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A genomic approach to understanding *Heliothis* and *Helicoverpa* resistance to chemical and biological insecticides

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Genomics is the comparative study of the structure and function of entire genomes. Although the complete sequencing of the genome of any insect pest is far in the future, a genomic approach can be useful in the study of mechanisms of insecticide resistance. We describe this strategy for *Heliothis* and *Helicoverpa*, two of the most destructive genera of pest moths (Lepidoptera) worldwide. Genome-wide linkage mapping provides the location of major and minor resistance genes. Positional cloning identifies novel resistance genes, even when the mechanisms are poorly understood, as with resistance to *Bacillus thuringiensis* toxins. Anchor loci provide the reference points for comparing the genomes and the genetic architecture of resistance mechanisms among related species. Collectively, these tools enable the description of the evolutionary response of related, but independent, genomes to the common selective pressure of insecticides in the environment. They also provide information that is useful for targeted management of specific resistance genes, and may even speed the search for families of novel insecticidal targets in Lepidoptera.

Keywords: insect genomics; linkage mapping; positional cloning; gene families

1. INTRODUCTION

During the past ten years, there has been unprecedented growth in one of the most paradoxical of all the biological sciences: genomics. In the sense that genomics aims at the comparative study of the structure and function of entire genomes, it is breathtakingly holistic. By analysing those genomes uniformly down to the resolution of single base pairs, it verges on being mindlessly reductionist. The tally of organisms whose genomes have been entirely sequenced has started an exponential growth phase, and biology will never be the same.

The current obsession with DNA sequences often obscures the fact that genomics is made possible by the convergence of two lines of research: genetic and physical. The genetic path began with the invention of linkage maps by Sturtevant and culminates in high-density maps saturated with molecular markers and constructed with data from crosses. The physical path started with Sutton's and Boveri's realization that chromosomes carried the units of heredity and ends in physical maps, which are collections of overlapping fragments of DNA, reassembled back into the same positions they naturally occupy along the chromosome. Points of correspondence between the genetic and physical maps are identified using the DNA sequences of the molecular markers. The two maps merge into one at the instant the entire DNA sequence (all possible markers) is known.

For the genomics of insect pests, this instant is far in the future. In the meantime, there are still aspects of a whole-genome approach that can inform our studies of mechanisms of insecticide resistance. Most useful at present are applications of linkage maps. Our development of a map for the tobacco budworm moth, *Heliothis virescens* (Lepidoptera: Noctuidae) was motivated by the need to analyse multiple resistance mechanisms to chemical insecticides. Currently, about 350 marker loci have been assigned to 31 linkage groups. These markers include 18 allozyme loci resolved by starch gel electrophoresis and enzyme-specific staining, ten restriction-fragment length polymorphisms (RFLPs) defined by anonymous single-copy genomic clones, nine RFLPs defined by known genes, 110 rapid amplified polymorphic DNA (RAPD) markers (Williams *et al*. 1990), and 206 amplified-fragment length polymorphism (AFLP) markers (Vos *et al*. 1995).

This linkage map has been used to determine the number and identity of linkage groups contributing to resistance to a particular insecticide in a particular strain (Heckel *et al*. 1997*b*). Linkage groups are identified using specific marker loci previously localized to them. Resistance can be measured directly by bioassay or indirectly by measuring some property of a resistance mechanism. Quantitative rather than qualitative measures of resistance can provide the extra information necessary to distinguish between resistance genes of major versus minor effect. Depending on the resolution of the mapping study, it may be possible to further discern the number of resistance genes within a linkage group.

We have recently initiated parallel genetic studies in the bollworm *Helicoverpa armigera*. We anticipate that comparison of these two species' maps will shed light on

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conservation of chromosome structure as well as shared resistance mechanisms: independent 'discoveries' of a common evolutionary solution to the same environmental stress. This comparative genomic approach is well established in mammalian genetics (especially human and mouse) and in the genetics of graminaceous crop plants (rice, wheat, sorghum, maize, etc.). It is starting to show its use in human pathogens, disease vectors and domesticated animals. It is our thesis that a genomic approach also has distinct benefits for the study of insect pests of crops, particularly in understanding and circumventing insecticide resistance, as well as in the discovery of novel insecticidal targets. Heliothine moths, which include some of the most destructive crop-pest species worldwide, offer a tractable and rewarding system for the application of a genome-wide perspective, three aspects of which are reviewed here.

2. GENOME SCANNING FOR RESISTANCE GENES

In our first example, we illustrate how a whole-genome approach adds increased resolution to the genetic characterization of pyrethroid resistance in *H. armigera*. In the standard approach to studying the genetic basis of resistance in a given strain, it is crossed to a susceptible strain, and the hybrids backcrossed to one or both parental strains. The dose–mortality curve of the hybrids is compared to the parental strains to estimate the degree of dominance. The hybrids, which are heterozygous at all resistance loci, transmit resistant and susceptible alleles in the ratio 1:1 to their progeny. The dose–mortality curve of these backcross progeny is examined for goodness-of-fit to a single-locus model. If the hypothesis is not rejected, it is usually concluded that resistance is monogenic; if the goodness-of-fit is poor, the data may be consistent with two or more loci affecting resistance. Although the analysis usually stops there, in certain cases the variance of the tolerance distribution in the F_2 and other generations can be used to estimate the number of effective factors, i.e. the number of loci affecting the trait if they all have the same effect and act additively.

