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Recombinant DNA Technology and Genetic Control of Pest Insects

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Introduction

The successful control of the screwworm *Cochliomyia hominivorax* in North America with radiation-sterilized males (Baumhover *et al.*, 1955) has stimulated interest in a range of genetic approaches to the control of pest insects (Waterhouse, LaChance and Whitten, 1974; for recent review *see* Whitten, 1984). This approach has been successfully extended to a few other species (LaChance, 1979). More sophisticated forms of genetic control, including homozygous translocations, meiotic drive, cytoplasmic incompatibility, hybrid sterility, compound chromosomes, sex-linked rearrangements coupled with conditional lethals, have also been developed, but their application has been restricted to a small number of pest species for which adequate genetic knowledge exists to isolate and characterize these phenomena.

The development and evaluation of many of the approaches to genetic control listed above have depended upon the extensive knowledge of the genetics of *Drosophila melanogaster*. This species, which has become the best-understood eukaryote in cytogenetic and genetic terms over the past six decades, now offers new potential to applied entomology because of recent advances in our understanding of its molecular biology.

This paper focuses on those recent advances in the molecular biology of *D. melanogaster* which have some bearing on the development of novel methods of controlling pest insects. We describe the methods currently available for cloning genes and for reinserting cloned genes into germ-line chromosomes. We then discuss how such techniques might be used in genetic control programmes. Whereas a serious limitation to applying classic genetic control

Abbreviations: ACE, acetylcholinesterase; ADH, alcohol dehydrogenase; bp, base pairs; kb, kilobases; SIRM, sterile insect release method; XDH, xanthine dehydrogenase.

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theory has been the absence of basic genetic information on most pest species, one of the attractive features of the molecular approach is that it may be applicable to pest species where little or no formal genetic information is available. For example, genes from *D. melanogaster* (or from other donors) might be transferred into particular pest species. Alternatively, cloned genes from *D. melanogaster* could be used as probes to isolate homologous genes from the pest insect, which could then be either modified by *in vitro* mutation or inserted at a new genomic site to create a specific genetic variant. In this way, geneticists should be able to exploit the enormous library of *D. melanogaster* mutants as a source of genetic variation for other species of insects.

Cloning and isolation of insect genes

In this section we concentrate on how specific genes can be cloned and identified, because the utility of the genetic control methods we will discuss later depends on the ease with which genetically characterized genes can be obtained. Descriptions of basic techniques such as the use of restriction enzymes to cleave DNA molecules at specific sequences, DNA ligase to join DNA molecules together, DNA hybridization to identify specific sequences, and other relevant techniques, can be found in basic texts on molecular biology. We recommend Old and Primrose (1981) for a general discussion of recombinant DNA technology, and Wu (1979) and Maniatis, Fritsch and Sambrook (1982) for specific experimental details.

CLONING VECTORS

In order to isolate a particular segment of DNA from any insect species, a library of cloned DNA sequences must be prepared. Such libraries are propagated in bacterial hosts, generally *Escherichia coli*. The essential step in the construction of such libraries is to attach the DNA segments to vector DNA molecules that can replicate independently in the bacterium. Three types of cloning vectors can be used in the construction of libraries: plasmids, bacteriophage, and cosmids.

Plasmids

Plasmids are small, circular DNA molecules which exist naturally within bacterial cells. Those used as cloning vectors have been selected by virtue of having the following features: capacity for self-replication, single target sites for cutting by certain restriction enzymes (cloning sites), and antibiotic-resistance genes which can be used to select cells containing the plasmid. One of the most commonly used plasmid vectors is pBR322 (Bolivar and Backman, 1979), the structure of which is shown in *Figure 1*. Naturally occurring plasmids can be very large, up to several hundred kilobases, but the plasmids used in library construction are generally much shorter (only a few kilobases), because there are technical difficulties in working with larger plasmids. Plasmids are seldom used to clone pieces of DNA longer than 10 kb. Once recombinant

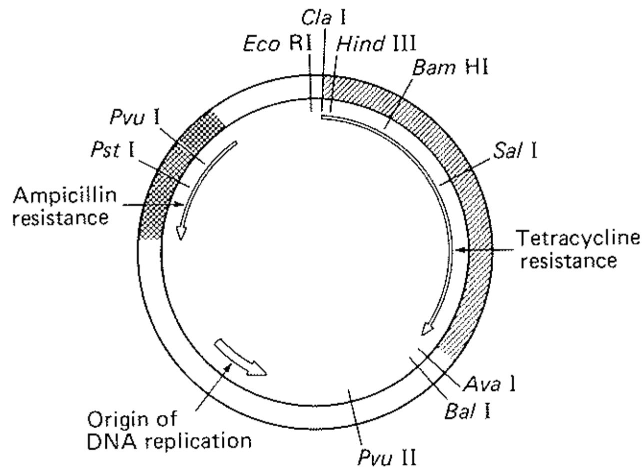


Figure 1. The genome of pBR322. The double circle represents circular double-stranded DNA and shows the positions of the antibiotic-resistance genes, the origin of replication, and the restriction enzyme sites that are commonly used as insertional cloning sites for cloning DNA fragments (*see* Old and Primrose, 1981).

plasmids consisting of the cloning vector plus passenger DNA fragment have been constructed *in vitro*, they are introduced by DNA transformation into the bacterial host strain for propagation.

Bacteriophage

Bacteriophage cloning vectors are derived from the λ bacteriophage. As with the plasmid vectors, they have been designed specifically to accept passenger DNA sequences. Most of the bacteriophage vectors are replacement vectors, in which a central region of the linear phage DNA is cut out and replaced with a passenger DNA fragment. These recombinant molecules are packaged *in vitro* using bacteriophage proteins to give infectious phage particles. The highly efficient process of infection can therefore be used to introduce the genetic material into the host cell. The need to package the recombinant DNA into the phage head puts strict limits on the length of the passenger DNA fragment — the recombinant DNA can be packaged only if the total length of the molecule is between 39 and 52 kb. A variety of λ replacement vectors is available and can be used to clone fragments of between 8 and 20 kb. Lambda insertional vectors, which contain a single cloning site, are also available. These accept small passenger fragments up to about 8 kb.

Cosmids

Cosmids, which are constructed from plasmids and are similar in size to the plasmid cloning vectors, are self-replicating minichromosomes that convey antibiotic resistance on the host cell, have insertional cloning sites for

passenger molecules, and also contain the λ *cos* sequence, which is the recognition site for the λ packaging system. Hence, recombinant cosmid molecules can be packaged *in vitro* into infectious particles provided that they are long enough (at least 39 kb); cosmids can be used therefore to clone very long pieces of passenger DNA (in the range 35–45 kb).

PREPARATION OF PASSENGER DNA MOLECULES

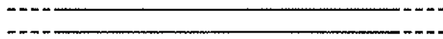
The passenger DNA molecules which are inserted into the vectors are generally of two types: genomic DNA or complementary DNA (cDNA). Genomic DNA is prepared from an organism and then randomly fragmented to give segments of the proper length for insertion. Fragmentation can be achieved mechanically (by shearing) or enzymatically (by partial digestion with a restriction enzyme). In the construction of genomic libraries, the aim is to propagate sufficient recombinant molecules to ensure a high probability of finding any genomic sequence represented in the library. Genomic libraries are usually constructed in either bacteriophage or cosmid vectors; these will accept longer insert fragments than plasmids, so fewer recombinant molecules are required to get a complete library. The procedures that we employed in the construction of a genomic library from the Australian sheep blowfly *Lucilia cuprina* are shown in *Figure 2*.

Complementary DNA (cDNA) is copied from messenger RNA. The synthesis is done using the enzyme RNA-dependent DNA polymerase ('reverse transcriptase'), which uses RNA as a template for DNA synthesis.

Figure 2. *(Opposite)*. Construction of a library of genomic DNA from *L. cuprina* in a bacteriophage λ-derived cloning vector.
(i) Preparation of *L. cuprina* DNA: High-molecular-weight DNA is isolated and partially digested with the restriction enzyme *Sau* 3A to an average size of 15 000 bp. The DNA is then treated with phosphatase to remove 5′-terminal phosphates. This prevents the subsequent religation of fragments and ensures that only one fragment can be inserted per recombinant phage.
(ii) Preparation of λ DNA: A detailed description of the cloning vector EMBL 3A is given in Frischauf *et al.* (1983). One feature of this vector is that polylinkers of 16 bp, containing cutting sites for *Sal* I, *Bam* HI and *Eco* RI, have been inserted at each end of the non-essential central region of the phage DNA: the *Bam* HI sites are used as the cloning sites. (For simplification the *Sal* I sites are not shown in our diagrams). EMBL 3A DNA is treated with DNA ligase to circularize the molecules, so protecting the *cos* sites at each end of the linear DNA. The non-essential region is cut out with the restriction enzyme *Eco* RI and the fragments then treated with phosphatase so that they cannot be religated to give intact vector. The mixture is then cut with the restriction enzyme *Bam* HI, which generates on the vector fragments the same overlapping end sequence as *Sau* 3A generates on the *L. cuprina* DNA. Thus the two types of fragments can be ligated together. The *Eco* RI–*Bam* HI fragments (9 bp) are too small to precipitate with ethanol and can therefore be eliminated.
(iii) Library construction: The *L. cuprina Sau* 3A-generated fragments are mixed with the EMBL 3A *Bam* HI-treated vector fragments and incubated with DNA ligase. The ligation reaction requires a phosphate group on at least one of the two ends being ligated together, so the genomic DNA fragments can join to the vector but cannot self-ligate. The non-essential region of EMBL 3A cannot reinsert because it has *Eco* RI overlapping end sequences, which do not match the *Bam* HI ends on the vector fragment. The ligation reaction produces high-molecular-weight concatameric DNA molecules consisting of alternating vector and insert (*L. cuprina*) DNA sequences. The ligated mixture is mixed with structural proteins *in vitro*; during the packaging reaction, which gives rise to infectious phage particles, the concatamers are cut at adjacent *cos* sites to give correctly sized recombinant DNA molecules.

