the relevance of molecular biology to pure and applied entomology

door dr. M.J. Whitten,
Canberra

THE RELEVANCE OF MOLECULAR BIOLOGY TO PURE AND APPLIED ENTOMOLOGY

door dr. M.J. Whitten,
Canberra

Tweede voordracht gehouden ter herdenking
van professor dr. J. de Wilde,
hoogleraar in de entomologie aan de
Landbouwhogeschool te Wageningen
(1954–1982) op vrijdag 29 april 1988.

THE RELEVANCE OF MOLECULAR BIOLOGY TO PURE AND APPLIED ENTOMOLOGY

It is a great pleasure and an undeserved honour to follow John Kennedy who presented the inaugural Jan de Wilde Memorial Lecture in 1986, and to deliver the second lecture in honour of the memory of this distinguished scientist.

We live in exciting times when we contemplate the analytical power of the research tools now available for studying a range of problems in biology, especially entomology, that have defied satisfactory analysis and resolution for many decades. If we successfully combine the tools of molecular biology and genetics with the more traditional disciplines of physiology, behaviour, developmental biology, ecology, taxonomy and evolutionary biology, we can truly come to grips with a range of processes and phenomena concerning insects. The knowledge that will emerge from such initiatives promises to have profound implications for both the cultural and material benefit of mankind.

I want to stress at the outset that the most immediate and obvious benefits of these new technologies will be in the area of fundamental science. Important practical benefits will undoubtedly follow. While we can spend some time anticipating the nature of these practical benefits in terms of improved applied entomology, we should really accept the philosophy of Pasteur, embodied in his statement 'There is no applied science; there is science and there is the application of science'. I believe Jan de Wilde (1982) was saying much the same thing when he advised students, in the words of his retirement lecture 'Here in Wageningen the students are trained to combine the two ways of thinking: the logical sequence from hypothesis to experiment and from there to the scientific conclusion, and the

social requirement always to keep in mind: what can I do with this knowledge on behalf of agriculture.' This viewpoint of the critical connection between fundamental and applied science was also shared by John Kennedy (1986), and persuasively developed by him in the first Jan de Wilde Memorial Lecture.

We are all entitled to ask the question 'Isn't molecular biology just another discipline, another set of analytical tools along with those of ecology, physiology, behaviour, genetics, cell biology and so on, to help the experimental scientist?' Worse still, is it another bandwagon capable of distorting our efforts by concentrating too many resources in a fashionable field; and might we be too willing to allow our budding scientists to abandon the traditional skills in favour of the glittering (and expensive) rituals of DNA sequencing and genetic engineering?

It might be argued that molecular biology is little more than a collection of techniques for manipulating and analysing nucleic acids and, accordingly, we could be tempted to put it into a pigeon hole alongside the traditional disciplines. To do so would be a serious mistake. Molecular biology is an extraordinarily powerful integrative discipline. It has already demonstrated its capacity to bring together areas of science previously disconnected, such as behaviour, developmental biology and neurobiology. It is forging research teams with a range of skills unheard of in the history of biological sciences. To ignore these developments must be at our own peril as effective research workers. And those charged with the responsibility to train the next generation of biologists have a particularly challenging task to advocate a

balanced perspective.

What should be particularly exciting for entomologists, both as educators and researchers, is the central role that insects are beginning to play in these new interactive sciences. We are all familiar with the various practical considerations favouring the use of insects as experimental organisms; and recently, animal welfare groups have further strengthened the potential importance of insects as experimental organisms by their expressed concerns over experimentation on vertebrates. Furthermore, Berta Scharrer (1987), in a recent review on the value of insects as model organisms, has argued that insect systems are not only valid models for cases where the organ or process has a common evolutionary origin, but are also valid for instances where the insect has found an independent solution, in an evolutionary sense. To make her point, Scharrer has compared the neuroendocrine system in mammals and its analogous counterpart in insects. Later, I will be making a similar point using insect vision which has both homologous and analogous components, when compared to vertebrate vision.

And so, if we endorse the integrative role of molecular biology, and also accept a more central role for insects as model experimental systems, then it seems prudent for entomologists at least to become literate about the procedures of molecular biology. I am certainly not advocating that we all become practising molecular biologists. But we need to understand what molecular biology can do for us in our various fields of endeavour; we also need to understand what it *cannot* deliver. In the longer term, the rare commodity, and the limiting resource

in exploiting molecular biology, is unlikely to be the availability of molecular biologists. Certainly, they are in strong demand just now. Rather, it will be the supply of scientists with a traditional and broad perspective in biology, both in the laboratories and the field.
Jan de Wilde was a scientist with this broad perspective. It therefore seems particularly appropriate that this Memorial Lecture explores the integrative role of molecular biology in entomology across disciplines, and from the basic to the applied.

What are the principal techniques of molecular biology?
Stripped to its essentials we could regard molecular biology as a set of techniques for the isolation, analysis and manipulation of nucleic acids and their protein products.
These include:

- availability of a set of restriction enzymes to cut DNA at specific sites and ligase enzymes to re-join the naked ends of the cut fragments;
- methods for the rapid sequencing of nucleotides of DNA or RNA and, to a lesser extent, amino acids of proteins;
- the capacity to synthesise specific short sequences of DNA or proteins;
- techniques for site-directed mutation of specific DNA segments;
- an ability to isolate genes, and tranfer them intact elsewhere in the genome or into other organisms with subsequent stable transmission and gene expression;
- a range of cytochemical techniques to identify

how, when and where genes are actually transcribed;

- a range of immuno and histochemical techniques to identify how, when and where a particular gene product functions.

Let us now look at the impact that tools such as these can or should have on various disciplines pertinent to entomology.

Molecular biology and basic entomology

Gene structure and function

The general picture of gene organisation as identified by the French workers F. Jacob and J. Monod in the 60's for bacteria is now known to be generally applicable to other prokaryotes, and to higher organisms largely as a result of insect studies. For our purposes, we can regard the eukaryote gene as comprising a 'structural' region which contains information coding for the gene product, usually a protein, and a promoter region, usually upstream to the direction of reading or transcribing the DNA into messenger RNA (mRNA). This promoter region determines the circumstances under which a gene becomes and remains active. There are important differences between the structure and expression of genes in higher organisms and micro-organisms but we can ignore these for our present purposes. Under specified conditions, the gene is transcribed into mRNA and this is then translated into a protein.