This standard approach has the advantages that it is simple, well-accepted by most researchers, and can provide convincing evidence for a single major resistance locus with a large effect if one exists. Disadvantages include low resolution in discriminating among different multigenic hypotheses within the same strain, and the inability to distinguish between different mechanisms in different strains unless they differ greatly in the magnitude of resistance conferred. A common abuse in practice, although not a disadvantage of the method *per se*, is to use the presence of any sort of inflection in the backcross dose–mortality curve to conclude that a major resistance locus is present, even if the monogenic hypothesis is rejected by goodness-of-fit. This conclusion can obscure the evidence for more than one resistance mechanism and may discourage further exploration to distinguish between two major genes of equal effect, as opposed to distinguishing between one major and several minor genes.

A useful complement to this standard approach uses the ability to score DNA or protein variation at the resistance loci themselves, or at marker loci linked to them. The segregation pattern of these loci in backcrosses can be compared with the resistance levels of the progeny at selected doses of the insecticide. Often a single dose proves to be diagnostic: a good correlation is found between survivorship at that dose, and possession of the 'resistant' genotype at the marker locus under study. This finding increases the resolution of the genetic analysis because the proportionate contribution of the major locus to the overall level of resistance can be quantified. Two examples from *H. virescens* in which the major locus accounted for some, but not all, of the resistance are the voltage-gated sodium channel (pyrethroid resistance; Taylor *et al*. 1993*a*) and acetylcholinesterase (organophosphate and carbamate resistance; Gilbert *et al*. 1996) mechanisms. However, it is rare to have two different markers, each linked to a different resistance mechanism, studied in the same cross, so that the relative contribution of each can be quantified. Moreover, an approach that focuses only on quantifiable variation in known resistance loci is unable to account for genetic variation at other, unknown loci, and to distinguish it from environmental variation.

A logical (although far from trivial) extension of the single- or double-marker approach is to score enough markers in the backcross so that the entire genome is scanned for segregating genes that contribute to the resistance. In most organisms, a saturated linkage map consisting of closely spaced markers would be needed to ensure that no portion of the genome escapes detection. In certain insects where crossing-over is confined to one sex, one or two markers on each chromosome are sufficient to scan the genome. In flies (Diptera) of the genera *Drosophila* and *Musca*, the genome is broken up into a few large chromosomes, so a few well-placed morphological markers suffice, but the resolution is low. In Lepidoptera such as *Heliothis* and *Helicoverpa*, there are many small chromosomes so the scanning resolution is much higher; but the challenge is to find markers for all the chromosomes.

We have applied the genome-scanning approach to the characterization of the mechanism of fenvalerate resistance in the ANO2 strain of *H. armigera*. Previous work (Daly & Fisk 1992) had implicated pyrethroid detoxification by cytochrome P450 as the major mechanism in this strain, as the resistance was semi-dominant and almost completely eliminated by piperonyl butoxide. Bioassays on backcrosses suggested segregation at a single, major locus, but interpretation was complicated by heterogeneity in different generations of the susceptible strain and in different replicate F_1 lines. We were particularly interested in whether we could find stronger evidence for the existence of a major locus, whether the heterogeneity could be explained by additional resistance loci, and if so what their relative contributions were.

Our initial approach was to use markers already mapped in *H. virescens*, one per chromosome, and to score the homologous markers in *H. armigera*. This would enable us to scan the latter genome for pyrethroid resistance genes and simultaneously establish the correspondence between linkage groups in the two species. This approach is still in progress, as described in more detail in § 4. However, we found that it was faster to develop new markers in *H. armigera* first, so that the scanning could be

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completed independently of the progress in converting markers from one species to another. To succeed, this alternative approach of 'scanning *de novo*' required a method capable of generating a large number of polymorphic markers so that all 31 chromosomes are marked, even if the identity of the chromosomes is unknown. We found the AFLP technique (Vos *et al*. 1995) very useful for this purpose.

Hybrids between the ANO2 strain and a susceptible strain were produced by single-pair crosses. A female hybrid was backcrossed to a susceptible male, and third-instar larvae were treated with 0.2 µg of fenvalerate in 1 µl of acetone. DNA was isolated from killed offspring and survivors, and 260 AFLPs scored using 11 primer combinations. These were found to group into 31 linkage groups, corresponding to the known number of chromosomes in *H. armigera*. No evidence of crossing-over was found within any of these linkage groups, confirming the earlier cytological observation of the absence of chiasmata in oogenesis in this species (Fisk 1989).

Figure 1 shows a preliminary analysis of the contribution of each of these 31 linkage groups to resistance in the backcross. Only group 13 shows an interaction between genotype and survivorship significant at the Bonferroni level $p < 0.0016$ (the nominal level of 5% adjusted for the fact that 31 independent tests were performed). The only offspring surviving the fenvalerate treatment were heterozygous for group 13; all offspring with two copies of this chromosome from the susceptible strain were killed (as were two heterozygotes). Groups 26 and 29 showed interactions that exceeded the single-test level of 5%, but this cannot be due to resistance alleles from ANO2 because the opposite pattern of mortality was seen. The most likely candidate for a minor resistance gene would be group 17, whose interaction is just significant at 5% and is in the predicted direction for resistance. However, because 31 independent tests were performed, a larger sample size would be required to confidently distinguish the minor locus hypothesis from chance.