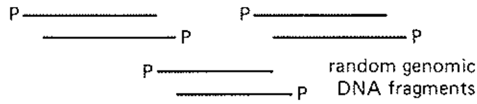
(i) high molecular weight *L. cuprina* DNA

1 genome $\approx 5 \times 10^8$ base pairs



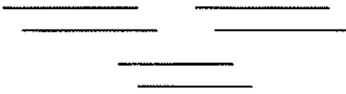
partial digestion with *Sau* 3A
to average size of 15,000 b.p.

1 genome $\approx$ 30,000 fragments

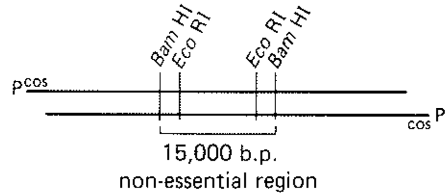


random genomic
DNA fragments

phosphatase

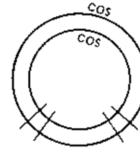


(ii) λ DNA (EMBL 3A)

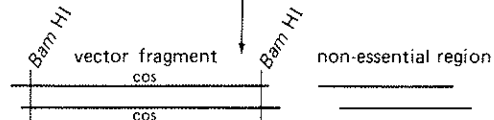


15,000 b.p.
non-essential region

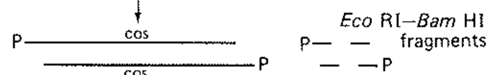
ligate



Eco RI cut, phosphatase



Bam HI cut

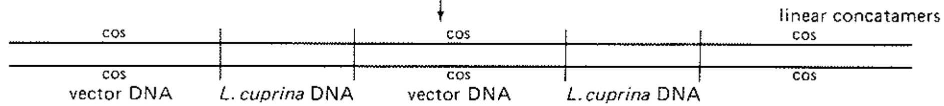


Eco RI–*Bam* HI
P— — fragments
— —P

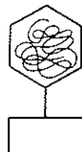
isolate large fragments

(iii) mix and ligate

linear concatamers



package into λ coat proteins



Infectious bacteriophage

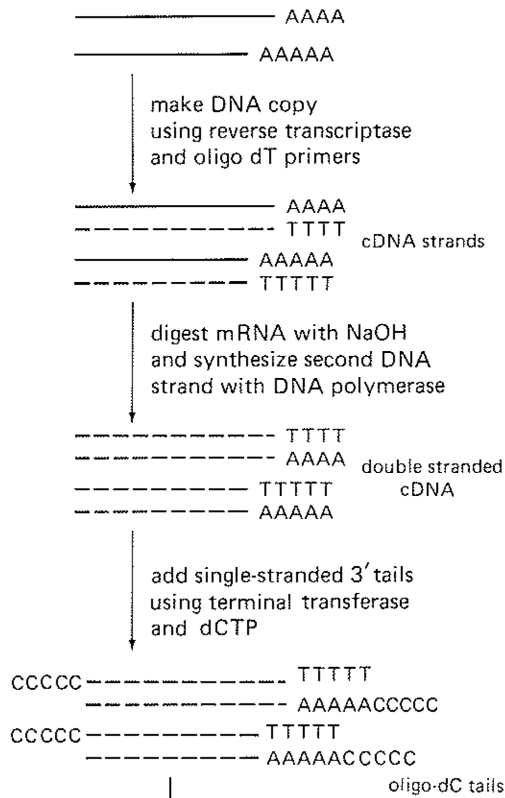
1 *L. cuprina* genome $\approx 3 \times 10^4$ phage

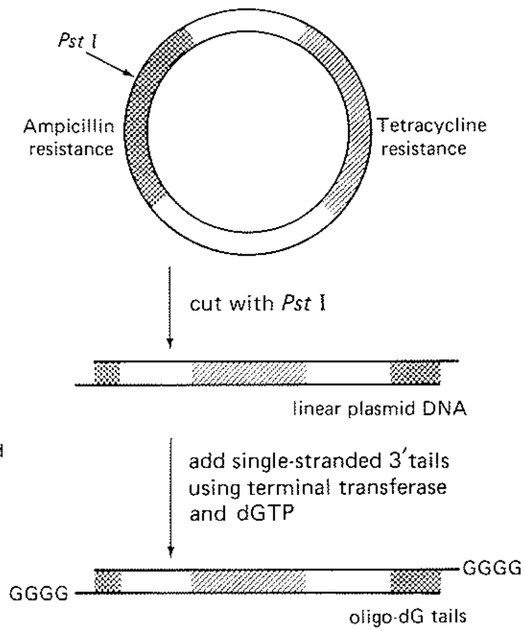
Since the average messenger RNA molecule is only about 1 kb long, cDNA libraries can be constructed using either plasmid or insertional bacteriophage vectors. As a cDNA molecule represents the protein-coding sequences of a gene, some cDNA cloning vectors have their cloning sites next to bacterial promoters so that the gene will be transcribed into RNA. Thus there is a possibility that the protein coded for in the inserted DNA will be produced in the bacterial cells. This enables the screening of such cDNA libraries to be carried out with antibodies (Broome and Gilbert, 1978; Erlich, Cohen and McDevitt, 1978). The steps involved in the construction of a cDNA library are summarized in *Figure 3*. It is also possible to clone chemically synthesized DNA molecules. Clones carrying the coding sequences for two human polypeptide hormones, somatostatin (Itakura *et al.*, 1977) and insulin (Crea *et al.*, 1978), have been produced in this way.

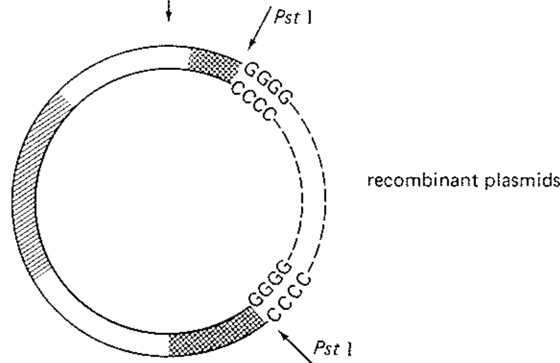
IDENTIFICATION OF SPECIFIC GENES IN DNA LIBRARIES

An insect genomic DNA library will contain between 10^4 and 10^6 different molecules, depending on the length of the inserts and the total genome size. Many methods which have been used to identify specific sequences in libraries involve the use of radioactively labelled DNA (or RNA) of a specific sequence as a hybridization 'probe'. Filter hybridization techniques have been developed for simultaneously screening large numbers of clones from plasmid, bacteriophage and cosmid libraries. Briefly, cells, or bacteriophage particles, containing several thousand recombinant molecules from a library are spread on a Petri plate and allowed to grow, giving colonies (or plaques). A nitrocellulose filter is placed on the surface of the plate, and some of the cells from each colony (or bacteriophage from each plaque) adhere to this. The filter is peeled off, treated to disrupt the cells (or bacteriophage), to denature the DNA, and to fix the DNA irreversibly to the nitrocellulose, giving a 'DNA print' of the original plate. Such filter-bound DNA is capable of hybridizing with radioactively labelled DNA of homologous sequence (the 'probe') and the colonies (or plaques) which hybridize can be identified by autoradiography.