Several important, and some quite unexpected, aspects of eukaryote gene expression relevant to pure and applied entomology have emerged from molecular studies. These include:

- Gene duplication or amplification manifested as a series of tandem repeats of the gene. This is one tactic used by insects to increase the titre of a specific gene product, for example where this is desirable for enhanced metabolism of a pesticide. *Myzus persicae* and *Culex pipiens*, for instance, have developed resistance to organophosphorus insecticides by gene amplification.
- Editing of mRNA before it is translated into protein. It was previously presumed that there was a fixed 1:1 relationship between the gene and its protein product. We now discover differential splicing of the mRNA product of the gene can occur before translation. In this way a single gene can produce several different versions of its protein product. Moreover, the versions can be produced in different tissues or at different times. Complex loci like *bithorax* produce a range of protein products through the course of *Drosophila* embryogenesis Kornfeld, et al. (in press). Differential splicing of mRNA also assists in explaining how relatively few genes in insect species (e.g. 14,000 in *Drosophila*) are sufficient to regulate development and behaviour.
- Synchronous production of different peptides through the cleavage of primary gene products into a number of smaller peptides. This finding has proved particularly valuable in understanding co-ordinated development and stereotyped insect behaviour. We return to this topic later.

Genome organisation.

Ever since T.H. Morgan described genome organisation in *Drosophila* as 'beads on a string', it has been assumed that the chromosomes of higher eukaryotes

have simply functioned as the physical carriers for genes. The spatial arrangement of genes, the number of chromosomes in the genome and the positioning of centromeres and 'inert' heterochromatin were invariably interpreted by geneticists, and inevitably from a genetical perspective. Furthermore, the actual molecular structure of chromosomal features such as centromeres, telomeres and chromomeres, was unknown. Not surprisingly, these organelles were always perceived as serving some narrow genetical function associated with storage, expression or transmission of hereditary information.

Many phenomena, not comfortably explained in the paradigm of the geneticist, such as co-ordinated centromere shifts across the chromosome set in closely related taxa, were given descriptive titles, such as orthoselection, which lacked any explanatory content; or often the phenomenon was ignored altogether. For example, jumping genes were not widely recognised in eukaryotes until their existence was put beyond dispute by bacterial geneticists. Even the term 'jumping gene' was an operational genetic tag for a phenomenon which we now interpret molecularly as mobilisation of a transposable element, and not literally as a gene that has altered its address. In a similar vein, the existence of viruses were inferred early this century by pathologists before their reality was confirmed. It is doubtful that they would have been regarded at all as microorganisms, especially retroviruses, if their existence had emerged from the endeavours of geneticists or molecular biologists (Whitten, 1985). Molecular biology has broadened our thinking on genome organisation beyond the strictly genetic view

of DNA as the bearer and transmitter of hereditary information. This wider perspective has permitted the introduction and acceptance of notions like selfish DNA, molecular drive (i.e. phylogenetic change at the DNA level not necessarily enhancing darwinian fitness), chromosomal organisation that is dictated either by intra-cellular spacing requirements or the need to synchronise gene action, differentiation processes or developmental rates, and so on.

One striking example of genomic reorganisation is the gradual but simultaneous shift of the centromere from a central to a terminal location in all chromosomes of one race of the Australian grasshopper, *Caledia captiva*, as the species extends down the eastern coast of NSW (Shaw et al., 1988).
It is now postulated by Shaw and his colleagues that the centromere shifts influence cell proliferation rates and developmental rates; and ultimately affect the insect's ability to survive in colder climates. There are many comparable examples amongst insects and mammals where the cytological observations are not readily explained by the narrow genetical interpretation.

Thus, molecular biology has proved an important element in generating the broader and more satisfying picture of genome organisation in eukaryotes, as well as providing tools for the detailed technical analysis.

Molecular Taxonomy
Taxa delineation and phylogenetic affinities have historically been based on the analysis of morphological characters and, to a lesser extent,

on physiological, cytological and behavioural parameters. More recently, immunology and electrophoresis have proved of value in determining kinship, especially at the sub-specific level where the morphological approach has proved inadequate. A range of molecular techniques has emerged in the past five years and it is informative to explore their potential to assist the insect taxonomist. Here, we briefly look at three of these techniques.

1. Restriction site polymorphisms

The addition, deletion or substitution of one or more nucleotide along a stretch of DNA can create a new restriction site or destroy an existing site which is recognised by a given restriction enzyme as a point where the DNA molecule can be severed. Accordingly, presence or absence of a restriction site will alter DNA fragment lengths once the DNA is digested with a restriction enzyme. The resulting restriction fragment length polymorphism (RFLP) can be used as a measure of similarity and, aggregated over a number of sites and for two or more restriction enzymes, the information allows us to decide how closely related two taxa are. This technique is particularly suited to determining phylogenetic relationships between closely related taxa, especially at the sub-specific level. Avise and colleagues (1983), using the RFLP approach in a study on genetic divergence within a particular gene located in mitochondrial DNA, were able to construct detailed phylogenetic lineages for the small rodent *Peromyscus* in the U.S.A. No previous technique, especially morphological, has approached the RFLP technique for ease of manipulation or for resolving power in the analysis of closely related taxa.

2. DNA–DNA hybridisation

Another technique which reveals the power of molecular taxonomy to challenge traditional thinking, is DNA–DNA hybridisation. If two single strands of DNA from different sources are allowed to combine, forming a double strand, which is then heated, the temperature at which the strands separate will depend on the degree of difference between them. It takes a higher temperature to separate strands of complementary DNA derived from the same or closely related taxa. Thus the temperature at which such hybrid strands dissociate is regarded as an accurate measure of the evolutionary distance between two taxa (Sibley and Ahlquist, 1987).

Let us look at the impact of this technique, as used by Sibley and Ahlquist, on the evolutionary origins of the Australian bird fauna.

