. Kofoid, *Annu. Rev. Genet.* **26**, 71 (1992); I. M. Ota and A. Varshavsky, *Science* **262**, 566 (1993).
13. C. Chang *et al.*, *Science* **262**, 539 (1993).
14. D. M. Kehoe and A. R. Grossman, unpublished results.
15. R. E. Kendrick and G. H. M. Kronenberg, *Photomorphogenesis in Plants* (Kluwer, Dordrecht, Netherlands, 1994).
16. J. Hua, C. Chang, Q. Sun, E. M. Meyerowitz, *Science* **269**, 1712 (1995).
17. J. Q. Wilkinson, M. B. Lanahan, H.-C. Yen, J. J. Giovannoni, H. J. Klee, *ibid.* **270**, 1807 (1995).
18. D. M. Kehoe and A. R. Grossman, data not shown.
19. G. E. Schaller and A. B. Bleecker, *Science* **270**, 1809 (1995).
20. H. A. W. Schneider-Poetsch, B. Braun, S. Marx, A. Schaumburg, *FEBS Lett.* **281**, 245 (1991); F. Thümmler, P. Algarra, G. M. Fobo, *ibid.* **357**, 149 (1995).
21. P. H. Quail *et al.*, *Science* **268**, 675 (1995).
22. D. M. Kehoe and A. R. Grossman, in *Photosynthesis: From Light to Biosphere*, P. Mathis, Ed. (Kluwer, Dordrecht, Netherlands, 1995), vol. III, pp. 501–504.
23. B. L. Wanner and W. W. Metcalf, *FEMS Microbiol. Lett.* **100**, 133 (1992).
24. B. L. Wanner and M. R. Wilmes-Riesenberg, *J. Bacteriol.* **174**, 2124 (1992); B. L. Wanner, in *Phosphate in Microorganisms. Cellular and Molecular Biology*, A. Torriani-Gorini, E. Yagil, S. Silver, Eds. (ASM Press, Washington, DC, 1994), pp. 215–221.
25. G. G. Chiang, M. R. Schaefer, A. R. Grossman, *Plant Physiol. Biochem.* **30**, 315 (1992).
26. Genomic DNA from wild-type *F. diplosiphon*, strain Fd33 [J. G. Cobley *et al.*, *Plasmid* **30**, 90 (1993)], was partially digested with Sau 3AI, size fractionated on a sucrose gradient, and ligated into the Bam HI site of the broad host range plasmid pPL2.7 (*25*). This recombinant library was introduced into FdBk by electroporation (*8, 25*), except that cells received a 2-day dark treatment before transformation. The plasmid pDK1 was rescued from transformants displaying normal CCA by transforming *Escherichia coli* strain DH5α with DNA isolated from these lines and selecting for kanamycin resistance (*25*). Curing of pDK1 from these lines restored the FdBk phenotype. The plasmid pDK2 was constructed by insertion of a blunt-ended Xba I fragment from pDK1 into the Sma I site of pGEM-7Zf(+) (Promega), exonuclease

III digestion (Promega Erase-a-Base System) of bases 4210 through 4405 (clone pDKΔ4210), followed by digestion and fill-in of the Bbs I site at nucleotide 1198 and the Apa I site in the polylinker outside of ORFIII. This fragment was cloned into the blunted Bam HI site of pPL2.7. The plasmid pDK3 is an Apa I–Pfl MI fragment from pDKΔ4210 inserted into the deletion clone pDKΔ2013 at the Apa I and Aat II sites of the polylinker. The outer four bases of the 3′ end of the Pfl MI end and the outer five bases of the 3′ end of the Aat II end were removed with T4 DNA polymerase (Pharmacia Biotech) before ligation. This resulted in an in-frame deletion of 525 amino acids and addition of a codon for an arginine residue. The insert was excised from this construct with Apa I and Bbs I and cloned into pPL2.7 (as for pDK2). For pDK4, pDKΔ4210 was digested with Bbs I and Bsa WI, and the ends of the excised fragment were end-filled and cloned into pPL2.7 (as for pDK2). Junctions of all constructs and pDK1 were sequenced with an ABI PRISM 310 genetic analyzer.

27. Cell lines were transformed as described in (*25*). After transformation, samples were divided in two, and each portion was plated onto solid BG-11 medium [M. M. Allen, *J. Bacteriol.* **96**, 836 (1968)] containing kanamycin (25 μg/ml). Plates were incubated at 25°C in 35 $\mu E\ m^{-2}\ s^{-1}$ of either constant red light (Westinghouse 20-watt red fluorescent tube, F20T12/R) or constant green light (Westinghouse 20-watt green fluorescent tube, F20T12/G) for 2 weeks.
28. Database searches were conducted with the BLAST Network Service at the National Center for Biotechnology Information [S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)]. Alignments were performed with the Bestfit program from the GCG Wisconsin Sequence Analysis Package, Madison, WI.
29. T. Clack, S. Mathews, R. A. Sharrock, *Plant Mol. Biol.* **25**, 413 (1994).
30. T. Seki, H. Yoshikawa, H. Takahashi, H. Saito, *J. Bacteriol.* **169**, 2913 (1987).