Figure 3. *(Opposite)*. Construction of a cDNA library. These diagrams simplify some of the steps involved, particularly in the synthesis of double-stranded cDNA. For further details see Goodman and MacDonald (1979).
(i) cDNA preparation: Messenger RNA is isolated from insects either at a specific developmental stage or from a specific tissue. Complementary DNA copies are made with reverse transcriptase, using short oligo-dT primers which hybridize to the poly A tail on the mRNA molecules. The mRNA is removed by NaOH digestion and the cDNA is made double-stranded using DNA polymerase. Single-stranded oligo-dC tails are added to the 3′ end of each strand using terminal transferase.
(ii) Plasmid vector preparation: pBR322 DNA is cleaved at the single *Pst* I site to give a linear molecule. Since the *Pst* I site is located in the ampicillin-resistance gene, the subsequent insertion of cDNA into this site will inactivate the gene. However, the tetracycline-resistance gene remains intact and is used for subsequent selection. Single-stranded oligo-dG tails are added to the *Pst* I-cleaved plasmid DNA molecules using terminal transferase (this recreates a *Pst* I site at each end of the vector and simplifies subsequent analysis of the cloned inserts).
(iii) Library construction: The two DNAs are mixed in approximately equal molar ratios and ligated (DNA ligase). The oligo-dC and oligo-dG tails hybridize to each other and ensure that each plasmid can have only one insert. The ligated DNA is used to transform $CaCl_2$-treated competent *E. coli* cells and the mixture is plated on tetracycline agar to select for cells which have received the tetracycline-resistance gene of pBR322.


(i) Isolate insect mRNA
AAAA
AAAAA
make DNA copy
using reverse transcriptase
and oligo dT primers
AAAA
TTTT
cDNA strands
AAAAA
TTTTT
digest mRNA with NaOH
and synthesize second DNA
strand with DNA polymerase
TTTT
AAAA
double stranded
cDNA
TTTTT
AAAAA
add single-stranded 3′ tails
using terminal transferase
and dCTP
CCCCC
TTTTT
AAAAACCCCC
CCCCC
TTTTT
AAAAACCCCC
oligo-dC tails


(ii) Isolate pBR322 DNA
Pst I
Ampicillin
resistance
Tetracycline
resistance
cut with Pst I
linear plasmid DNA
add single-stranded 3′ tails
using terminal transferase
and dGTP
GGGG
GGGG
oligo-dG tails


(iii)
mix and ligate
Pst I
GGGG
CCCC
recombinant plasmids
GGGG
CCCC
Pst I
transform CaCl2-treated E. coli cells
and select on tetracycline agar
tetracycline-resistant colonies

SCREENING TECHNIQUES FOR ISOLATING SPECIFIC CLONED GENES FROM *D. MELANOGASTER*

At least 200 genes (approximately 2% of the total genome) have been isolated and identified from *D. melanogaster*, making its genome the best characterized of any higher eukaryote. It is worth while, therefore, to describe the types of successful cloning strategies that have been used with *D. melanogaster* and then to discuss how these might be applied to other species of insect.

The use of cloned genes from other organisms

As the amino-acid sequences of certain proteins have been highly conserved during evolution, the relevant genes also show a high degree of sequence homology, even between distantly related species. Cloning strategies based on this homology have been used to isolate a number of genes from *D. melanogaster*. For example, the histone genes were identified using histone-gene probes from sea urchin (Lifton *et al.*, 1977) and the actin genes by use of an actin-gene probe from slime mould (Fyrberg *et al.*, 1980). Such conservation of sequences appears to apply even to genes for some enzymes. Thus the gene complex in *D. melanogaster* which codes for the first three enzymes of the pyrimidine biosynthetic pathway (carbamylphosphate synthetase, aspartate transcarbamylase and dihydro-orotase) was isolated using sequences from the homologous gene complex from the Chinese hamster as the probe (Segraves *et al.*, 1983). As the nucleotide sequences of such genes have been conserved over the evolutionary time-span which separates insects from rodents, it seems reasonable to suggest that many insect genes will have been conserved over the relatively shorter time it has taken for the Insecta to diverge. If this surmise is correct, then it may be possible to isolate many insect genes using the homologous gene from a well-characterized insect such as *D. melanogaster* as a probe.

cDNA screening

For those cases where a particular gene is strongly expressed in a particular tissue during some stage of development, its mRNA is one of the most abundant in that tissue. Hence, radioactively labelled cDNA (prepared from total mRNA from that tissue) can be used as the hybridization probe. Because the probe contains labelled sequences in proportion to their abundance in the mRNA, those plaques or colonies which become most highly labelled during hybridization contain the most highly transcribed genes. A more powerful variation of the above procedure is applicable to genes differentially expressed in different tissues, at different stages of development, under different physiological conditions, or when a mutant strain in which the gene is not expressed is available. cDNA probes are synthesized both from total mRNA which contains transcripts of the gene (the 'plus' probe) and from total mRNA which lacks such transcripts (the 'minus' probe). Colonies or plaques which hybridize to the plus, but not the minus, probe are selected and further characterized to identify the gene of interest. Genes that are present at

frequencies of as low as 10^{-4} of the total cDNA in the plus probe can be isolated. This strategy was used to obtain the genes coding for the heat-shock proteins (Livak *et al.*, 1978), for larval cuticle proteins (Snyder, Hirsh and Davidson, 1981), and the enzyme dopa decarboxylase (Hirsh and Davidson, 1981).

Chromosome walking

Because genomic DNA libraries are usually prepared from randomly fragmented chromosomal DNA, a specific sequence will be carried in the library in several overlapping inserts. Therefore, once any single-copy sequence has been isolated it can be used as the starting point for a 'walk' along the chromosome. Sequences from the ends of the original clone are used as probes to isolate clones carrying overlapping inserts. The ends of these can be used in turn to isolate further clones and so on. The procedure is illustrated in *Figure 4*.

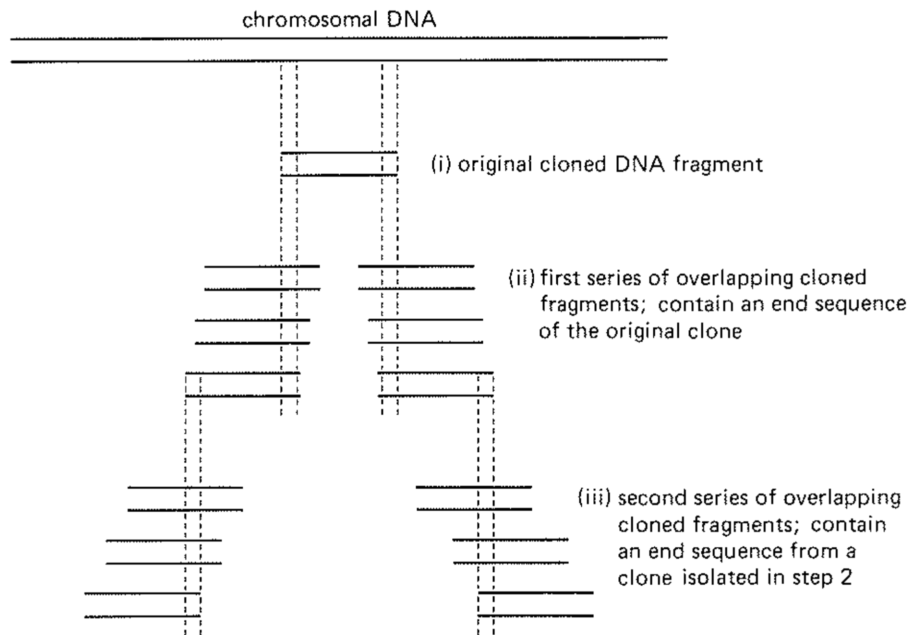


Figure 4. Chromosome walking.
(i) Short pieces of terminal sequence are isolated from each end of the original cloned DNA fragment that serves as the starting point of the 'walk'.
(ii) Each of these terminal pieces is used as a hybridization probe to select overlapping clones from the library.
(iii) Short fragments from the outermost end of the first series of clones are used to select a second series of overlapping clones. This process can be continued indefinitely, until the end of the chromosome or a region of repetitive sequences is reached. (It is not possible to 'walk' through repeated sequence DNA). *See* Bender, Spierer and Hogness (1983) for a detailed discussion of this technique and its refinements.

The significance of chromosome walking in relation to gene cloning is that it is possible to walk from one previously cloned gene to another that maps genetically nearby on the chromosome. The rate of walking is slow: when using a bacteriophage library, each step gives an advance of about 10 kb and an average polytene chromosome band in *D. melanogaster* contains about 30 kb of DNA (there are about 5000 bands in the genome). However, by the use of chromosome deletions and rearrangements which make it possible to 'jump' from one region of the genome to another, the rate of progress of a 'walk' can be greatly accelerated. These procedures are particularly powerful in *D. melanogaster* because the cytogenetic locations within the chromosomes of many genes are known and because numerous chromosome deletions and rearrangements have been characterized. Furthermore, the chromosomal position of cloned genes can be determined by hybridizing radioactively labelled DNA to polytene chromosome squashes *in situ* (Wensink *et al.*, 1974). Such an approach was used to isolate the *bithorax* complex, the *rosy*$^{+}$ gene, and the gene for acetylcholinesterase (*Ace*$^{+}$) in one 'walk' on the third chromosome (Bender, Spierer and Hogness, 1983).