When European settlers first came to Australia, many birds were observed to be quite similar to the more familiar European or Asian birds. In general, the Australian birds slotted comfortably into the existing European avian families, even according to an extensive range of morphological criteria. Sibley and Ahlquist, however, using DNA–DNA hybridisation have argued that this interpretation is completely false. They contend that most of the Australian bird lineages diverged in Australia. If the DNA–DNA hybridisation technique is valid, then we are left with an extraordinary example of convergent evolution. Indeed, it implies that Australia is not simply a recipient of species that have evolved elsewhere; it must be a centre of evolution. This picture has now been supported by subsequent electrophoretic

and chromosomal studies (Schodde and Christidis, in press).

The morphologist, confronted with these new phylogenies has been forced to revisit the morphological data; and now many characters previously regarded as unreliable (e.g. humeral fossae), because they did not fit the preconceived evolutionary picture are, on closer scrutiny, considered to be consistent with the molecular interpretation.

It is surprising that much of the effort in applying molecular techniques such as RFLP's and DNA-DNA hybridization has focused on mammals and birds, with relatively little effort on invertebrates. Recent successful studies on insects (e.g. Bishop and Hunt, 1988) suggests that this position will change in the years to come, as insect taxonomists who are not overwhelmed with a massive undescribed fauna as we have in Australia, have time and opportunity to master the techniques of molecular analysis.

3. Ribosomal genes

The area of molecular taxonomy which promises to be most exciting and which perhaps has greatest relevance for insect systematics comes from the study of ribosomal genes. The RNA molecules within a ribosome are transcribed from a cluster of three genes of which there are multiple copies. The segment containing this cluster also contains 'spacer' DNA which is either not copied into RNA or is deleted after transcription but before the edited RNA is incorporated into the ribosome. There are several reasons why the molecular study of ribosomal genes should revolutionise whole areas of invertebrate

taxonomy. Essentially, all animals contain ribosomal genes and ribosomal RNA occurs in abundant supply in insect tissue. Some regions of the ribosomal genes are highly conserved while others have undergone more rapid evolution. Therefore the same system can be readily used to look at evolutionary relationships across phyla or within a single species complex.

For example, the ribosomal RNA might assist in solving the following higherorder taxonomic problems:

1. Is the Arthropoda monophyletic or has it arisen on separate occasions from different ancestors?
2. Are orders like the Heteroptera sensible evolutionary entities or arbitrary creations of taxonomists?
3. What is the evolutionary relationship between orders like the Coleoptera and Strepsiptera? Where do the Peloridiidae belong, with the Homoptera or the Heteroptera?
4. Can we construct more realistic phylogenies within economically important families such as the tortricid leafrollers, a group where palaearctic taxonomy had set the scene before extensive material from the centre of origin in the sourthern hemisphere had become available.

It is interesting to note that our confidence in the higher order classification of the Insecta has eroded over the past 50 years. For example, a chart depicting phylogenetic relationships between insect orders as presented by Kristensen (in press) has many more question marks and dotted lines than Jeannel's (1949) presentation. Clearly, existing methods of delineating relationships continue to leave room for improvement.

Valuable insights have resulted from some of the first efforts to apply a range of molecular techniques to defining the evolutionary relationships between the orders of Insecta. Wheeler for example has supported Kristensen.
Contention for the monophyly of the Odonata and Neoptera, contrary to the views of Hennig and Boudreaux (Wheeler, in press). Equally, Wheeler concludes that the molecular evidence supports Kristensen's claims for monophyly of the Neuroptera and Coleoptera against the views of Boudreaux and Ross. The relative strengths of the various molecular approaches, as assessed by Wheeler, indicates some very interesting debate is inevitable in the years to come.

A survey of workers, techniques and problems being addressed in insect systematics, ecology and evolution by molecular techniques is presented in Simon (1988).

Molecular taxonomy does not threaten to displace conventional taxonomy.
In fact the two approaches should complement beautifully. However, the taxonomist needs to know what technologies are available and to seek opportunity to influence the molecular biologist, especially in the area ofhigher order systematics or at the sub-specific level for invertebrates where morphology is weakest and where molecular techniques excel. Without the initiative being taken by the taxonomist, the molecular biologist is likely to gravitate towards the more topical areas of developmental biology, physiology and behaviour for many years to come.

Since giving the lecture, and while preparing a written text for publication, considerable controversy has erupted over the procedures used by Sibley and Ahlquist and the validity of their interpretation. Students of the history and philosophy of science have an excellent opportunity to observe the human dimension in the scientific process and to observe a heated battle as it continues to unfold. A critical and balanced review of these developments can be found in Lewin (1988).
It is ironical to note that the distinguishing feature and saving grace of molecular taxonomy was expected to be its high level of objectivity – it was expected to raise taxonomy above subjective opinions, to transform the Art into a Science! However, the hostile reaction to Sibley and Ahlquist (see Lewin, 1988) by some systematists, including molecular biologists, strongly suggests that objectivity is still a long way down the track!

Developmental Biology

Let us move to developmental biology where there has been considerable research activity, and where insects have returned to center stage after some decades in the wings. One prominent theme has been 'what causes segmentation during embryogenesis and how is each segment differentiated from its neighbours? For example, what tells one insect segment to become the 3rd thoracic segment, while its neighbour becomes the first abdominal segment?' Clearly, this question can be generalised to include most animal groups from earthworms to man.
In fact, William Bateson as far back as 1894 predicted that if we could understand homeotic variation, or how one organ develops where another

normally would, we might have the key to understanding development. Bateson's (1894) treatise illustrates one such homeotic variant in a crustacean with an antenna replacing an eye. A modern day version created by Ed Lewis of Caltec using Drosophila, and depicted on the cover of a recent Science (1 July, 1983: Vol 221 (4605)), displays a fly with an additional thorax complete with a second set of wings, developing in place of the first abdominal segment. Genetical analysis of mutants at the bithorax locus giving rise to various bithorax phenotypes has assisted in determining how the wild type gene functions during normal differentiation. In particular, it established the role of differential splicing of mRNA from complex loci like Btx in normal segmentation processes Kornfeld, et al. (in press).