Two of the ten AFLPs identified on group 13 were also informative in another backcross between an F_1 male and a susceptible female. Male crossing-over between these markers and the resistance locus, as well as a third AFLP identified in that backcross, permits a linkage map to be constructed (figure 1*c*). The most likely location of the resistance gene, which we have named *FenR1*, is 9 centiMorgans (cM) from the AFLP marker *atcag1c*.

The results of the analysis to date suggest that a single locus does in fact confer most of the metabolic resistance in the ANO2 strain, and suggests that the heterogeneity found in the earlier studies does not have a genetic basis. We are presently trying to identify which *H. virescens* linkage group corresponds to *H. armigera* AFLP group 13, and to compare the location of *FenR1* with the genomic locations of cytochrome P450 genes that are implicated in resistance in this strain (Pittendrigh *et al*. 1997).

3. POSITIONAL CLONING: FROM GENOME TO RESISTANCE GENE

Once a genomic scan has revealed the location of one or more resistance genes, how can their identity be determined? Most resistance genes that have been cloned were first identified using previous knowledge of the insecticide target site (e.g. acetylcholinesterase or sodium channel) or mechanism of detoxification (e.g. esterases, cytochromes P450 or glutathione transferases), rather than their genomic location. An outstanding exception was the cloning of *Rdl*, a γ-aminobutyric acid (GABA)-gated chloride channel in *Drosophila melanogaster* in which a specific amino-acid substitution confers resistance to dieldrin (ffrench-Constant *et al*. 1991). Although the deletion stocks and other powerful tools of *Drosophila* genetics used to identify *Rdl* as a resistance gene cannot be applied to most other insects, the basic principle can: i.e. positional cloning.

Map-based or positional cloning is a strategy for identifying a gene based primarily on the measurement of its phenotypic effect and knowledge of its genomic location. Although early positional cloning efforts, particularly in humans, were enormous, expensive and time-consuming, recent advances have made it a manageable and feasible strategy more applicable to other organisms. These include faster techniques for screening the thousands of loci needed to find flanking markers and easier methods for constructing genomic libraries with very large inserts.

The steps in positional cloning aim towards continually narrowing the interval of DNA in which the gene of interest must lie; first by linkage analysis and second by construction and analysis of physical maps. The first step is to identify the chromosome or chromosome segment containing the gene, by classical linkage analysis. Second, a saturated map of the region is constructed with as many markers as possible, and their order and spacing relative to the gene of interest is determined by additional linkage analysis. Third, a genomic library is constructed in a vector capable of containing very large DNA fragments. Fourth, the library is screened with markers closest to the gene of interest and flanking it on either side to identify large fragments of DNA that are near to, or contain, the gene. A combination of 'chromosome walking' and additional linkage analysis is employed to construct a contig, an overlapping set of clones that spans the region from one flanking marker to other, in which the gene of interest must lie. Fifth, expressed sequences in the contig are identified, i.e. those that are translated and expressed in the cell, as opposed to repetitive, non-coding or 'junk' DNA. Finally, screening of the remaining candidates based on appropriate expression patterns, gene products, sequence analysis, and other evidence, yields the gene of interest (figure 2).

Positional cloning has been used successfully to identify a wide array of genes of diverse structure, function and phenotypic effect: for example, the gene responsible for cystic fibrosis in humans (Rommens *et al*. 1989); *BRCA1*, which increases breast and ovarian cancer susceptibility in humans (Miki *et al*. 1994); *obese*, which affects obesity in mice (Zhang *et al*. 1994); *nude*, which causes the nude mouse phenotype (Segre *et al*. 1995); *RPS2*, which confers resistance to bacterial infection in *Arabidopsis thaliana* (Bent *et al*. 1994); and *ABI3*, which affects responsiveness to the plant hormone abscisic acid in *A. thaliana* (Giraudat *et al*. 1992). In most cases, the genes found have been completely novel, and shed unexpected new light on the biological process under study. Often it seems in

