Development time and resistance to *Bt* crops

Crop plants genetically engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (*Bt*) are being grown on millions of hectares, but their success will be short-lived if pests adapt to them quickly[1,2]. The primary strategy for delaying insect resistance to transgenic *Bt* plants is to provide refuges of host plants that do not produce *Bt* toxins. This potentially delays the development of insect resistance to *Bt* crops by providing susceptible insects for mating with resistant insects. But our laboratory results with a worldwide pest of cotton, pink bollworm moths (*Pectinophora gossypiella*)[3], contradict an important assumption of the refuge strategy. We find that a resistant strain of larvae on *Bt* cotton takes longer to develop than susceptible larvae on non-*Bt* cotton. This developmental asynchrony favours non-random mating that could reduce the expected benefits of the refuge strategy.

The refuge strategy has two critical assumptions: that inheritance of resistance is recessive, and that random mating occurs between susceptible and resistant insects. If resistance is recessive, hybrid first-generation (F_1) offspring produced by matings between susceptible and resistant adults are killed by eating *Bt* plants. If mating is random, initially rare homozygous resistant adults emerging from *Bt* plants are likely to mate with the more abundant homozygous susceptible adults emerging from non-*Bt* plants, producing hybrid F_1 progeny that cannot survive on *Bt* plants. Mathematical models and limited data from laboratory and greenhouse studies indicate that resistance can be delayed substantially when these assumptions are valid[4–7].

Previous work on the feasibility of the refuge strategy has focused on inheritance of resistance, spatial proximity of refuges relative to transgenic crops, and refuge size[4–7]. To achieve random mating, however, resistant adults from *Bt* plants and susceptible adults from non-*Bt* plants must emerge synchronously[4,7]. We tested the inheritance of resistance and synchrony for the pink bollworm by measuring survival and developmental rates of a laboratory-selected resistant strain, a susceptible strain, and their hybrid F_1 progeny on *Bt* cotton and non-*Bt* cotton.

Consistent with one of the assumptions of the refuge strategy, we find that pink bollworm resistance to *Bt* cotton is recessive. Survival of the hybrid F_1 progeny (2%) was not higher than survival of the susceptible strain (6%), and both were markedly lower than survival of the resistant strain

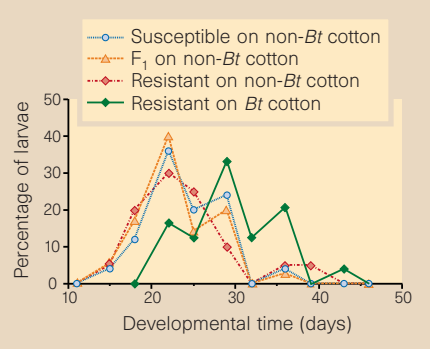

Figure 1 Development of pink bollworm larvae on *Bt* and non-*Bt* cotton plants. Pieces of paper, each with 20–40 eggs, were placed under the bracts of 74 bolls of 16 *Bt* cotton plants (Delta Pine 50B) and 10 non-*Bt* cotton plants (Delta Pine 50) in a greenhouse. After a week, we counted a total of 1,411 entrance holes made by neonates. After two weeks, bolls were caged, and twice a week we counted mature larvae that had exited from bolls. For each strain, survival on *Bt* cotton was estimated by adjusting for mortality on non-*Bt* cotton using Abbott's correction. The resistant strain (APHIS-98R) developed significantly more slowly in bolls of *Bt* cotton (29.8 ± 1.1 days) than the susceptible strain (APHIS-S) did in bolls of non-*Bt* cotton (24.1±0.9) (*t*-test, $t = 3.89$, d.f. = 47, $P < 0.001$). Developmental time on non-*Bt* cotton did not vary significantly among the resistant strain, the susceptible strain and the F_1 progeny.

(37%) (*G*-test, $G_{adj} = 24.8$, d.f. = 1, $P < 0.001$). In the only other case in which inheritance of resistance was studied using *Bt* plants, resistance was also recessive[8]. These results differ from the non-recessive resistance to *Bt* toxins in artificial diet seen in a laboratory-selected strain of European corn borer[9].