I wish to give just one example showing how mathematicians working with molecular biologists can give us a better insight into the genesis of segmentation. Barry Nagorcka (1988), in my Division, has demonstrated that one can use a simple reaction-diffusion system to generate a segmental pre-pattern to which developmental genes might respond. According to Nagorcka's model, segmentation is initiated during the syncytial blastoderm stage when all the nuclei reside in a single layer below the egg surface, and just before embryonic gene action commences. By assuming the existence of two maternal substances that diffuse through the cytoplasm and react with each other, Nagorcka has postulated that, with successive cell divisions, wave-like distributions of the substances create a series of "segments" which double in number with successive mitotic cycles. Furthermore, each segment of the final set of

segments can be individually characterised during the process of segmentation. Nagorcka argues that key developmental genes respond to the pre-patterning, which is essentially laid down by physico-chemical processes, rather than to some epigenetic sequence of events. For example, the gene loci, *futz* and *eve* in Drosophilashow a corresponding pattern of action from embryonic cell cycles 10-14.
Another gene, *paired* also becomes active, co-incident with the 8- and 16-segmented stage (see Nagorcka, 1989). According to Nagorcka, these genes differentially express, in response to the pre-pattern, rather than acting as inducers of segmentation themselves.

Molecular biologists have exploited to great advantage the fact that a conserved DNA sequence can be used as a probe to detect genes with similar sequences from the genome of the same or unrelated species. You are probably aware that many key developmental genes isolated in insects appear to have a common evolutionary origin with developmental genes in other segmentally organised animals, including man. These genes, or parts thereof, can be highly conserved. Accordingly, they sometimes share a DNA segment which contains common nucleotides sequences. One such set of genes produces a class of proteins called 'zinc-finger' proteins which bind to specific genes and regulate their expression (Klug and Rhodes, 1987). The stereo-chemistry of these DNA-binding proteins, and their sequence specificity is beginning to be understood through the use of appropriate developmental mutations in *Drosophila.* Rob Saint and his colleagues in my Division have succeeded in using the 'homeo-box' sequence, a

conserved DNA-binding domain, characteristic of genetic loci controlling early developmental events, to isolate *rough*, a gene implicated in pattern formation in the developing eye of *Drosophila* (Saint et al., 1988). Other conserved sequences have enabled the isolation of large families of genes otherwise not recognisable as having common ancestry or related functions. For example, until recently little was know about the protein kinase genes, many of which mediate cellular responses to external signals. Now, over 100 members of the family have been identified and investigated because of their conserved catalytic domains (Hanks et al., 1988). An explosion of such valuable knowledge would have been unimaginable even 10 years ago.

Physiology and Behaviour

Many of you will be familiar with the idea that fixed pattern or stereotyped behaviour can be regulated by peptide molecules called neuropeptides (for review see O'Shea, 1985). Let us look at the role of molecular biology in reaching this conclusion, using egg laying behaviour in an 'honorary' insect, the marine mollusc, *Aplysia californica*, and then let us explore its relevance to *real* insects. Once a female *Aplysia* has become gravid, egg-laying behaviour consists of a set of actions executed in fixed sequence following an appropriate environmental stimulus, i.e. encountering a suitable oviposition surface. Careful studies by neurophysiologists and biochemists over a period of years had previously demonstrated that a peptide, 36 amino acids in length, was implicated in egg laying. This egg laying hormone (ELH) was known to be synthesised in a group of cells, the bag cells, near the main abdominal

ganglion. Molecular biology entered the proceedings with that background knowledge.

The molecular techniques greatly facilitated the demonstration that ELH derives from a precursor, 271 amino acids in length. This longer precursor was then shown to be cleaved into a set of 10 peptides, whose individual roles are to stimulate or repress specific neurones or to act as a neuromuscular hormone, all in connection with elements of the egg laying behaviour. In effect, the gravid female is 'soft-wired' for a related set of responses to the external stimulus, appropriate with her physiological status. This perspective is unlikely to have been reached without the intervention of molecular biology since it provided the critical linkage between ELH and the other nine peptides. Conversely, it was the 'traditional' disciplines with a well defined phenomenon before them which represented a *sine qua non* for the integrative tools of molecular biology to exploit so powerfully. Since the publication of the *Aplysia* 'story', there has been considerable discussion amongst my entomological colleagues as to its validity as a model for stereotyped behaviour in insects generally. Clearly, the model is not appropriate to explain instantaneous responses to external stimuli such as avoidance behaviour where there is insufficient time for *de novo* gene action to be implicated. However, an earlier co-ordinated release of several neuropeptides, following some endogenous signal could well be relevant to understanding why the reaction should differ between physiological states or be dependent on whether the insect is actively engaged in some stereotyped behaviour or not. For example, a

blood sucking tabanid or an ovipositing calliphorid reacts much more slowly to a waving hand than a resting insect or one that is not gravid, when similarly challenged.

In other words the *'Aplysia'* model suggests a mechanism for explaining different sets of behavioural response to the same external stimulus, dependent upon and consistent with the physiological status of the individual insect under scrutiny. In a similar manner, where the response time itself need not be rapid, but where synchronised and sequential steps are necessary for an orderly outcome, (say, during a series of developmental events or behavioural responses characteristic of a particular developmental stage), we could well expect some prior internal signal had been triggered. The signal would initiate expression of a gene that codes for one or a number of neuropeptides which, in turn, 'soft wire' or program the individual to respond in an appropriate manner.
Although, I am not aware of any study on insects that demonstrates the simultaneous production of neuropeptide subunits that act in quite the same manner as the 'egg laying' gene in *Aplysia*, the recent work of Tublitz and colleagues (1986) supports the contention that neuropeptides will prove to be one important mechanism in the regulation of insect development and behaviour. Monsma and Wolfner (1988) have established that one accessory gland protein of *Drosophila* transferred to females during copulation includes a sequence in which 11 of 17 amino acids are identical to the ELH of *Aplysia*. It seem reasonable to suppose that the *Drosophila* protein containing the ELH-related sequence might be cleaved at some later

stage within the female to give rise to one or more neuropeptide that influence subsequent female behaviour.