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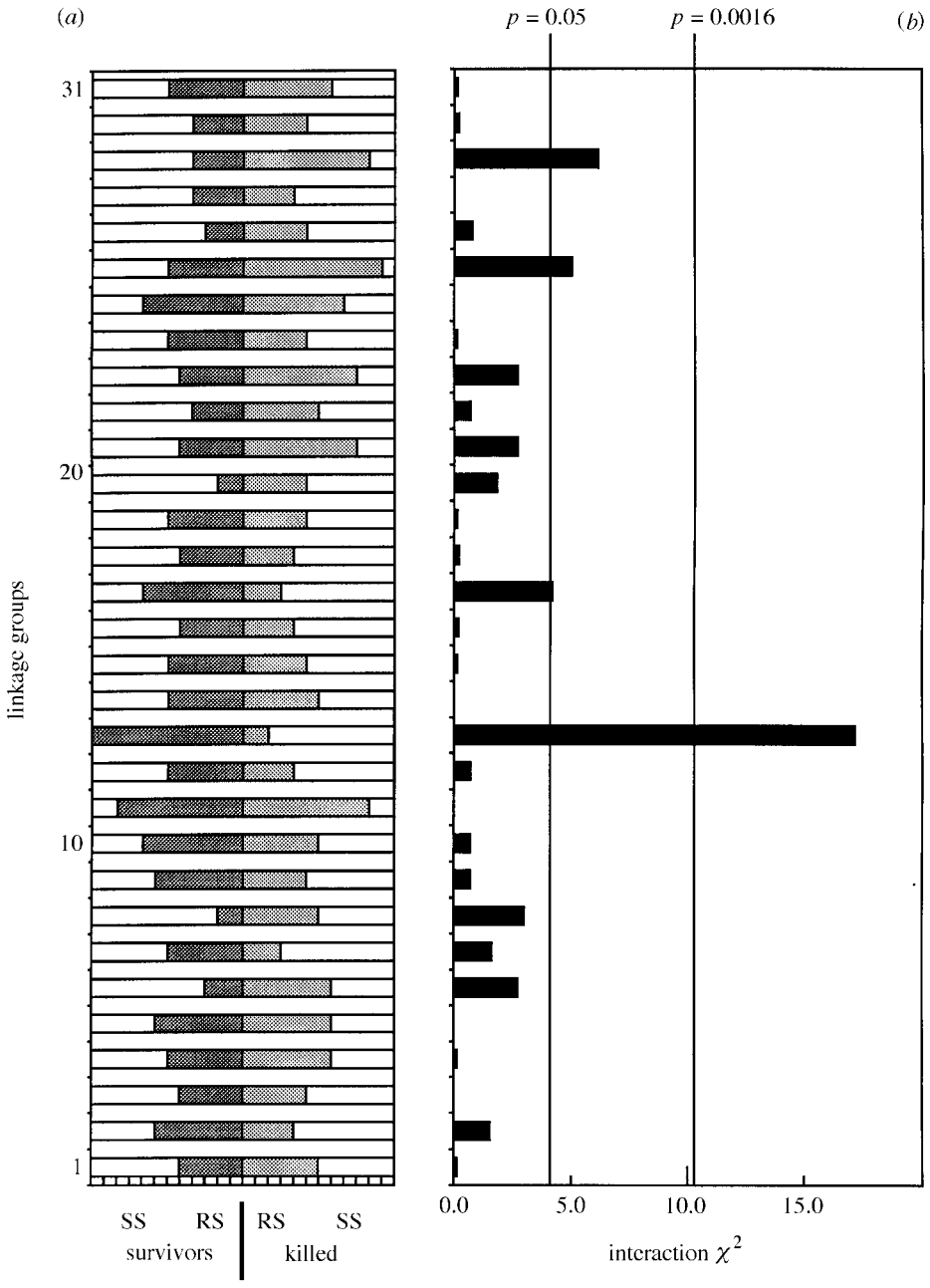

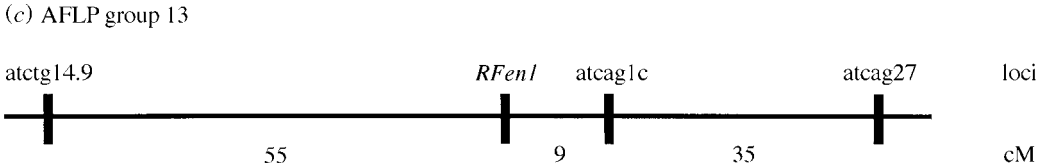

Figure 1. Scanning the *H. armigera* genome for pyrethroid resistance genes in the ANO2 strain. (*a*) Distribution of backcross progeny killed by 0.2 µg of fenvalerate and survivors, for each of the 31 chromosomes identified as AFLP linkage groups. Because there is no crossing-over in the F_1 mother of the backcross progeny, she transmits intact to each of her offspring either the susceptible-strain (S) or the resistance-strain (R) homologue of each chromosome. Her mate is from the susceptible strain and always transmits the S homologue. AFLP scores for 12 surviving offspring and 12 killed offspring (*a*) were used to determine the strain origin of both homologues for each linkage group. RS offspring have one chromosome from the resistant strain and one from the susceptible strain (shaded bars); SS have both from the susceptible strain (open bars). At AFLP group 13, for example, all 12 surviving offspring scored were RS and none were SS, whereas two killed offspring were RS and ten were SS. (*b*) Value of the χ^2-statistic testing for an interaction between genotype and mortality. A 2×2 table was constructed for each linkage group and the standard χ^2-test was performed for independence between rows (SS or RS) and columns (killed or survived); $p = 0.0016$ is the Bonferroni confidence level calculated from the nominal 5% level with 31 independent comparisons. Only AFLP group 13 shows a significant departure from independence by this criterion. (*c*) Location of the major resistance locus, *RFen1*, on AFLP group 13. Crossing-over in another cross between an F_1 male and a susceptible female enabled recombination fractions among the AFLP markers and the resistance locus to be measured. Distances are given in centimorgans (cM) using the Haldane mapping function.