Microdissection and microcloning

Perhaps the most impressive demonstration of the combined skills of the cytogeneticist and the molecular biologist is the microdissection and cloning of single bands from polytene chromosomes (Frey, Koller and Lezzi, 1982). Enough DNA can be recovered from a few dissected bands to construct a 'mini-library' of sequences from that band. This technique was recently used to conduct a detailed study of the 3C region of the X chromosome which contains the *white* gene (Scalenghe *et al.*, 1981). Microdissection and microcloning therefore provide a general procedure for obtaining any gene that has been mapped cytologically.

SCREENING TECHNIQUES APPLICABLE TO OTHER INSECT SPECIES

Of the strategies outlined in the preceding sections, the approach involving use of a previously isolated gene from another species to isolate the homologous gene is probably the most promising and should be applicable to any insect species. We are currently using a number of genes from *D. melanogaster* to identify their homologues in *L. cuprina*. Given the rapidly increasing number of genes which have been isolated from *D. melanogaster*, we believe that this should be the first approach to evaluate when attempting to clone a gene from any other insect species. The cDNA strategies are also general techniques that should be applicable to any insect species.

Chromosome walking will probably not be widely applied in other species although it may be useful in some particular cases. Its general usefulness in *D. melanogaster* is dependent upon the availability of detailed genetic and cytogenetic information on the location of genes and on the presence of polytene chromosomes, which permit cloned genes to be localized precisely by *in situ* hybridization. It is also greatly aided in this species by the large number

of cloned genes already available (which provide numerous starting points for walks) and by the availability of a large collection of chromosome rearrangements. No other insect species has such an array of advantageous features.

Chromosomal microdissection is also likely to have fairly restricted application to other species. It can be used only in species that have polytene chromosomes (which does include dipteran pests, such as *L. cuprina* and many mosquito and blackfly species). A second prerequisite for this procedure to be useful is that the genes of interest have been cytologically mapped on the polytene chromosomes. There are very few genes (in species other than *D. melanogaster*) that meet this criterion at present. In *L. cuprina*, some 11 mutations potentially useful in a genetic control programme have each been localized to one or a few polytene bands (Foster *et al.*, 1980, 1981; G.G. Foster, personal communication).

A number of other strategies have been used to identify genes from *D. melanogaster* and other organisms. Among these are screening of cDNA clones using antibodies directed against a specific protein (Broome and Gilbert, 1978; Erlich, Cohen and McDevitt, 1978), complementation of mutants in yeast (Henikoff *et al.*, 1981), and use of transposable elements as mutators (Bingham, Levis and Rubin, 1981). These techniques may have specialized applications, but probably will not be widely used to isolate insect genes.

There is, however, one further approach which has been used successfully with a range of organisms (although not yet, to our knowledge, with insects). This involves utilizing the amino-acid sequences of purified proteins to derive probable mRNA sequences and then chemically to synthesize regions of complementary DNA sequence. Reasonably short synthetic complementary oligonucleotide sequences (10–20 nucleotides), when added to preparations of mRNA, act as primers for the production of specific cDNA probes. For example, in the cloning of the rat gene for the polypeptide hormone relaxin, a synthetic 11-nucleotide primer, complementary to a region of predicted mRNA sequence, permitted the preparation of a labelled cDNA probe which specifically hybridized to DNA from clones carrying the relaxin gene sequences (Hudson *et al.*, 1981). Other examples illustrating the use of amino-acid sequences, derived mRNA sequences and synthetic complementary oligonucleotides in the production of specific probes include human leukocyte interferon (Goeddel *et al.*, 1980) and mouse β-nerve growth factor (Scott *et al.*, 1983). As techniques are now available for determining the amino-acid sequence of small (microgram) amounts of purified protein or polypeptide (Hunkapiller and Hood, 1980), this provides a further general approach to the cloning of genes. It is applicable to any gene (from any organism), the protein product of which can be purified.

In concluding this section, we wish to emphasize, firstly, that it is now a relatively straightforward task to isolate genes from *D. melanogaster* and, secondly, that it may often be possible to use the cloned *D. melanogaster* gene to identify its homologues in genomic DNA libraries prepared from other insect species. Furthermore, in those cases where the above approach is unsuccessful, a variety of other strategies is available. Any insect gene can be isolated if something is known about its expression into RNA or protein, its

genetic location, if a mutant is available or if its protein product can be purified and partially sequenced. In the next section we will discuss how isolated genes can be reintroduced into insect chromosomes.

Transformation of *D. melanogaster* with cloned DNA

Although several methods are available for introducing DNA into individual cells in culture, it is much more difficult to transform intact multicellular organisms, such as insects, particularly if one wishes to obtain germ-line transformation. However, successful germ-line transformation of *D. melanogaster* was achieved recently by taking advantage of the P transposable element. We will therefore begin this section by briefly describing the general properties of transposable elements.

GENERAL PROPERTIES OF TRANSPOSABLE ELEMENTS

The molecular structures and properties of prokaryotic transposable elements (transposons) are well established (*see* reviews by Calos and Miller, 1980; Kleckner, 1981). They are discrete sequence elements of DNA that can exist in many different locations in the bacterial chromosome or plasmids. A variety of elements is known, varying in length from simple structures of about 0·7 kb to large composite structures of at least 40 kb. Their characteristic feature is that they can transpose from one site in a chromosome to another. In doing so they cause mutations by inactivation of the genes into which they insert. The frequency of transposition varies greatly between transposons, from about 10^{-4} to 10^{-7} per cell division. A general feature of their structure is that they have the same nucleotide sequence at each end, either in inverted or direct orientation. These terminal repeats, which are quite short (10–50 bp), are recognition sequences for the enzymes ('transposases') that are involved in mobilizing the element. Most prokaryotic transposons are transcribed and presumably code for one or more transposases, and often carry other genes such as ones conferring antibiotic resistance.

Transposons which are similar in structure and properties to those in prokaryotes have been found in many eukaryotes (Calos and Miller, 1980). In *D. melanogaster*, transposons represent about 75% of the moderately repeated DNA (about 10% of the total genome) (Spradling and Rubin, 1981). These elements may fall into as many as 100 families of related sequences. The different families do not share any sequence homology but do have similarities in overall structure. Like the prokaryotic transposons, the frequency of transposition of those in *D. melanogaster* varies greatly, from those which transpose very rarely (copia-like elements) to those, such as the P element, which can transpose at very high frequencies under certain genetic conditions. When transposition does occur, mutations can follow due to the insertional inactivation of the gene at the target site. Naturally occurring mutant alleles of *D. melanogaster* genes are often the consequence of the presence of a transposable element within the gene (Spradling and Rubin, 1981). For example, of the 13 mutant alleles of *white* investigated by Zachar and Bingham

(1982), seven were found to contain a transposon within the gene. In the case of *bithorax*, every mutant allele so far investigated has been found to be due either to a chromosomal rearrangement or to the insertion of a transposable element (Bender, Spierer and Hogness, 1983). The structure of the best-characterized transposon of *D. melanogaster*, copia, is shown in *Figure 5a*.

THE P TRANSPOSABLE ELEMENT AND HYBRID DYSGENESIS

The P element of *D. melanogaster* was originally characterized genetically because it induces a syndrome known as hybrid dysgenesis (Kidwell, 1983). The symptoms of hybrid dysgenesis include a greatly increased mutation rate (due mainly to the insertion of the element into genes), increased recombination in the male germ line, increased germ line chromosome breakage, and gonadal dysgenesis. The syndrome is seen only when a male carrying the elements (generally from a wild strain) is mated to a female lacking them (generally from a laboratory strain). Their progeny exhibit the hybrid dysgenic phenotype, and are normally identified because many of them are sterile. The progeny of the reciprocal cross (females carrying elements mated to males lacking them) are completely normal. It has been proposed that these P^+ females carrying the elements transmit a repressor to their progeny via the egg cytoplasm, which represses both maternal and paternal elements (Engels, 1979a).

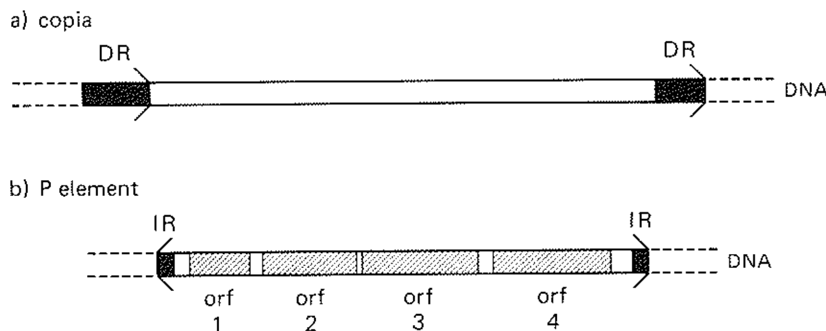


Figure 5. Structures of transposons from *D. melanogaster*.
(a) The copia element. Total length, approx. 5000 bp. Flanked by 276-bp direct repeats (DR). Number of proteins encoded is unknown (*see* Calos and Miller, 1980).
(b) The P element. Total length, 2907 bp. Flanked by 31-bp inverted repeats (IR). Contains four open reading frames (orf) and may encode four proteins (*see* O'Hare and Rubin, 1983).