Let us now turn to *Manduca sexta*, the tobacco hornworm, and the pioneering studies by Tublitz and his colleages. I wish to make two simple points:

- The key role of neuropeptides in controlling development and behaviour is well demonstrated by their work.
- The eclosion hormone (EH), a neuropeptide, was identified as far back as 1970, but peptides, then, were chemically too difficult to work with, compared to juvenile hormone and the ecdysteroids. Consequently, further study of EH was effectively set aside. In contrast, peptides are a preferred medium for molecular biologists rather than the chemically more simple downstream metabolites, like steroids. We can now see attention concentrating on mechanisms involving proteins like EH. As so often happens in science, the questions asked and the course of action plotted, reflect the techniques available.

We can now confidently predict that the application of molecular biology to key life history events like eclosion, wing expansion and hardening, and the behaviours that accompany these events, will lend a new lease of life to this field, and not just with *Manduca*. In passing, I might observe that Wageningen's Department of Entomology, through staff like Dr. Schooneveld and Dr. de Kort, is particularly well placed to break new ground on the role of neuropeptides, both in fundamental and applied entomology.

Several of my staff are especially interested in

solving the problem of how the act of mating in the Australian sheep blowfly stimulates oviposition by females and dampens their enthusiasm for further mating. A component of the secretion of the male accessory gland is responsible for these two behavioural effects in the female Smith et al. (in press).

During copulation, the male deposits accessory gland material direct into the wall of the bursa copulatrix Merritt (in press) of the female which then enters the haemocoel. What this material is and how it functions is yet to be determined. The molecular biologists are working with the physiologists, Dr. Keith Binnington and Dr. Peter Smith, to answer these queries. An enzyme analogous to the esterase 6 of *Drosophila*, may be implicated. Produced in the male accessory gland this enzyme appears to be transferred to females during copulation. Esterase 6 is one of a family of esterases which are now being isolated and studied because of their conserved DNA sequences. Each esterase features in quite different, but important areas of entomological research in the Division ranging from insecticide resistance to pheromone reception. Once again, it illustrates how molecular biology builds unexpected bridges between research teams.

Hybridoma libraries – Man and flies

A recent technique developed by Fujita (1988) offers great promise for fundamental entomological research and strengthens the model role of insects. The advent of the monoclonal antibody (MAb) technique has permitted "the construction of panels of MAb's by immunization with complex mixtures of unidentified antigens such as tissue homogenate" (Fujita, 1988).

These MAb panels, or hybridoma libraries, can be used to identify the histological distribution of an individual gene's product. Fujita's library of 148 MAb's, each with a specific staining pattern in head sections of *Drosophila*, has been used by Miller and Benzer (1983) to show that 50% of genes in the brain of *Drosophila* are related to genes in the human brain as defined by immunological affinity. No doubt many of these genes produce proteins concerned with ion-transport channels (e.g. Na, K, Ca), with neuropeptide synthesis, or are genes concerned with laying down of neural networks, cell-cell recognition, protein phosphorylation associated with short term memory processes, gene activation during long term memory deposition, etc. And that is the point – we can expect to learn much about human behaviour and physiology from the molecular analysis of insects because many of these functions are common to man and fly. It won't be long before the action of human genes will be studied in defective *Drosophila* by transforming the latter with wild type genes from man to restore normal functions; this step has already been foreshadowed by Miller and Benzer (1983).

Insect Vision

From studies on bacteria and mammals, we know that the principal photoreceptor molecule is a membrane-binding protein moiety coupled with a chromophore. The protein derived from cows had already been isolated and the corresponding gene coding for the opsin protein cloned and sequenced. In the insect eye we know there are three species of opsin with different absorption spectra. Alan Cowman and colleages (1986) used the bovine gene to isolate the principal opsin gene from *Drosophila* and showed that

the gene is expressed in each of the 6 full length photoreceptor cells; whereas a related opsin which differentially absorbs blue light is only synthesised in the truncated 8th photoreceptor cell. Furthermore, Cowman's group proposed that the critical region for attachment of the chromophore was the variable region of the protein, projecting outside the membrane. They were able to test and disprove this idea by constructing a mutant which lacked precisely the region to which the chromophore was presumed to attach. They showed that the defective molecule still functioned despite deletion of the region in question. Although their prediction had proved incorrect, the outcome was an unequivocal advance in scientific knowledge and suggested the course of further experimentation.

Just imagine what Wigglesworth or de Wilde might have done with such powerful tools at their disposal instead of the crude ligature experiments which nevertheless yielded so much information!

I cannot resist the temptation to talk about several applications of our knowledge of insect vision in the field of fibre optics and robotics.
Allen Snyder at the National University in Canberra has, for some years, studied the loss of energy by polarised light as it passes through the photoreceptor cell in the insect eye (Synder, 1979). In so doing, he devised a way to reduce the energy loss as light travels along optical fibres by some 100-fold. This understanding potentially has enormous ramifications for the fibre optics industry. He has also used the photoreceptor cell as a successful model for low energy sensors in medicine and security

systems. Adrian Horridge, in the same Department, has shown the merits of insect vision as a more appropriate model system for artificial vision in robotics, instead of vertebrate vision which, till then, had been the preferred model (Horridge, 1987).

While the compound eye has evolved independently in vertebrates and insects, many vital genes in the two groups concerned with vision, such as the photoreceptor proteins, share a common ancestry. As Berta Scharrer has advocated, if we are to derive full value from insects as models, we must feel comfortable in combining analogous and homologous systems when we use insects as tools in fundamental research.

Unfortunately time does not permit detailed analysis of the contribution of insect vision to high-technology fields, but the examples I have sketched do illustrate the serendipitous nature of science and the extraordinary spin-offs which frequently flow from first class science – so long as the researcher retains a healthy attitude to seeking applications for the knowledge generated. I believe this was one of the distinctive hallmarks of Jan de Wilde's successful career in pure and applied entomology.