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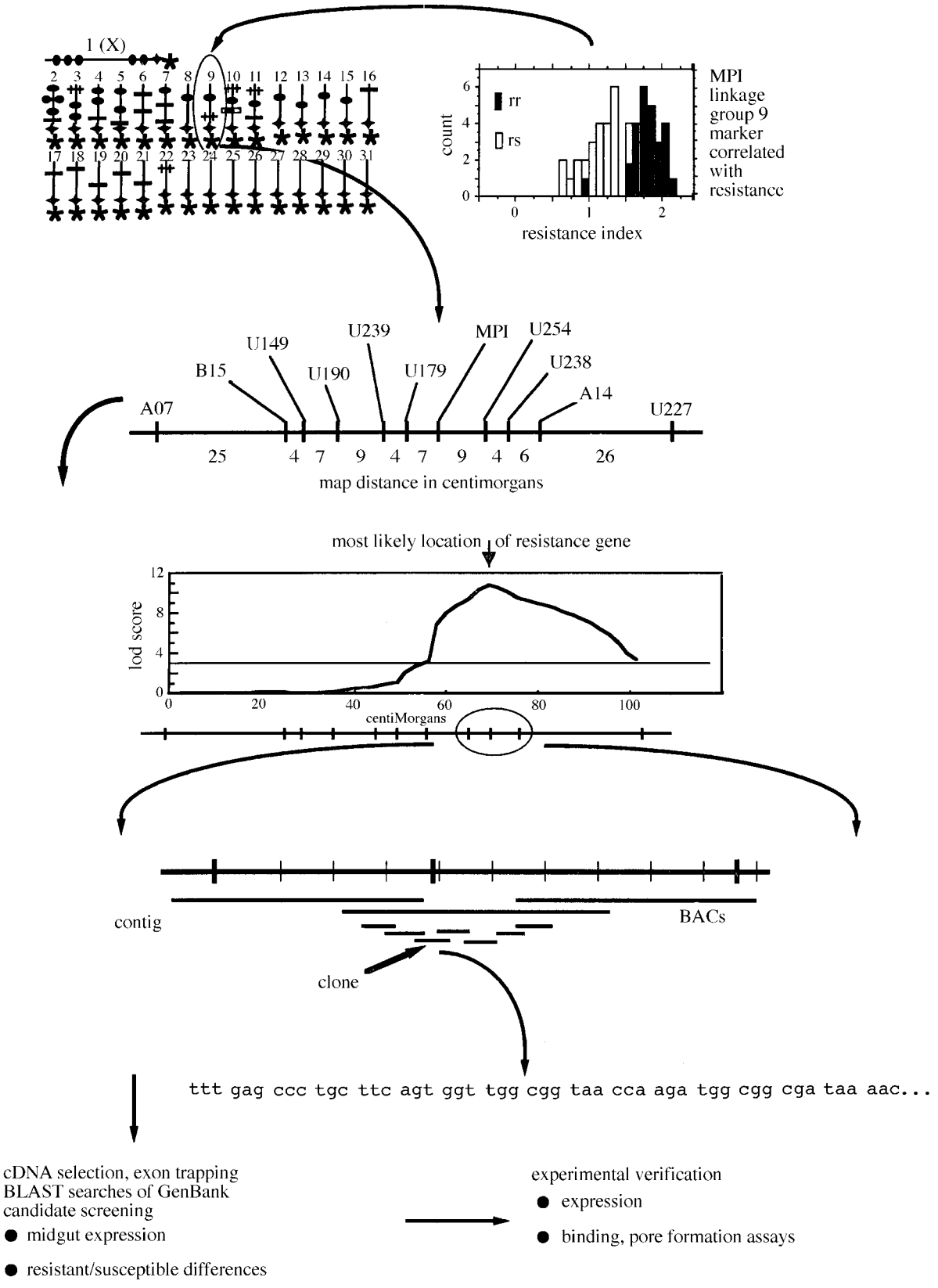

Figure 2. Strategy for positional cloning of the *BtR-4* gene conferring resistance to *Bacillus thuringiensis* Cry1Ac toxin in *H. virescens*. Correlation of the resistance phenotype with the marker locus MPI (mannose phosphate isomerase) enables linkage assignment of *BtR-4* to linkage group 9. Quantitative trait locus (QTL) mapping using ten additional markers on linkage group 9 identifies the most likely location of *BtR-4*. A collection of overlapping bacterial artificial chromosome (BAC) clones is identified that covers the relevant area, and fine-scale and physical mapping of this contig identifies appropriate subclones for sequencing. The sequences are analysed to identify suitable candidates. Finally, *BtR-4* is identified among these candidates by experimental approaches.

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retrospect that the gene could not have been cloned in any other way.

A prime candidate for the positional cloning approach is a major gene conferring resistance to *Bacillus thuringiensis* (Bt) crystal protein toxin CrylAc in *Heliothis virescens*. Although Bt resistance has not yet been detected in field populations of *H. virescens*, several strains have been selected in the laboratory. The most resistant strain produced to date is YHD2 (Gould *et al*. 1995), which has attained up to 10 000-fold resistance to CrylAc. Recently, we have shown that nearly 80% of the CrylAc resistance in YHD2 is due to the effects of a single autosomal gene on linkage group 9 of *H. virescens* (Heckel *et al*. 1997*a*). This gene, *BtR-4*, is the most potent Bt-resistant gene known in any insect. The resistant allele *r* is recessive, thus *rs* heterozygotes are nearly as susceptible as *ss* homozygotes. However, *rr* resistant homozygotes can survive high doses of CrylAc that are lethal to all susceptibles, and can grow and complete development at lower doses that completely inhibit growth of susceptibles. Now that Monsanto's transgenic 'Bollgard' cotton, expressing the CrylAc toxin, is commercially grown in the United States, we can expect to see the resistant allele *r* at *BtR-4* favoured by selection.