At least two families of transposable elements cause hybrid dysgenesis in *D. melanogaster*. These are called 'P' (for paternal) and 'I' (for inducer); strains lacking the elements are called 'M' (for maternal) and 'R' (for reactive) respectively. These two elements do not interact and seem to be parts of independent systems. The dysgenic phenotypes are slightly different: P–M dysgenesis affects both males and females, while I–R dysgenesis affects only females. Both P and I elements have been cloned and there are no apparent sequence similarities between the two.

In order to clone the P-element sequences, Rubin, Kidwell and Bingham (1982) selected a number of mutants of the *white* locus that had been caused by insertion of P elements during hybrid dysgenesis. The mutant *white* genes were found to have homologous inserts of different sizes. When the DNA of one of these inserts was used as a hybridization probe, it was found that the DNA of P^+ flies contain about 50 copies per genome of variously sized P-element sequences. The longest element (2·9 kb) has been completely sequenced (O'Hare and Rubin, 1983) and the general features of its organization are given in *Figure 5b*. It has 31-base-pair inverted repeats at its ends, causes an 8-base-pair sequence at the site of insertion to be duplicated when it transposes and, within the internal sequences, contains four long non-overlapping open reading frames, suggesting that it could code for four proteins (O'Hare and Rubin, 1983). The shorter elements were found to be deleted versions of the longest one.

Part of the I element has also been cloned by using a similar strategy. It has proved to be unstable when cloned in bacteria, and has not been well characterized to date (Bucheton *et al.*, 1982).

TRANSFORMATION USING P-ELEMENT DNA

The procedure for germ-line transformation of *D. melanogaster* developed by Rubin and Spradling (1982) (*Figure 6*), involves injecting the P-element DNA (cloned in a bacterial plasmid) into the posterior region of syncitial blastoderm embryos. There it can become incorporated into the pole (embryonic germ line) cells and occasionally transpose from the bacterial plasmid (in which it is injected) to a chromosome. The embryos must be from M strains, so that the injection of P elements mimics a P–M mating. The injected embryos then become genetic mosaics: some of their germ cells contain the integrated P elements and some do not. In the initial experiments, the presence of integrated P elements was detected in the next generation by scoring the instability of a *singed* bristle mutant $singed^{weak}$ ($singed^{w}$). This mutant arose by the insertion of a defective P element into the $singed^{+}$ gene. In the absence of an intact P element within the genome the mutant is stable, but in the presence of an intact P element it becomes unstable and changes to a more extreme allele ($singed^{e}$) or to wild type at a high frequency (Engels, 1979b). The destabilization of the $singed^{w}$ allele is believed to be due to the mobilization of the defective P element which can generate either a small deletion ($singed^{e}$) or

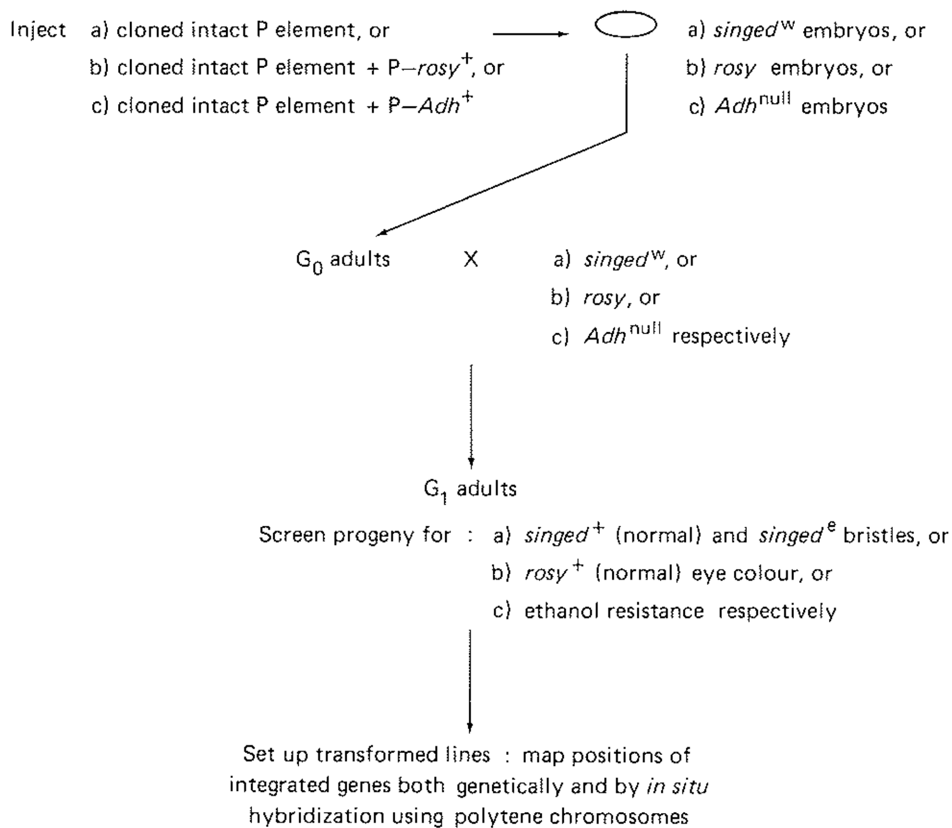


Figure 6. Transformation of *D. melanogaster* using P-element DNA. Cloned P-element DNA is injected into early (less than 3-hour) embryos (of different genotypes) either (a) by itself or (b) and (c) in conjunction with the DNA of a P-element plasmid carrying a marker gene. P-*rosy*$^+$ carries the *rosy*$^+$ (xanthine dehydrogenase) gene (Rubin and Spradling, 1982) and P-*Adh*$^+$ carries the *Adh*$^+$ (alcohol dehydrogenase) gene (Goldberg, Posakony and Maniatis, 1983).
The adults which develop from the injected eggs are mated with non-injected adults from the appropriate genetic stock. The progeny of these crosses are screened for evidence of transformation as follows:
(a) the integration and expression of an intact P element destabilizes the *singed*^{w} (weak) bristle mutant, giving rise to *singed*$^+$ (normal) and *singed*^{e} (extreme) progeny; (b) the integration and expression of the *rosy*$^+$ gene changes the mutant *rosy* eye colour giving *rosy*$^+$ (normal) progeny; (c) the integration and expression of the *Adh*$^+$ gene renders the recipients more resistant to ethanol.
Transformed lines are established from individual transformed flies for further analysis (*see* Rubin and Spradling, 1982).

restore the normal gene. Spradling and Rubin (1982) found that about half of the flies which developed from the surviving injected embryos produced *singed*^{e} or wild-type progeny, indicating that these had been transformed with P element.

Rubin and Spradling (1982) then showed that the P-element system could be used to transform *D. melanogaster* with other genes. The *rosy*$^+$ gene from *D. melanogaster*, which codes for the enzyme xanthine dehydrogenase (XDH) (Yen and Glassman, 1965) was used because only about 5% of the wild-type activity is necessary to give normal eye colour and because it is not cell autonomous (Hadorn and Schwink, 1956). Thus, even if the inserted gene was expressed poorly, there was the possibility that it might still give a recognizable change in phenotype.

An 8·1 kb DNA fragment containing the *rosy*$^+$ gene (Bender, Spierer and Hogness, 1983) was inserted into a cloned, internally deleted (defective) P element. This plasmid was then injected into *rosy*$^-$ embryos in conjunction with a plasmid containing an intact P element. The injected flies were backcrossed to *rosy* flies and the eye colour of their progeny examined (*Figure 6*). About half of the individuals which survived the injection produced some wild-type progeny, indicating successful transformation. DNA analysis showed that while the P element and *rosy*$^+$ sequences became integrated into recipient chromosomes, no bacterial plasmid sequences were transferred. The sites of integration of the introduced genes were mapped both genetically and by DNA hybridization *in situ* to polytene chromosomes. Of approximately 30 independent transformants analysed, none had integrated at the normal *rosy*$^+$ locus and no two had integrated at the same site; the sites seem to be relatively randomly distributed throughout the genome. The levels of expression and the tissue specificity of the integrated genes were near to normal in all cases, so neither the chromosomal sequences at the regions of integration nor the presence of the flanking P-element sequences interfere with transcription of the integrated *rosy*$^+$ gene (Spradling and Rubin, 1983).

Several other genes have been reintroduced into *D. melanogaster* using this system, including alcohol dehydrogenase (*Adh*$^+$) (Goldberg, Posakony and Maniatis, 1983), dopa decarboxylase (*Ddc*$^+$) (Scholnick, Morgan and Hirsh, 1983), *white*$^+$ (Hazelrigg, Levis and Rubin, 1984), and some of the chorion protein genes (A.R. Spradling, personal communication). In all cases the genes seem to be normally expressed, even though the lengths of the DNA fragments used in the construction of the transformation plasmids are only a few thousand base pairs longer than the transcription units in some cases. Thus these genes do not seem to require long adjacent regulatory regions to function, an important point if transformation is to be useful for introducing new genes into insects. The largest transformation plasmid used so far (containing the *rosy*$^+$ and *white*$^+$ genes) carried an insert of about 20 kb (Hazelrigg, Levis and Rubin, 1984). The frequency of transformation obtained with this construct was only about one-tenth of that obtained with the *rosy*$^+$ gene alone (8·1 kb insert), suggesting that larger plasmids transform much more poorly than smaller ones. Thus it is important that the fragments to be inserted into the vectors be as small as possible, especially if several genes are to be inserted into a single P-element vector.