Molecular Biology and Ecology

Let me give one example to show how molecular biology of insects can be pertinent to the ecologist. Dietz and Barettino (1984) in Germany developed a cytological method for detecting when genes are actively transcribing messenger RNA. In normal salivary gland cells using their technique, we find that many genes fluoresce along the polytene chromosome indicating active expression. However,

following heat-shock of the whole insect, several new genes are switched on, and their products quickly suppress normal gene action. And so, following heat shock, only 3 or 4 bands are 'lit-up', the rest cease fluorescing.

Thus, under stress conditions, which can be heat or chemically induced, gene action is reduced to a minimum, presumably to lessen errors in synthesis of non-essential proteins. The genes that control this co-ordinated switch-off of gene expression are called heat shock protein genes (HSP) and have been found in all organisms so far examined, from bacteria and plants to man. However, the temperature profiles stimulating the response, and the lag time, vary between species. Thus, under field conditions, ecologists can observe that some populations will crash on exposure to high temperature – at other times they survive. Awareness of the heat shock response can assist in unravelling what can appear to be inconsistent behaviour between populations, or varying behaviour across species. For example, Thompson (1987) who studied two related species of Australian *Drosophila*, could better explain unpredictable population crashes following periods of high temperature and why the two species responded differently to variation in ambient temperature. Such a perspective should at least give the observer some intellectual satisfaction knowing something about the underlying mechanisms when something happens, or fails to happen. It could even make us better ecologists!

Another example I like to quote to ecologists comes from *E. coli* which has a range of genes concerned

with carbohydrate metabolism. Some sugars are more readily metabolised than others in terms of energy budgets. Consequently, there is an orderly sequence in which these genes are activated, despite a mix of carbohydrates in the diet. Genetic variation can cause differences in carbohydrate utilisation between *E. coli* populations.

Surely the *E. coli* ecologist can cope with this situation more intelligently and with greater intellectual satisfaction if, as Dethier (1962) would argue, you know your organism better. Unfortunately, to some ecologists, their beast is a typological 'black box', every individual being genetically and phenotypically equivalent in all regards.

The relevance of molecular biology to biotechnology and applied entomology

So far, this lecture has focused on the impact that molecular biology can have on the fundamental side of entomology and, through the use of insects as models, on science more generally. I have stated several times, but it bears repeating: the more spectacular applications to economic entomology are likely to arise serendipitously and with little forewarning from the laboratories that are at the forefront of the basic research, particularly where there exists an open minded approach to the broader relevance of the new discoveries. The balance of this lecture looks at some of the more specific applications of molecular biology in industry generally and, more particularly, to applied entomology.

Biotechnology

The term "expression system" has arisen from the ability to transfer specific genes, coupled to

suitable regulatory DNA sequences, into foreign organisms so that the gene produces a desired product under prescribed conditions. Here the object is to generate quantities of what was previously a rare compound in sufficient amounts for further research purposes, or to make it available in pure form in commercial quantities, e.g. interferon, insulin, epidermal growth factors etc. Often the host organism for such expression systems is a debilitated bacterium such as *E. coli*, a yeast strain or an animal cell, which is mass produced under fermentation or cell culture conditions. More recently, research on insect cell lines (e.g. lepidopteran) and insect viruses (e.g. nuclear polyhedrosis viruses) has enabled construction of expression systems using promoters from non-essential genes (e.g. the polyhedrin gene) coupled with the coding regions of the foreign genes of interest (e.g. human interferon gene) (Luckow and Summers, 1988). So far, the insect system has not offered significant advantages over some of the other expression systems (though commercial secrecy makes it difficult to know how advanced the art is); but it is probable that insect-derived systems will prove as efficient as those based on *E. coli* or other prokaryotes. The insect system will generally have advantages over prokaryotes with respect to processing and stability of product, but not necessarily over yeast systems. It should give pride to the University of Wageningen to acknowledge the pioneering role of Dr. Just Vlak of the Virology Department in the development of the polyhedrin promoter system from the NPV of *Autographa californica* in Max Summers' laboratory at Texas A & M, and his continued research in identifying other promoters useful in expression systems.

Improved management of pesticide resistance
Increased effort is being focused on the management of pesticide resistance as resistance to the major classes of chemicals continues to evolve in many important pest species (for review see Roush and McKenzie, 1987). For these efforts to succeed we need to identify robust models which have realistic estimates of mutation rates from the wildtype to the resistant allelic state, and to have a good understanding of the field circumstances which favour survival of the resistant over the susceptible phenotypes. Models for explaining the emergence of resistance (e.g. Whitten and McKenzie, 1983) have relied on traditional estimates of mutation rates associated with spontaneous mutations. Unfortunately such estimates can no longer be regarded as reliable. Molecular analysis of resistance genes will indicate whether we are dealing with point mutations, single or multiple mutational events, mutations associated with the mobilisation of transposable elements, tandem repeats of DNA sequences, etc. Each of these categories of mutation is likely to be subject to quite different mutation rates.

While it might appear a daunting task to identify the generalities needed if resistance management models with broad applicability are to be devised, fortunately some of the genes widely implicated in pesticide resistance do belong to known gene families, e.g. the esterases (Russell et al., 1989). Just as molecular biology has helped identify and classify the dozens of protein kinase genes, we can expect key resistance genes to be thoroughly categorised and sequenced in the next few years. Such knowledge should prove valuable in the further

development of robust resistance management strategies. The knowledge will also allow us to determine the feasibility of using site directed mutations to alter genes in natural enemies to confer resistance to pesticides which would otherwise reduce their effectiveness under operating field conditions.

Gene transfer systems and applied entomology

Rubin and Spradling (1982) were the first to develop a practical system for introducing foreign genes into an animal genome in such a way that they are capable of normal expression and stable inheritance. In their case, of course, the experimental organism was *Drosophila melanogaster*. The initial hopes that the same or similar systems, all utilising the transposable P-element as the vehicle for effecting the transformation, would represent a general mechanism for gene transfer in insects have yet to be realised, despite serious attempts on a range of insect pests.

Blackman et al. (1989) have demonstrated that another transposable element *hobo* can act as a vehicle for germ-line transformation of *Drosophila*. Its activity outside *Drosophila* is not yet known.