Resistant larvae on *Bt* cotton required an average of 5.7 days longer to develop than susceptible larvae on non-*Bt* cotton (Fig. 1). Field data suggest that the median longevity of male pink bollworms is less than a week[10], and laboratory results show that 80% of moths mate within three days of emergence[2]. This developmental asynchrony therefore favours assortative mating among resistant moths from *Bt* plants. In the field, the extent of developmental asynchrony and assortative mating would be affected by variation in toxin expression, weather and overlap between generations.

Assortative mating would generate a disproportionately high number of homozygous resistant insects, accelerating the evolution of resistance. This effect would be diminished if the slower development of resistant larvae increased mortality associated with overwintering or other factors. Computer simulations show that interactions between developmental asynchrony and season length increase uncertainty because they either hasten or slow the evolution of resistance[11]. There are no reports of resistance to *Bt* crops in the field, but our results indicate that developmental asynchrony must be considered in efforts to sustain this technology.

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1. Butler, D. & Reichhardt, T. *Nature* **398,** 651–656 (1999).
2. Mellon, M. & Rissler, J. *Now or Never, Serious New Plans to Save a Natural Pest Control* (Union of Concerned Scientists, Cambridge, MA, 1998).
3. Ingram, W. R. in *Insect Pests of Cotton* (eds Matthews, G. A. & Tunstall, J. P.) 107–148 (CABI, Wallingford, 1994).
4. Gould, F. *Annu. Rev. Entomol.* **43,** 701–726 (1998).
5. Liu, Y.-B. & Tabashnik, B. E. *Proc. R. Soc. Lond. B* **264,** 605–610 (1997).
6. Shelton, A. M., Tang, J. D., Earle, E. D. & Roush, R. T. in *Pest Management – Future Challenges: Proc. 6th Australasian Appl. Entomol. Res. Conf. Vol. 1* (eds Zalucki, M. P., Drew, R. A. I. & White, G. G.) 258–266 (Univ. Queensland, Brisbane, 1998).
7. Tabashnik, B. E. *Proc. R. Soc. Lond. B* **255,** 7–12 (1994).
8. Metz, T. D., Roush, R. T., Tang, J. D., Shelton, A. M. & Earle, E. D. *Mol. Breed.* **1,** 309–317 (1995).
9. Huang, F., Buschman, L. L., Higgins, R. A. & McGaughey, W. H. *Science* **284,** 965–967 (1999).
10. Henneberry, T. J. & Keaveny, D. F. III *Suppression of Pink Bollworm by Sterile Moth Releases* (US Department of Agriculture, Springfield, VA, 1985).
11. Peck, S. L., Gould, F. & Ellner, S. P. *J. Econ. Entomol.* **92,** 1–16 (1999).

How was the *Sdic* gene fixed?

Nurminsky *et al.*[1] have described a new gene of *Drosophila melanogaster*, termed *Sdic* (for sperm-specific dynein, intermediate chain), which appears to have evolved recently as a result of fusion between duplicated copies of two adjacent genes, *Cdic* and *AnnX*. They consider that the low DNA sequence variation at *Sdic* and *Cdic* is consistent with a recent 'selective sweep' associated with the fixation of *Sdic*. (In a selective sweep, new favourable mutations are incorporated so rapidly that linked alleles can 'hitchhike' and become fixed.) Here we evaluate the evidence for this proposal.

Sdic and *Cdic* are located in the centromere–proximal region of the X chromosome, where the frequency of genetic recombination is greatly reduced[2]. A relation between reduced recombination and reduced occurrence of natural polymorphisms in *Drosophila* is well established[3], which Nurminsky *et al.* argue implies frequent selective sweeps[1]. But there are other explanations[4,5], such as background selection, which can eliminate recurrent deleterious mutations by selection and have the effect of removing variants at linked neutral sites[4]. This model quantitatively fits the data on the relation between recombination rate

and the amount of genetic variation in *D. melanogaster*[6].

To justify using evidence from DNA sequence variation to show that there has been a selective sweep involving *Sdic* and *Cdic*, it is necessary (if not sufficient) to demonstrate that there is less variation in these genes than would be expected from the background selection model for genes in this region of the X chromosome. This was not done by Nurminsky *et al.*[1], and indeed it is difficult to do. For example, although these genes are in a region where recombination is reduced in frequency compared with the middle of the X chromosome, the relation between the rate of recom-bination and the location is not known with any accuracy[2]. It is therefore not easy to predict the expected degree of variation under any model, in contrast to the tip of the X chromosome where the gradient of recombination frequency is better known[6].