Prospects for DNA transformation of other insects

There are at least two major technical problems to be overcome in developing transformation systems for other species. Firstly, suitable transformation vectors need to be developed; secondly, methods for selecting transformants have to be established.

TRANSPOSABLE ELEMENT VECTORS

The P element from *D. melanogaster* codes for its own transposase system. Consequently, it seems possible that it might function as a transposable element in other species. If such is the case, the P-element-derived transformation vectors developed for *D. melanogaster* might be generally useful in insect transformation. Successful transformation using such vectors has apparently been obtained with one species of insect in addition to *D. melanogaster* so far — *D. hawaiensis*, a Hawaiian picturewing species of *Drosophila* (M. Brennan, personal communication).

In the event that P-element vectors do not prove to be useful, then it may be necessary to attempt to develop specific vectors for each insect pest under consideration. Given the widespread occurrence of transposable elements in nature (they have been characterized in bacteria, yeast, nematodes, maize, fruitflies and mice), it seems probable that they exist in the genomes of most organisms. The cloned DNA of the transposable elements of any organism would be the obvious starting point for the development of a transformation vector for that organism.

OTHER APPROACHES TO TRANSFORMATION

An alternative approach to obtaining germ-line transformation of insects would be to attempt to transform insect cells in culture and then to insert such cells into developing embryos. For example, it might be possible to remove germ-line cells, transform them *in vitro* and then transplant them back into recipient embryos. Such cell transplantations have already been achieved with embryos from *D. melanogaster* (Van Deusen, 1976; Zalocar, 1981).

Many different types of vectors have been developed to transform eukaryotic cells in culture. In yeast, for example, both plasmids and minichromosomes have been used. These types of vector replicate within the host cell but cannot integrate into the genome (Hinnen, Hicks and Fink, 1978; Hicks, Hinnen and Fink, 1978). In yeast, plasmids are lost during meiosis (Stinchcomb, Struhl and Davis, 1979). It therefore seems unlikely that non-integrative plasmids will be useful as transformation vectors with insect cells.

Minichromosome vectors differ from plasmids in that they contain centromeric sequences (Clarke and Carbon, 1980; Murray and Szostak, 1983). These are much more stable during mitosis and meiosis than plasmids and so do not require continuous selection. Such vectors are not yet available for cells of higher eukaryotes but might be developed in the future. Various viral transformation vectors have been used in mammalian cells. Minichromosomes based on the monkey virus SV40 are the most commonly used (Elder, Spritz and Weissman, 1981). Insect viruses could be investigated for this purpose, but a major problem with virus-based vectors is the danger of the vector spreading by infection to other, non-pest, species. Of course, it might be possible to genetically engineer certain insect viruses to make them non-pathogenic. However, a discussion of the properties of insect viruses and of the prospects for manipulating their genomes will not be attempted in this review. The use of viruses as pest-control agents is discussed by Carter (1984) in volume 1 of this series.

Mammalian-cell transformation is often done without a specific vector. Under certain conditions tissue-culture cells will take up added DNA and incorporate it into high-molecular-weight complexes, which are then inserted into the genome (Perucho, Hanahan and Wigler, 1980). The frequency of transformation is usually low, so selectable markers need to be available to monitor transformation. However, much higher frequencies of transformation can be obtained by the microinjection of DNA directly into cells (Capecchi, 1980; Gordon *et al.*, 1980).

IDENTIFICATION OF TRANSFORMANTS

The identification of transformants in the P-element transformation experiments in *D. melanogaster* took advantage both of detailed knowledge about hybrid dysgenesis and of the availability of certain mutants of this species. Although neither the mutants nor the other detailed genetic information are readily available in other insects, there are a number of alternative possibilities for monitoring successful transformation.

DNA hybridization

The most reliable (but tedious) method is to use DNA hybridization. Progeny of injected insects can be individually ground up, their nucleic acids extracted, bound to a filter support, and hybridized with radioactive vector DNA. An advantage of this approach is that transformation (when it occurs) would be detected irrespective of whether any marker gene carried by the vector was expressed. (The only genes which would have to be expressed would be those necessary for vector transposition). In addition, it is applicable to any species regardless of the availability of mutant stocks or biochemical information. If the transformation frequency in other species is near that achieved with *D. melanogaster* (e.g. 1–10%), then DNA hybridization will be a practical monitoring system; in fact it would be easier to use this method than to spend time isolating mutants and constructing suitable recipient stocks. However, at

present there are no data on which to base predictions about either the transformation frequencies or the optimal transformation conditions for other species.

Visible markers

The first genetic marker used to detect P-element transformation was the ability of an intact P element to destabilize the *singed*^{w} mutation. Mutants of this type are only available in *D. melanogaster* at present, and the chances of finding defective P-element induced mutants in other species appears to be small. The first marker actually introduced with the P element into *D. melanogaster* was the xanthine dehydrogenase gene, *rosy*$^+$. In *D. melanogaster* this was a good choice of marker because it affects eye colour and is, therefore, easy to score (Yen and Glassman, 1965); in addition, it can be selected because *rosy* flies are sensitive to added purine in their diet (Glassman, 1965). However, XDH-mutants are not known in other insects. Because XDH affects the level of the drosopterin eye pigments (which are unique to species of *Drosophila*), its absence would not cause an easily recognizable eye-colour phenotype in other insects (Summers, Howells and Pyliotis, 1982). Nevertheless, it may be possible to identify XDH-mutants in other species, either on the basis of their purine sensitivity or by screening for reduced enzyme activity.

Another eye-colour gene which has been introduced into *D. melanogaster* using the P-element system is the *white*$^+$ gene. Although this is not as attractive as *rosy*$^+$ as a marker in *D. melanogaster* as it is cell autonomous, and its gene product is uncharacterized, there are equivalent mutants in many species of insects: *Musca domestica* (Hiraga, 1964; Milani, 1975); *Calliphora erythrocephala* (Langer, 1967); *L. cuprina* (Foster *et al.*, 1981), and *Ephestia kuhniella* (Caspari and Gottlieb, 1975). Because the *white*$^+$ gene of *D. melanogaster* appears to contain a very complex control region (Judd, 1976), it is possible that it will not function in other species. However, it may be possible to isolate the *white*$^+$ gene from the species to be transformed, using the *D. melanogaster* gene as a probe, and to insert that into the vector as the marker. A clone which cross-hybridizes with sequences in the *D. melanogaster white*$^+$ gene has been isolated from a *L. cuprina* genomic DNA library (A. Vacek and A.J. Howells, unpublished work) and is currently being characterized. If it carries the *white*$^+$ gene of *L. cuprina*, we plan to test it as a transformation marker in this species.

Selectable markers

The alcohol dehydrogenase gene (*Adh*$^+$) is effective as a selectable marker in P-element transformation of *D. melanogaster* (Goldberg, Posakony and Maniatis, 1983). There are excellent schemes for selecting both *Adh*$^+$ individuals (using ethanol; Vigue and Sofer, 1975) and *Adh*$^-$ individuals (using 1-pentyne-3-ol; O'Donnell *et al.*, 1974). Hence it may be possible to select the necessary *Adh*$^-$ recipient strains, as well as being able to select for *Adh*$^+$ transformants, in other species. The *D. melanogaster Adh*$^+$ gene is extremely

active, so it may be possible to use this gene in other species. Alternatively, if necessary, the gene could be isolated from each species to overcome specificity problems. Since ADH converts ethanol into acetaldehyde, which is also toxic, the insect must have an acetaldehyde detoxification system. In *D. melanogaster* ADH itself detoxifies acetaldehyde (Heinstra *et al.*, 1983) by converting it into acetate.

The insecticide-resistance genes constitute a large group of potentially selectable markers. As yet, no insecticide-resistance gene has been cloned, but the acetylcholinesterase (*Ace*$^{+}$) gene has been implicated in organophosphorus-pesticide resistance in some arthropods, e.g. the cattle tick *Boophilus microplus* (Stone, Nolan and Schuntner, 1976). This gene has been cloned from *D. melanogaster* and is being used as a probe to isolate and characterize resistance alleles at the *Ace* locus in the cattle tick (J. Nolan and P. Riddles, personal communication). In time, other resistance genes will probably be isolated and characterized, and could then be used as markers. An advantage of using such genes in transformation is that they do not require a special recipient strain, since the resistance is a novel function (although in the case of many serious pests this is unfortunately no longer true). Obviously, careful consideration would be necessary before pesticide resistance is introduced into a pest species simply as a marker of successful transformation.