31. We acknowledge the Carnegie Institution of Washington for providing an enlightened research environment and, in particular, W. Briggs, who has always shared his enthusiasm and encouragement with us and has been instrumental in bringing this work to fruition. We also thank E. Casey, D. Bhaya, and B. Kehoe for thoughtful discussions and K. Bump for help in preparing the manuscript. Supported by a NSF 1993 Postdoctoral Fellowship in Plant Biology to D.M.K. and NSF award MCB 9513576 to A.R.G. The GenBank accession number for *rcaE* is U59741. This is Carnegie Institution of Washington publication number 1303.

28 February 1996; accepted 8 May 1996

TECHNICAL COMMENTS

Evolution of Insect Resistance to *Bacillus thuringiensis*–Transformed Plants

More than 30 crop species have been genetically engineered to express *Bacillus thuringiensis* endotoxins which are highly toxic to specific insect pests (*1*). However, several insect species have evolved resistance to *B. thuringiensis* toxins, and resistance evolution could seriously compromise the success of *B. thuringiensis*–transformed crops in controlling pests (*2*).

Recently, D. N. Alstad and D. A. Andow (*3*) proposed a strategy to slow the rate of resistance evolution in the European corn borer to *B. thuringiensis*–transformed maize. Below, I demonstrate that their conclusions are based on an inappropriate comparison of models. Then I use a general model to demonstrate why their strategy does not substantially reduce resistance evolution. I conclude that changing the distribution of toxic plants among fields is not a silver bullet to combat resistance evolution.

A critical feature of corn borer natural history is its preferential migration into the most mature stands (the "preferred crop") during the first of its two annual generations. Alstad and Andow state that resistance evolution can be slowed by using *B. thuringiensis*–toxic plants in the preferred crop, thereby creating a "trap crop." Insect densities predicted by Alstad and Andow's model (Fig. 1A) are compared in their report to those obtained in a model without preference-biased migration (*4*). Because preference-biased migration concentrates insect densities, it increases density dependent mortality and reduces insect abundance. The improvement Alstad and Andow attribute to the "trap crop" strategy is actually caused by preference-biased migration itself (*5*).

The correct comparison of densities would be among cases having different distributions of toxic plants among fields, but retaining preference-biased migration. For example, consider the case when 72% of the fields contain toxic plants, regardless of whether they are preferred or nonpreferred (Fig. 1B), or the case when mixtures of toxic and nontoxic plants are used to reduce insect survival to the same rate in both preferred and nonpreferred crops (Fig. 1C) (*6*). In all three cases, the reduction in the total insect density is the same, so strategies can be evaluated in terms of the rate of the evolution of resistance (*7*). Alstad and Andow's strategy is only slightly better than the second (Fig. 1B), and worse than the third (Fig. 1C) example.

I developed a general model, appropriate for a large number of insect pests, to ask how the distribution of toxic plants among fields affects resistance evolution (*8*). The rate of resistance evolution increases with the average per capita reproductive potential of resistant insects, R, at the reduced insect density, n_{min}, created by mortality of susceptible insects (*9*). R is calculated as

$$R = rf\mathrm{F}[fK_1 n_{\min}] + r(1-f)\mathrm{F}[(1-f)K_2 n_{\min}] \quad (1)$$

where F is a function giving density-dependent survival, r denotes the insect's intrinsic rate of increase, f is the fraction of insects in the preferred crop, and K_1 and K_2 are the fractions of susceptible insects surviving the toxic plants in preferred and nonpreferred crops. This equation demonstrates the trade-off between reducing insect density and slowing resistance evolution. Because F is a decreasing function, lower $n_{\min}$ produces higher R and more rapid resistance evolution. It is possible to mitigate this trade-off by changing values of K_2 and K_1. In the nonpreferred crop, density dependence is weak (because low immigration produces smaller populations), and therefore the per capita reproductive potential of resistant insects is greater than in the preferred crop. Thus, R is lowest when the proportion of insects killed by toxic plants is higher in the nonpreferred crop ($K_2 \leq K_1$) (*10*).

An example for the rate of resistance

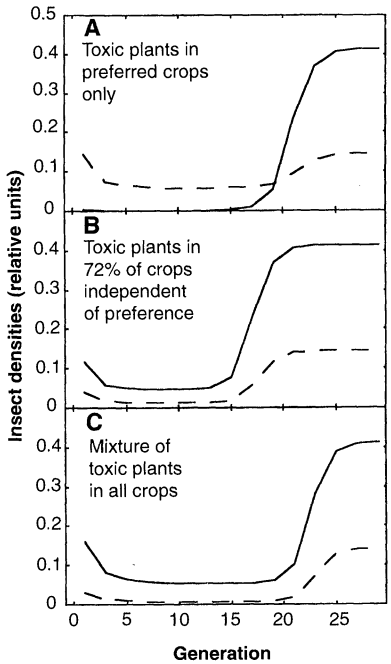

Fig. 1. Insect densities during the first annual generation in preferred (solid) and nonpreferred (dashed) crops. Densities increase around generation 20 because of the increase in resistance allele frequency. (**A**) From the model presented by Alstad and Andow (*1*) [with the typo in equation 9 in the report corrected: $X'''' = X'''(1 + aX''')^{-b}$] in which toxic plants in preferred crops reduce survival to 0.1%. (**B**) A modified model in which 72% of both preferred and nonpreferred fields contain toxic plants that reduce survival to 0.1%. (**C**) The case with density-independent survivals of $K_1 = K_2 =$ 15.25% in both preferred and nonpreferred crops.