The selection response may well be immediate, because the resistant YHD2-type *r* allele is already present at a frequency of *ca*. 10^{-3} in field populations. When *H. virescens* males were captured in the field using pheromone traps and mated to laboratory YHD2 females (all homozogous *rr* for the resistant allele), 3 out of 1025 field-caught males were found to be *rs* heterozygotes based on high CrylAc resistance in their progeny (Gould *et al*. 1997). By conventional expectations, this is an extraordinarily high preselection field frequency for resistance alleles. With this information, it is clearly fortunate that a resistance-management strategy has been implemented from the very first commercial release of Bollgard cotton. Under the terms of the licence agreement with Monsanto, each farmer growing Bollgard must also provide for a 'refuge' where any budworms that develop do so without Bt exposure. The intent is to provide a pool of susceptible insects for mating with the few resistant survivors of selection from the Bollgard crop, to dilute resistance alleles and keep as many of them as possible in the heterozygous state. But how successful will this strategy be, and how long will it delay resistance? No successful resistance-management strategy for tobacco budworm on transgenic cotton can ignore the effects of *BtR-4*. It would be very useful to monitor the frequency of the resistant YHD2-type allele in the field, to determine how well the strategy is working. Yet the only method available at present is the extremely laborious one of conducting thousands of single-pair matings and progeny tests as done by Gould *et al*. (1997). Cloning *BtR-4* would make this much easier by enabling the development of gene-specific markers for field monitoring.

In preparation for positional cloning of *BtR-4*, we have already mapped it to a 10-cM region of linkage group 9 and isolated flanking RAPD markers. In future, we will be working on finer localization with AFLPs, construction of a library in a bacterial artificial chromosome (BAC) vector and assembly into a set of overlapping clones covering the *BtR-4* region, isolating candidate genes from these clones by exon trapping and hybrid selection with complementary DNA, and confirming experimentally that one of these candidates is the *BtR-4* gene (figure 2).

4. ANCHOR LOCI FOR COMPARING RELATED GENOMES

Scanning a genome and positional cloning are activities conducted for a single species. We may want to use a comparative approach to learn more about the occurrence of common resistance mechanisms in different species. How would we perform an interspecific comparison of genome scans? Can we use positional information from one species to assist in cloning a resistance gene from another? Both endeavours require that we have anchor loci to serve as reference points for the comparison.

Ideal anchor loci are present in exactly one copy per haploid genome, and can be easily detected, scored and mapped in all species to be compared. When chromosomal rearrangements have occurred after the divergence of two species from a common ancestor, they will be revealed by the different mutual linkage relationships of the anchors in the two species. In conserved regions of the genome not affected by such chromosomal rearrangements, the position relative to anchors will define the interspecies correspondence between the intervening genes. This approach can be used to provide indirect evidence that resistance genes in two different species are homologous, if they occur in the same relative place in the genomes of both species.

When the resistance genes are already known, of course, there is more direct evidence of homology. The finding that the same mutation in the voltage-gated sodium channel confers pyrethroid resistance in houseflies (Williamson *et al*. 1996), and cockroaches (Miyazaki *et al*. 1996; Dong *et al*. 1997), is a powerful example of this. Within each species, the resistance phenotype is genetically linked to molecular variation at the sodium channel. The resistance locus itself serves as the anchor connecting the results of the two species. It shows that resistance in both is conferred by modification of the same target, and that the target was modified in the same way in both species. It also serves as an anchor in comparisons with *H. armigera* and *H. virescens*, in which different modifications of the sodium channel appear to have occurred (Park & Taylor 1997; Head *et al*. 1998). Gene duplication can complicate the comparisons, even for anchor loci that are also resistance genes. Recent evidence points to the existence of two acetylcholinesterase genes in the mosquito *Culex pipiens*, only one of which appears to be modified to confer resistance to organophosphates (Malcolm *et al*. 1998).

When the resistance genes have not yet been cloned, the indirect approach can be used to obtain evidence for homologous mechanisms in different species. Resistance to Bt toxins in Lepidoptera provides a timely example of the need for anchor loci for this purpose. The homology-based approach is needed because physiological and biochemical comparisons are still inconclusive. Both *Plodia interpunctella* (Van Rie *et al*. 1990) and *Plutella xylostella* (Ferré *et al*. 1991) show a striking correlation

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between high CrylAc resistance and greatly reduced CrylAc toxin binding to midgut vesicles. Yet it is not known whether the same binding target is involved in the two species. Moreover, *H. virescens* (Lee *et al*. 1995) as well as many other species show a much less convincing relationship between resistance and toxin binding. On the other hand, suggestive evidence for homologous Bt targets among Lepidoptera is provided by the finding that midgut aminopeptidase N has been shown to bind CrylAc toxin in many species (Knight *et al*. 1994; Sangadala *et al*. 1994; Gill *et al*. 1995; Valaitis *et al*. 1995; Luo *et al*. 1997). But even here, there is no evidence to date of homologous resistance mechanisms, i.e. modified aminopeptidases with reduced toxin binding or indeed any effect on resistance levels.

We believe that a comparison of *Heliothis* and *Helicoverpa* genomes will reveal homologous resistance mechanisms. Bt resistance genes have already been shown to occur on five linkage groups in *H. virescens* (Heckel *et al*. 1996). As *H. armigera* develops Bt resistance, we will map those loci as well. For comparison, though, it may be faster to develop anchor loci for the *H. virescens* Bt resistance loci and score them in *H. armigera* strains currently undergoing selection for resistance, than to independently isolate putative Bt resistance genes from *H. armigera* first. More broadly, we plan to compare *H. virescens* to other Lepidoptera that have evolved Bt resistance. Tabashnik *et al*. (this issue) have suggested that some (but not all) resistant strains of *P. xylostella*, *P. interpunctella* and *H. virescens* may have a common genetic mechanism of CrylAc resistance involving a major gene. This hypothesis would be strongly supported if resistance in these strains (but not necessarily others) was shown to be linked to the same anchor locus for all three species.