Although the lack of progress to date on transformation in insects has been disappointing, there is little doubt that practical means will be devised within 5 to 10 years for transferring genes, unaltered or bearing specific site-directed mutations, into different species. It is not appropriate to canvas these various approaches here (for recent review see Walker, in press). Instead, what we need to consider are the possible advantages of this new technology in applied entomology.

I suspect that it will be the lack of vision and imagination as to how the technology can be used to advantage, rather than deficiences in the technology itself, which will limit progress. Below are a few examples which have already received some attention.

1. *Host resistance to viral diseases in honeybees and other beneficials insects.* Viral and other pathogen related diseases of honeybee and silkworms are responsible for major economic losses to these industries. What prospect, therefore, is there for genetically engineering resistance to pathogens using 'resistance' genes derived from the pathogen's own genome? Sandford and Johnston (1985), using the host *E. coli* and the QB bacteriophage, an RNA virus containing only 4 genes as a model system, have shown the extensive range of possibilities for genetic modification of the host to prevent the parasitic virus completing its life cycle. Their model exercise illustrates the importance of having a comprehensive understanding of the biology of the pathogen since, in their analysis, each viral gene presented one or more possibilities for use as a resistance gene. The number of options identifiable and the plausibility of each option, are direct functions of the knowledge of the host/pathogen relationship. Although it may be a long time before we have a comparable level of knowledge in the much more complicated honeybee/pathogen systems, whether the latter be a virus, a bacterium, a fungus or a protozoan, it remains a worthy long term goal to seek ways of genetically engineering the honeybee or silkworm to confer immunity to a range of debilitating pathogens. A first step is to develop the enabling

technology, such as devising gene transfer systems for these beneficial insects.
Secondly, we need to focus on the general biology of the major pathogens, possibly commencing with the *Bacillus* species that cause widespread brood disease in honeybees.

2. *Genetic engineering of beneficial natural enemies.* In a similar manner, we may want to introduce pesticide resistance or otherwise induce a phenotypic shift in a beneficial insect, (e.g. natural enemy) which would be difficult to generate by conventional genetic improvement procedures. A range of possibilities has been discussed by Beckendorf and Hoy (1988) and Cockburn et al. (1984). These might include altered host range, ability to function at higher or lower temperatures, sex ratio manipulation, diapausing (or non-diapausing) and aestivation capacity, and so on.

While it might seem an advantage to transform beneficial insects to enhance their efficacy or modify their specificity, we should also be conscious of the 'downside' of introducing genetic engineering into biological control practice.
In brief, in many countries it could attract increased scrutiny about safety and certainty of outcome for 'traditional' biological control projects. Bearing in mind that we can never even safely predict success in biological control, we may experience considerable difficulty in demonstrating to a concerned and possibly hostile layman or decision maker the safety of a process we intuitatively regard as being of acceptable risk level.

3. *Improved autocidal control of insect pests by genetic manipulation.* The control of the screw-worm, Chrysomyia hominivorax by the sterile male technique during the 1960's by E. Knipling and colleagues in the USA, demonstrated a totally novel method of pest control. The technique has been successfully extended to include some other significant pests, but it would be true to say that the early expectations have not been fully realised. Attempts to improve the efficacy of the sterile male technique using a wide range of genetical trickery have been made in different countries since the 1970's. For example, classical genetics and cytogenetics have been used on the Australian sheep blowfly *Lucilia cuprina* to construct strains of this pest which, on release into the field, can induce genetic death on a scale capable of causing population collapse (Foster et al., 1985). One system currently being field evaluated by Drs. Geoff Foster and Rod Mahon, in my Division, involves the use of chromosomal rearrangements and conditional lethal genes which induce high levels of genetic death, especially in female descendents of released males. Cockburn et al. (1984) have shown how genetic engineering could increase the options and lead a given release:field ratio to generate higher levels of genetic death. Attempts to develop a method of transferring genes into *L. cuprina* strains using the *Drosphila* P-element systems have so far proved unsuccessful. However, this has only firmed the resolve of the molecular biologists to systematically explore a wider range of techniques to identify one that will work. Several laboratories around the world have

accepted the challenge issued by the Joint FAO/IAEA Division of the IAEA (1985) to develop practical methods of genetic sexing of the medfly and other fruitflies. Related research is yielding valuable background information on the molecular basis of sex determination (McKeown et al., 1988) and it is conceivable that within 10 years it will be possible, under the conditions of mass-rearing, to transform females into males or selectively kill one or other sex of the major fruitfly species. The availability of such options should enhance the utility of the sterile male approach for controlling fruitfly pests which are currently not satisfactorily suppressed by other means.

4. *Genetic engineering of agriculturally important plants.* Much has been publicised about the potential of improved plant protection through the introduction into plants of genes which confer resistance to herbicides, fungal and microbial pathogens or to invertebrates. Much can be said about the scientific, economic and social implications which flow from application of this revolutionary technology. I wish to restrict my observations to the attempts to introduce genes which confer resistance in crop plants to invertebrate herbivores.

To date, almost all attempts have focused on the use of endotoxin genes from *Bacillus thuringiensis (Bt)*. Much of the effort has concentrated on developing the enabling technologies such as cell culturing, transformation procedures, and whole plant regeneration from individual cells. However, a major cause for concern should be the extremely narrow range of genes that the genetic engineers

presently have to choose from for their transformation attempts. It is inevitable that resistance to the *Bt* endotoxins will develop in most, if not all major pests which are exposed to this class of chemical. Undoubtedly, additional protein toxins will be identified and introduced either separately or with *Bt* toxin genes during the transformation process. However, it would be a serious mistake to assume that differing modes of toxin action represent a guarantee against a single genetic mechanism in the pest providing multiple resistance to the complex of toxins being presented to it by the host plant. What we need to do under this heading, in order to broaden the options available to the molecular biologist, is to identify genes whose protein products have enzymic activity on some available substrate in the transformed plant, resulting in a new metabolite which deters or kills the herbivore. One way to achieve this objective more rapidly is to strengthen our biochemical research effort on intermediary metabolism in related groups of plants which show differing tolerances to insect damage or display varying levels of antibiosis. I do not deny that such research is long term and risky. However, genetic engineering technology is so powerful and pervasive, that it is imperative we increase the options available for the new technology to exploit its potential. Again, I repeat my earlier claim: molecular biology should create opportunities for the traditional disciplines; it cannot rationally be viewed as a threat.