Bacterial antibiotic-resistance genes could also be tried in insects as selectable markers. Kanamycin-resistance (*kan*^{R}) genes from *E. coli* have been used (after being spliced to a eukaryotic promoter) as transformation markers in yeast (Jimenez and Davies, 1980), in mammalian tissue-culture cells (Southern and Berg, 1982; Colbere-Garapin *et al.*, 1981), and in the slime mould (Hirth, Edwards and Firtel, 1982). It may be necessary to splice the *kan*^{R} gene to an efficient insect promoter sequence. Efficient promoters from *D. melanogaster* are already available and similar sequences can undoubtedly be obtained from genes of other insects.

We conclude this section by noting that the prospects for manipulating the genomes of insects by introducing cloned DNA appear to be good. Several approaches are available for inserting the cloned DNA into embryos and, as far as transformation markers are concerned, there is a wide variety of cloned genes already available for this purpose. Consequently, in the final section of this paper, we will consider ways in which such genome manipulation might contribute to insect-pest control.

Recombinant DNA techniques and genetic control

The aim of a genetic control programme is to manipulate the hereditary apparatus of individuals in a target population such that a high proportion of individuals in some ensuing generations will not survive. Genetic control therefore requires the laboratory propagation and release of genetically modified individuals which, by mating with residents of the target population, will serve to introduce the modified genetic material into the population. The number of individuals and number of releases required are determined by the mechanism used to spread the genetic modification through the target

population. The sterile insect release method (SIRM) relies on swamping the target population with large numbers of released insects, hence the need for large production factories and the development of sophisticated rearing and release technologies. The ultimate success of SIRM is realized when all field females in the release areas are inseminated by males treated with ionizing radiation or chemosterilant such that all gametes produced by these individuals carry one or more dominant lethal conditions. The Y-chromosome–autosomal translocation system (*see below*) also relies on an initial swamping of the target population, and on the effects of the genetic load induced while the released genetic material is being eliminated by natural selection. The application of recombinant DNA techniques to this system of genetic control is discussed in the next section.

Some systems of genetic control involve a transporting mechanism, e.g. meiotic drive or negative heterosis, which are devices for enabling the genetic condition to spread through the target population despite the genetic disability which it bestows on its carrier (Whitten, 1984). The techniques of molecular biology may increase options open to applied entomologists working in this area. These possibilities are considered in the final section.

ENHANCEMENT OF 'CLASSIC' GENETIC CONTROL

It is not intended here to give a comprehensive treatment of this subject but simply to provide one example of classic genetic control, i.e. one which draws upon traditional cytogenetics and chromosome mechanics, and to indicate how its efficacy might be enhanced by use of recombinant DNA techniques.

The use of reciprocal translocations between the Y or male-determining chromosome and some autosomes, as a means of limiting conditional lethals to the female sex, has been outlined by Whitten (1979). This system relies on the absence of crossing over during male meiosis to effect complete linkage between the maleness factor and the conditional lethal loci. In species in which crossing over occurs during male meiosis, crossover suppressors such as a chromosome inversion have been used to secure adequate coupling. For example, this approach has been used in developing sex-killing systems in certain mosquito species (LaChance, 1979). The rationale for the Y–autosome translocation system for inducing high genetic loads in the generations following a period of release is outlined in *Figure 7*. The genetic load in this system derives from two sources. Firstly, the released males give rise to a proportion of inviable offspring as a direct result of the production of aneuploid gametes. All their viable male offspring inherit the rearrangement and consequently perpetuate this source of genetic load. The recessive mutations m_1 to m_6 (*see Figure 7*) yield phenotypes which are lethal under field conditions, but are viable in the laboratory. If these loci assort independently during meiosis in the female progeny of the cross between field females and released males, then the female progeny in the next generation will be inviable, unless they receive the wild-type allele for each of the six loci. If the loci are unlinked, the probability of a female offspring being viable is thus $(1/2)^6$ (*see Figure 7*). It is greater if a degree of linkage exists between the loci, and the consequent

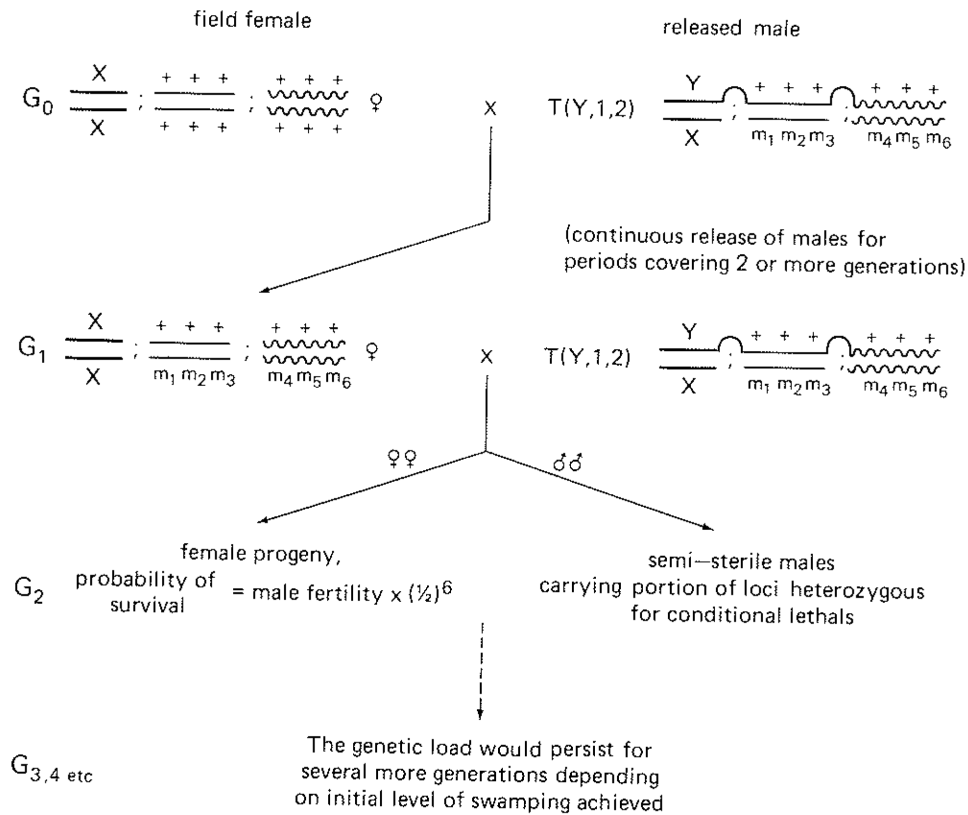


Figure 7. A scheme for using Y–autosome translocations coupled with conditional lethals to control the Australian sheep blowfly, *Lucilia cuprina*. Genetically manipulated males are mass reared and released into a target population, ideally in numbers to ensure that most field females are inseminated by the released males. Females from the released strain are either eliminated during production by a genetic sex killing system (Whitten, 1984), or express mutations which prevent survival in the field. Releases are maintained such that the first-generation daughters (G_1) are largely inseminated by released males. In the following generation (G_2), the only daughters to survive are those not expressing chromosomal duplication/deficiencies which have a wild-type allele (+) at each of the recessive conditional lethal loci (m_1, m_2 etc.).

genetic load is correspondingly lower. The reduced load with linked loci follows from the fact that the conditional lethals are more likely to be coupled, i.e. doubly kill their bearer and therefore be eliminated without additional cost. This system is currently being evaluated in *L. cuprina*.

Two features of the system limit its usefulness. It may be desirable to avoid the male sterility which is necessarily associated with the translocation rearrangements. For example, partially sterile males can create limitations in mass rearing the strain. Secondly, only conditional lethals which are located on the translocated chromosomes can be used. In the case of *L. cuprina* this limits our choice to a maximum of three or six loci depending on whether one or two

autosomes are involved in the Y–autosome translocation. This latter argument assumes a chromosomal length of approximately 100 map units, which seems to represent an upper size for many insect chromosomes (e.g. Foster *et al.*, 1981).

Access to cloned genes and a transformation system would permit us to examine the possibility of transferring the wild-type allele of any number of loci to the male-determining chromosome. Alternatively, if the maleness element is itself a discrete entity, it might be cloned and then relocated to another chromosome. In the longer term, it may be feasible to construct a mini-chromosome carrying the maleness factor and the wild-type allele of as many loci as we wish to include in the scheme. It would then suffice to construct multiple marker stocks carrying the desired combination of conditional lethals masked by the wild-type allele on the modified or synthetic Y chromosome. These males would be fully fertile, phenotypically wild type and would be used as a vehicle for introducing a genetic load that would be borne by the female sector of the population. The magnitude of the genetic load depends on the number and location of the genetic loci and on the ratio of wild:released males. The load would decay during ensuing generations, leading to population collapse and extinction in particular cases. Thus the opportunity exists of combining conventional cytogenetic methods with molecular techniques, to induce a change in the genetic information of a target population which is detrimental to the genetic fitness of that population. Sex limitation of conditional lethals represents one application of the molecular techniques that should become available.