Among the various classes of marker loci in use, there is unfortunately a trade-off between suitability for genome scanning and suitability as anchors. RAPDs and AFLPs, for which it is easiest to score large numbers of polymorphisms within species, are the hardest to establish with confidence as homologous between species. Yet Lepidoptera with many small chromosomes provide exacting demands for large numbers of markers. Any chromosome that lacks an anchor cannot be compared among species. Increasing densities of anchor loci permit increasingly refined comparisons (figure 3). One anchor locus per chromosome would suffice to detect chromosomal fusions, but most translocations would go undetected. Two anchor loci per chromosome could detect some translocations and provide an overall test of conserved synteny, but would not provide any information about conservation of gene order. Three anchor loci per chromosome would enable coarse-grained tests of conserved order between species, but would miss micro-scale rearrangements. Detection of these might best be accomplished by comparison of physical maps, as described in the next section.

An extensive list of anchor loci has been proposed for mammals (O'Brien *et al*. 1993), but unfortunately most of these seem unlikely to have easily identifiable homologues in other taxa. As a first step in developing a set of anchor loci for genomic comparisons in insects, we have chosen to focus on the gene–enzyme systems studied by population geneticists since the 1960s. These enzymes catalyse

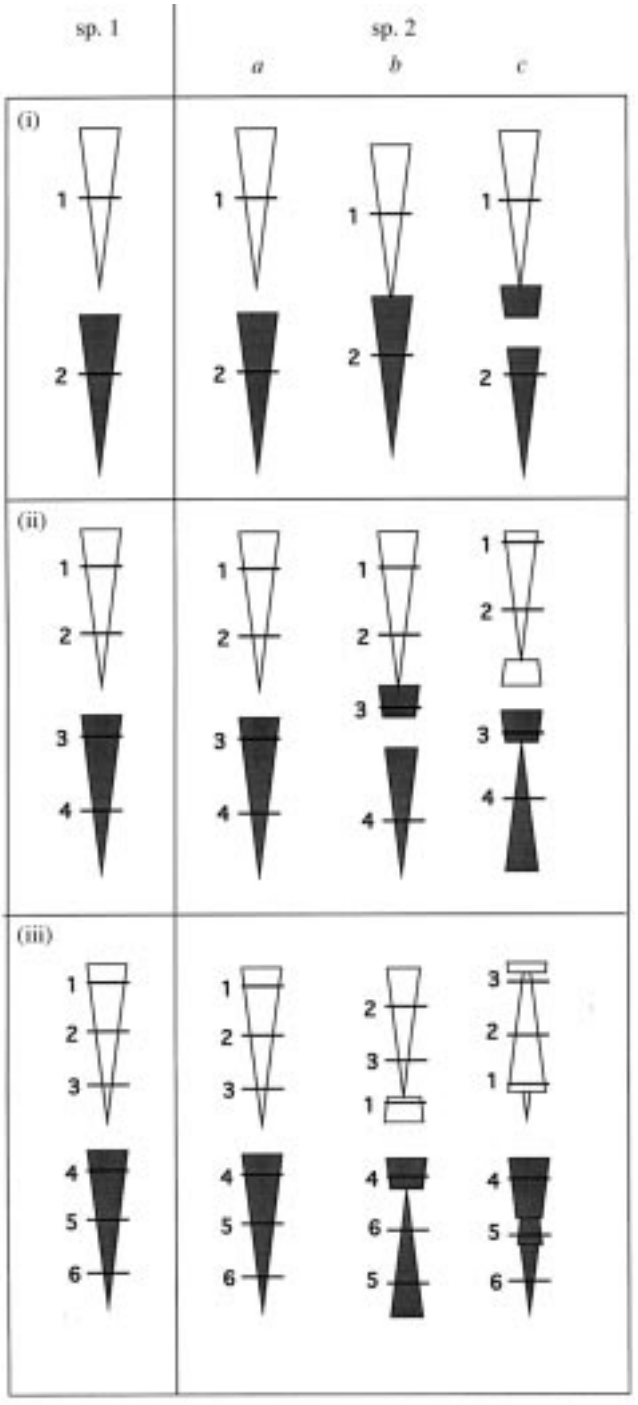

Figure 3. Use of anchor loci to detect chromosomal rearrangements. Two non-homologous chromosomes are depicted as wedges. Anchor loci are shown on the chromosomes of species 1. Scoring the same anchor loci in species 2 will detect some but not all chromosomal rearrangements, depending on the number of anchors per chromosome. In condition (*a*), species 2 has no rearrangements relative to species 1. The anchors shown enable this situation to be distinguished from condition (*b*), but not from condition (*c*). (i) One anchor per chromosome can detect chromosomal fusions, but not certain translocations. (ii) Two anchors per chromosome can detect gross translocations, but not inversions. (iii) Three anchors per chromosome can detect gross inversions, but not micro-inversions.

steps in glycolysis, the citric acid cycle, and nucleotide and amino-acid metabolism that are universal to all living organisms. Variation in electrophoretic mobility of the allozymes—allelic forms of the same enzyme—has

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Table 1. *Proposed anchor loci based on allozymes mapped in* H. virescens