The gene *Zw* is located at position D1 on the X chromosome, just distal to *Sdic* and *Cdic*, and has a nucleotide-site diversity (π) of 3.8×10^{-3} (ref. 7); the gene *su(f)* is located at 20E–F and has a π value of 0.5×10^{-3} (ref. 8). The π values for *Sdic* and *Cdic* (0.89×10^{-3} and 0.45×10^{-3}, respectively) are closer to those of *su(f)* than to that of Zw, but the large standard deviations of these estimates (0.73×10^{-3} and 0.50×10^{-3}, respectively) mean the true π values may lie between those for *Zw* and *su(f)*. Differences in selective constraints on different genes may also contribute to differences among loci; these can be accounted for by calibrating with respect to interspecific sequence comparisons[9], which was not done here[1].

The effects of selective sweeps can be detected from departures of frequencies of variants from neutral expectation, as measured by statistics such as Tajima's *D* (ref. 10). The small numbers of variant sites at *Sdic* and *Cdic* mean that such tests lack power in this case. Although Tajima's *D* values are negative for both loci (-0.085 and -0.104, respectively), indicating an excess of rare variants (as expected after a selective sweep[10]), their magnitudes are far below those needed for statistical significance.

We believe, therefore, that the data presented by Nurminsky *et al.* do not provide convincing evidence for a recent selective sweep. This does not imply that *Sdic* has not been fixed by selection. It is possible that the fixation event took place relatively soon after the divergence of *D. melanogaster* and *D. simulans*, and that variability in this region has since returned to that expected for its degree of recombination. Only much better characterization of the levels of genetic diversity and recombination frequencies in this region of the X chromosome can resolve this question.

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1. Nurminsky, D. I., Nurminskaya, M. V., De Aguiar, D. & Hartl, D. L. *Nature* **396,** 572–575 (1998).
2. Schalet, A. & Lefevre, G. in *The Genetics and Biology of* Drosophila (eds Ashburner, M. & Novitski, E.) 847–902 (Academic, London, 1976).
3. Begun, D. J. & Aquadro, C. F. *Nature* **356,** 519–520 (1992).
4. Charlesworth, B., Morgan, M. T. & Charlesworth, D. *Genetics* **134,** 1289–1303 (1993).
5. Gillespie, J. H. *Gene* **205,** 291–299 (1997).
6. Charlesworth, B. *Genet. Res.* **68,** 131–150 (1996).
7. Eanes, W. F., Kirchner, M. & Yoon, J. *Proc. Natl Acad. Sci. USA* **90,** 7475–7479 (1993).
8. Langley, C. H., MacDonald, J., Miyashita, N. & Aguadé, M. *Proc. Natl Acad. Sci. USA* **90,** 1800–1803 (1993).
9. Hudson, R. R., Kreitman, M. & Aguadé, M. *Genetics* **116,** 153–159 (1987).
10. Simonsen, K. L., Churchill, G. A. & Aquadro, C. F. *Genetics* **141,** 413–429 (1995).

Nurminsky and Hartl reply — One hallmark of persistent strong background selection is a severe diminution of codon usage bias[1–3]. An example is the *Drosophila* gene *rolled* (gene 1 in Fig. 1), which is located in the centromeric heterochromatin of chromosome 2, where recombination is severely restricted. Other genes shown in Fig. 1 are located near the base of the X chromosome. Genes 10 and 11 are *AnnX* and *Cdic*, respectively, which flank the *Sdic* gene[4]. As pointed out by Charlesworth and Charlesworth, gene 2, which is *su(f)*, has much less DNA sequence variation than gene 17 (*Zw*). This difference is consistent with the discordant levels of codon usage bias and suggests strong background selection at *su(f)* but not at *Zw*. The degree of codon usage bias shows an extremely sharp increase as the gene locations progress outwards from *su(f)*. The transition is near genes 5 (*sol*) and 6 (*slgA*), which are proximal to the *Cdic–AnnX* region.