DEVELOPMENT OF NOVEL GENETIC CONTROL STRATEGIES

Meiotic drive

As outlined above, genetic control attempts to reduce the genetic fitness of the target population by the injection of inappropriate genetic information into that population. Large releases aimed at swamping the target population may be cost effective in some instances, e.g. SIRM for screwworm. However, it will always remain the dream of geneticists to devise methods which simply require a single seeding of the target population with genetic material that spreads by some infectious mechanism. One possible means of achieving such a spread is to use meiotic drive (Zimmering, Sandler and Nicoletti, 1970), where one chromosome or chromosomal segment enjoys a segregation advantage during meiosis. This transmission advantage ensures its spread through the population despite the fact that it may be propagating some deleterious condition. Little or nothing is yet known about the precise underlying molecular mechanism(s) of meiotic drive and, until more information is available at this level, its application to genetic control is likely to remain limited. Molecular techniques might assist in the elucidation of the mechanisms of meiotic drive systems such as segregation distorter in *D. melanogaster* (Ganetzky, 1977). This knowledge may, in turn, enable the eventual cloning and transfer of such genetic conditions from *D. melanogaster* or facilitate the identification of similar phenomena in pest species.

Transposable elements as control agents

Transposable elements have several properties that might make them useful in genetic control. In particular, when they transpose from one chromosome to another they replicate at the same time, thus increasing their copy number in the genome. Because they can spread in this way they may have a selective advantage over other DNA sequences that are restricted to a single genetic location (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). These authors have pointed out that transposable elements can be considered to be genetic parasites, so using them as control agents is in theory similar to the use of pathogens such as viruses, although it is not thought that transposons can spread by infection (but *see* 'Safety considerations' on page 94).

The P element causes phenomena that reduce the fitness of individuals and could be useful in a genetic control programme: gonadal dysgenesis, an increased mutation rate, and increased chromosome breakage. The sterility of hybrid dysgenic flies is temperature dependent (Kidwell and Novy, 1979). Most flies reared at 18°C are fertile, although not completely so (due to chromosome breakage), but almost all flies reared at 25°C are sterile, because of a failure of the gonads to develop. The mutations generated by P elements (deletions, insertions, translocations) are of the type that completely inactivate genes (and hence cause the loss of the gene product) rather than altering the structure of the gene product (as point mutations might); thus, they are not likely to cause an improvement in fitness. As discussed earlier, the hybrid dysgenesis phenotype is expressed only when the P element becomes derepressed, i.e. when a P male is mated to a M female, because of the absence of the P-element repressor in the cytoplasm of the eggs of such females. Thus, by site-directed mutagenesis of P-element DNA *in vitro*, it might be possible selectively to inactivate the gene for the repressor, leaving the transposase functions unaffected. Such an irrepressible transposon might be very effective in reducing the fitness of populations into which it is introduced.

The critical variables for the spread of a disadvantageous transposon through a population are the rate at which it transposes, the fitness cost to the organism harbouring it, and the initial frequency at which it occurs in the population (Hickey, 1982). Because the initial frequency is limited by the number of individuals that it is practical to release in a genetic control programme, the damage that can be done to a wild population is dependent mainly on the transposition rate of the vector. It would be worth while, therefore, to have available additional high-frequency transposing elements. Elements that transpose rapidly will probably cause an increased mutation rate, so strains of insects that harbour mutator systems, such as those known in *D. melanogaster* (Green, 1976), should be screened for such elements.

Transposable elements for transporting deleterious genes

Mutant genes which have dominant effects on viability or fertility are potentially useful in genetic control. Genes introduced using this system must be dominant, since the P-element vectors randomly introduce genes into the genome and do not replace existing genes. An example of a biochemically

well-characterized dominant mutant, which might be introduced into recipients to reduce their fitness, is the defective testes-specific tubulin gene ($B2t^D$) that causes male sterility in *D. melanogaster* (Kemphues *et al.*, 1979). This mutant gene produces a defective tubulin that can copolymerize with normal tubulin, forming aberrant microtubules. The use of highly conserved genes like the tubulin genes increases the probability that they will have the same effects in other species as in *D. melanogaster*. It might be possible to produce similar mutations in genes for other proteins that have a structural role (e.g. actins, myosins, collagens) or are part of multimeric enzyme complexes. An important problem to be overcome when considering the introduction of genes with dominant effects, is the rearing of stocks carrying such genes in the laboratory. It would be desirable to have mutants which express the phenotype only under a certain set of experimental conditions, e.g. temperature-sensitive mutants. Dominant, temperature-sensitive lethal mutants of *D. melanogaster* are known (Suzuki *et al.*, 1976). Unfortunately, few of them have been characterized biochemically and so it will be difficult to isolate the genes involved. However, it may be possible to construct temperature-sensitive dominant genes using recombinant DNA techniques. For example, a temperature-sensitive dominant male-sterility gene could be constructed by fusing the promoter sequence from the 70 kilodalton *D. melanogaster* heat-shock gene (*hsp70*) to the $B2t^D$ tubulin gene. The *hsp70* gene is actively transcribed at 30°C but not at 20°C, and the promoter responsible for this temperature sensitivity has been isolated and sequenced (Corces *et al.*, 1981). Promoters from other types of conditionally activated genes, e.g. those induced by hormones, metabolites, or metal ions, might also be useful in the construction of genes conferring conditional lethality or sterility.

Sex-determining elements

The isolation and use of sex-determining elements to enhance a classic control scheme has been discussed earlier. However, the introduction of additional male-determining genes, as a means of altering the sex ratio of a target population, can also be considered as a genetic control strategy in its own right. A wide variety of sex-determining systems are known in insects (for examples *see* King, 1975), so different genes might have to be isolated for different pest species. With *Musca domestica* (Wagoner, McDonald and Childress, 1974) and some other dipterans (Green, 1980), the maleness element behaves as a single gene, and this may also apply to species which have a typical heterochromatic Y chromosome. For species in which the male-determining element is carried on an autosome and its genetic location is known, e.g. *Chironomus* (Martin *et al.*, 1980), it might be possible to obtain the appropriate DNA by chromosome microdissection followed by microcloning.

SELECTION AGAINST GENETIC CONTROL

The swamping systems outlined earlier depend upon natural selection to exert their controlling influence on target populations. They work because they

introduce deleterious genetic information into natural populations, which is lost from the population with the death of its carrier. The genetic load effect is therefore limited in time and amount by the genetic cost of eliminating the carriers of the information.

By way of contrast, systems such as meiotic drive or mobilized transposable elements that would rely on some transmission advantage, will necessarily be opposed by natural selection, should suitable variability exist on which selection can operate. Selection operates to counter meiotic drive systems both in laboratory populations and in natural populations where suppressors of drive are widespread (Lyttle, 1979). A similar reaction in relation to sex determination has occurred in *M. domestica* where the spread of the M maleness factor has been emasculated by an overriding F system. For example, in the Bowhill population in Queensland, the M element is present on three autosomes but the 1:1 sex ratio is still preserved by the existence of yet another sex-determining system, the F system (Ff are female and ff male), which is fully epistatic to the M system (Wagoner, McDonald and Childress, 1974). Thus, where genetic information is spread surreptitiously bringing with it a reduction in Darwinian fitness, its spread may ultimately be thwarted by the generation and spread of some counteracting information to restore normality. As with the development of pesticide resistance, the track record suggests a high probability of resistance developing to the 'infectious vector', but clearly this is not a logical necessity.

SAFETY CONSIDERATIONS

Although genetic engineering is less controversial than it was a few years ago, the use of recombinant DNA technology to control wild insect pests must be preceded by careful analysis of its impact on the environment (Levin, 1979). A potential advantage of the approaches outlined here, over the release of genetically modified insect pathogens such as bacteria or viruses, is that once the novel genetic information becomes stably integrated in an insect genome, there should be no more chance of an inserted gene escaping to cause damage to another species than there is of a naturally occurring insect gene doing so.

The possible use of transposons as genetic control agents must also be evaluated with caution. Transposons share a number of structural features with the mammalian retroviruses and it has been proposed that the two are evolutionarily related (Finnegan, 1983). Certainly, the DNA forms of retroviruses, which integrate stably into mammalian genomes, behave in some ways like transposons. Whether any insect transposon can spread via infection, like retroviruses, will have to be determined carefully. Clearly, transposons must be treated initially as pathogens and tested for infectivity and effects on other insects before the release of any engineered species carrying transposons is undertaken.

Conclusion

Work on the possible impact of recombinant DNA technology on insect-pest control is still in its infancy. It may be several years yet before the correct

combinations of transformation vectors and deleterious genes are assembled for any pest species, or before some of the suggestions we have made in this article are tested on a small scale in the laboratory. It is likely that, as molecular genetic research increases our understanding of the structure and functioning of the eukaryotic genome, we will be better able to predict what other sorts of manipulation might ultimately prove useful in controlling insect pests.

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