TPI	triosephosphate isomerase
MPI	mannose-6-phosphate isomerase
PGM	phosphoglucomutase
G6P	glucose-6-phosphate dehydrogenase
SOD	superoxide dismutase
PGI	phosphoglucose isomerase
Fum	fumarate hydratase
MDHm	malate dehydrogenase (mit)
SoDH	sorbitol dehydrogenase
IDHm	isocitrate dehydrogenase (mit)
AATm	aspartate aminotransferase (mit)
AATc	aspartate aminotransferase (cyt)

been used to map such loci in a wide variety of species. Scoring nucleotide variation in the genes encoding these enzymes would make them informative as anchors even in species where the allozymic variation is rare or absent. With the increasing amount of information from genome sequencing projects, genes for the same enzyme can be identified in several different species and aligned to identify evolutionarily conserved regions. We have designed degenerate polymerase chain reaction (PCR) primers that are complementary to these regions for amplifying a set of genes that have already been mapped in *H. virescens* on the basis of allozymic variation (table 1). We will use these to begin to establish the correspondence between the *H. armigera* linkage groups identified in the genome-scanning for pyrethroid resistance, and the linkage groups of *H. virescens*. We encourage the use of these primers by workers on other insects as well, because the most useful anchors are those that have been scored in a large number of species.

5. TOWARDS A PHYSICAL MAP OF THE HELIOTHINE GENOME

So far, our main approach has been genetic, and our only venture into the physical realm was discussion of a collection of contiguous clones covering a portion of linkage group 9 for positional cloning of *BtR-4*. Extension of this coverage to all the chromosomes would constitute a first-generation physical map. Technological advances have brought this step within reach. We have thus begun to investigate the benefits a physical map would bring, and the feasibility of constructing one in *Heliothis* or *Helicoverpa*.

H. virescens has a genome size of about 403 million base pairs (Taylor *et al*. 1993*b*). A BAC library with 60 000 clones containing inserts of 100–150 kilobases would provide about a 20-fold coverage of the genome. Colonies of all clones can be spotted onto filters in a high-density array for efficient detection by hybridization. With every region of the genome covered by multiple overlapping BAC inserts, fingerprinting techniques based on matching restriction fragment profiles of the clones can be used to piece them together into locally contiguous groups that correspond to different chromosomal regions. Hybridization of genetically mapped AFLP fragments to the array would provide the correspondence between the genetic map and the physical map.

Such a physical map would offer both short-term and long-term benefits. In the short term, it would speed up mapping of newly cloned genes, by replacing RFLP linkage mapping with hybridization to the BAC filters. The whole-genome coverage would facilitate faster and more complete characterization of tandemly repeated gene families involved in resistance, such as cytochromes P450 and esterases. It would speed up positional cloning of novel resistance genes by providing immediate access to clones covering the chromosomal area harbouring the resistance gene, rather than having to 'walk' *de novo* across the region spanned by the flanking markers each time.

In the longer term, a physical map of BAC clones permits a very efficient strategy for large-scale sequencing, whether the whole genome is being contemplated or attention is limited to areas with multigene families. BAC libraries have already been constructed for the silkworm *Bombyx mori*, another Lepidopteran. Comparison of the bombycid and heliothine physical maps would provide the first detailed information on genome rearrangement within Lepidoptera at a smaller-scale than linkage mapping with anchor loci can detect. Comparing both maps to the physical map of *Drosophila melanogaster* will reveal whether any gene collinearity has been preserved at the fine-scale, as a vestige of the gene order of their common insect ancestor.

The degree of collinearity found will indicate how feasible it will be to directly clone novel insecticidal targets using conserved synteny. This could bring discoveries in insect model systems directly to bear on biorational insecticide discovery. *Bombyx mori*, which has been studied intensively on the genetic, physiological and biochemical levels, has been proposed as a model Lepidopteran (Goldsmith 1995). *Drosophila melanogaster* is currently being used to elucidate evolutionarily conserved signalling pathways as targets for novel drugs in the pharmacogenetic approach (Scangos 1997). The *Heliothis* homologues of genes discovered in either model system could be cloned by synteny, providing the fraction of the genome collinear in all three species is high. Although this fraction is currently unknown for insects, extensive gene collinearity has been discovered within mammals (e.g. human and mouse, where it has been used to assist positional cloning) and within crop plants of the grass family (e.g. rice, wheat, sorghum and maize).

6. CONCLUSION

In closing, we emphasize two features of the genomics approach that are hard to quantify but extremely important. First, and most elusive, is the property of 'completeness' or 'exhaustiveness' that is just manifesting itself in the study of the dozen or so organism whose genomes have been entirely sequenced. Not only do we know all the genes such an organism has, we also know which genes it does not have. If genes crucial for a particular biochemical pathway are absent from the genome, we know that pathway is not used by that organism, and if the result of that pathway is achieved, it must be done some other way. Second, is the paramount nature of the comparative approach. There is now far more information on structural genomics (i.e. DNA sequences) than on functional genomics (the biological function of genes).

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The vast majority of sequenced genes resulting from the genome projects have not been studied directly to ascertain their function. Instead, their function is inferred on the basis of sequence similarity to genes whose function has been experimentally determined. If circularity can be avoided, the combination of completeness and globally applied sequence comparisons provides a powerful new tool to study how the evolutionary process has resulted in the current diversity of life from a single common ancestor. It is a tool we must learn to use, and to apply to problems of practical importance such as understanding and circumventing insecticide resistance—the multiple 'solutions' produced by evolution in insect pests to the selective challenge of pesticides in their environment.

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