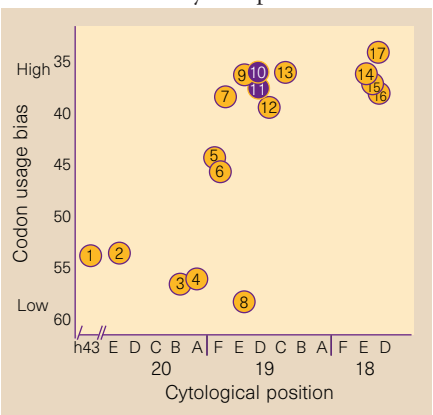

Figure 1 Codon usage bias is scaled according to the effective number of codons[7]. The vertical axis is inverted because a smaller effective number of codons corresponds to a greater bias in codon usage. A similar pattern is seen with other measures of codon bias, such as the χ^2/L statistic[8] (data not shown). The genes and their accession numbers are: 1, *rl* (M95124); 2, *su(f)* (X62679); 3, *S6kII* (L28945); 4, *fog* (U03717); 5, *sol* (M64084); 6, *slgA* (L07330); 7, *dod* (U35140); 8, *shakB* (U17330); 9, *run* (X56432); 10, *AnnX* (M34069); 11, *Cdic* (AF070699); 12, *Pbprp2* (U05981); 13, *Pp4-19C* (Y14213); 14, *Mer* (U49724); 15, *Cdc42* (U11824); 16, *Bap* (X75910); and 17, *Zw* (M26673, M26674).

The result is that *AnnX* and *Cdic* are located in a region of codon usage bias similar to that of *Zw*. The cytological region 19DE might therefore support a level of DNA sequence variation of *Sdic* and *Cdic* comparable to that of *Zw*. However, based on the amount of polymorphism observed for *Zw*, the probability of obtaining a value as low or lower than that for *Sdic* and *Cdic* is about 0.043 and 0.008, respectively. These estimates are based on 10,000 simulations using Watterson's formula[5] for pairwise mismatches in the infinite-alleles model with no recombination, so they should be conservative. There seems to be a statistically significant difference between *Zw* and the other two genes.

The evolution of *Sdic* required an initial duplication and gene fusion accompanied or followed by three deletions, two more insertions/deletions (including one that created a new splice junction), 11 nucleotide substitutions (including reversal of a chain-terminating codon), and a ten-fold tandem reiteration of the *Sdic* coding sequence. Although all these changes may have occurred shortly after the divergence of *D. melanogaster* and *D. simulans*, the similarity in degree of codon usage bias of *Cdic* and *Zw*, contrasted with the significant discrepancy in their levels of nucleotide diversity, provides independent evidence in support of our original inference of at least one relatively recent selective sweep. The negative values of Tajima's *D* statistic[6] for *Sdic* and *Cdic* also support this idea, notwithstanding their lack of statistical significance.

Charlesworth and Charlesworth are correct in pointing out that all the genes in the region 19DE might have limited DNA sequence variation as a result of background selection, despite the higher than predicted level of codon usage bias indicated in Fig. 1. We agree that a much more complete characterization of the levels of genetic diversity and recombination in this region would be informative.

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1. Kliman, R. M. & Hey, J. *Mol. Biol. Evol.* **10,** 1239–1258 (1994).
2. Munte, A., Aguadé, M. & Segarra, C. *Genetics* **147,** 165–175 (1997).
3. Kreitman, M. E. & Antezana, M. in *Evolutionary Genetics from Molecules to Morphology* (eds Singh, R. S. & Krimbas, C.) (Cambridge Univ. Press, New York, in the press).
4. Nurminsky, D. I., Nurminskaya, M. V., De Aguiar, D. & Hartl, D. L. *Nature* **396,** 572–575 (1998).
5. Watterson, G. A. *Theor. Popul. Biol.* **7,** 256–276 (1975).
6. Tajima, F. *Genetics* **123,** 585–595 (1989).
7. Wright, F. *Gene* **87,** 23–29 (1990).
8. Shields, D. C., Sharp, P. M., Higgins, D. G. & Wright, F. *Mol. Biol. Evol.* **5,** 704–716 (1988).