5. *Genetic engineering of insect pathogens.* A useful darwinian premise to hold when we contemplate

altering the efficacy or specifity of a pathogen is the following: pathogens "strive" to maximise their own evolutionary fitness, not to minimise the fitness of their hosts. Accordingly, when opponents of genetic engineering of insect pathogens raise the challenge, 'why are we trying to improve on nature?' we have good reason to acknowledge that that is precisely what we are attempting to do. 'Nature' has no brief to serve the interests of *Homo sapiens*. It makes sense for us to seek ways to minimise the fitness of the host, which is not the 'intention' of the pathogen. Admittedly, by increasing the virulence of the pathogen we are probably impairing its own evolutionary fitness. In reality that outcome may prove to be beneficial, because continued and effective control is likely to require repetitive and inundative releases of the engineered pathogen.

Such a prospect, unlike the inoculative one–off releases of natural enemies in classical biological control, makes the technology more attractive to private enterprise and therefore increases the likelihood of industry funding and collaboration for the relevant research and development.

Genetically engineered pathogens can reasonably be viewed as delivery systems for taking a gene or its product into the relevant pest and ensuring the product reaches its target site. For example, a modified Bt might deliver a toxic protein, or even a toxic metabolite into the gut of the pest. Still more specifically, several viruses could take the gene across the gut membrane and directly into a tissue where the gene product will exert

its debilitating effect. Unlike the narrow range of suitable genes currently available to the plant genetic engineer, a relatively wider range of genes therefore may be suitable for delivery by some insect pathogens, particularly viruses. This might include genes coding for: neuropeptides, protein toxins (various venoms), protease inhibitors, proteins with immunological properties etc.

Efforts should not be restricted to engineering viruses normally prominent in insect pathology. For example, viruses not usually capable of generating epizootics because they presently lack virulence in their natural state might, nevertheless, have more appropriate host specificy ranges, or even have delivery capabilities more suited to genetic engineering. Thus entomopox-viruses could prove more suitable than nuclear polyhedrosis viruses for particular pests in certain field situations (J. Oakshott, pers comm). At this early stage of genetic engineering of plants and insect pathogens, it would seem prudent to keep an open mind as to which direction the winners will come from. Whatever the outcome, it is improbable that the new technologies will remove the need for pesticides, biological control or some integration of these. More likely, successful development of the new technologies will add to the repertoire of tomorrow's pest manner.

Concluding remarks

I have cast widely during this lecture. Undoubtedly I have overlooked areas important to some of you and have certainly treated some topics too superficially

for some with a greater knowledge of the topic than myself. Despite these obvious deficiencies, I hope the perspective I have presented and the directions of research I have tried to outline befit the memory of Jan de Wilde, the eminent mentor of many here today.

References

AVISE, J.C., CHAPIRA, J.F., DANIEL, S.W., AQUADRO, C.F., and LANSMAN, R.A. (1983). Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. Mol. Biol. Evol. 1:38–56.

BATESON, W., (1894). Materials for the Study of Variation.

BECKENDORF, S.K. and HOY, M.A. (1985). Genetic improvement of arthropod natural enemies through selection, hybridization or genetic engineering techniques, pp. 167–187. In M.A. Hoy & D.C. Herzog (eds.) Biological Control in Agricultural IPM Systems. University of California, Berkeley, and Natural Science Foundation.

BISHOP, J.G. and HUNT, J.A. (1988). DNA divergence in and around the alcohol dehydrogenas locus in five closely related species of Hawaiian *Drosophila*. Mol. Biol. Evol. 5(4): 415–431.

COCKBURN, A.F., HOWELLS, A.J. and WHITTEN, M.J. (1984). Recombinant DNA technology and genetic control of pest insects, pp. 69–99. In G.E. Russell (ed.) Biotechnology and Genetic Engineering Reviews – Vol. 2, Intercept Ltd, Newcastle upon Tyne.

COWMAN, A.F., ZUKER, C.S., and RUBIN, G.M. (1986). An opsin gene expressed in only one photoreceptor cell type of the *Drosophila* eye. Cell. 44: 705–10.

DE WILDE J., (1982), Insect control in retrospect and in prospect. Ent. exp. & appl. Ned. Entomol. Ver. Amsterdam. 31: 121–131.

DETHIER, V.G. (1962). To know a Fly. Holden–Day, San Francisco, Calif. 119 pp.

DIETZ, J.L., and BARETTINO, D. (1984). DNA–RNA hybrids and transcriptional activity in *Chironomus* polytene chromosones. Chromosoma (Berl). 90: 103–110.

FOSTER, G.G., VOGT, W.G. and WOODBURN, T.L. (1985). Genetic analysis of field trials of sex–linked translocation strains for genetic control of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann). Aust. J. Biol. Sci. 38: 275–93.

FUJITA, S.C. (1988). Use of hybridoma libraries in the study of the genetics and development of *Drosophila*. Ann. Rev. Entomol. 33: 1–15.

HANKS, S.K., QUINN, A.M. and HUNTER, T. (1988). The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science. 241: 42–52.

HORRIDGE, G.A. (1987). The evolution of visual processing and the construction of seeing systems. Proc. R. Soc. Lond. B230: 279–92.

INTERNATIONAL ATOMIC ENERGY AGENCY. (1985). Application of genetic engineering and recombinant DNA technology in the development of genetic sexing mechanisms for the Mediterranean Fruit Fly, *Ceratitis capitata* (Wied.) (Rep. Con–sultants Mtg Vienna, 1985), IAEA, Vienna, pp. 1–18

JEANNEL, R. (1949). Les Insectes. Classification et phylogenie les insectes fossiles. Evolution et geonemie, pp. 1–110. In P.–P. Grasse (ed.), Traité de Zoologie: Anatomie, Systematique, Biologie. Tome IX. Insectes: Paleontologie, Géonemie, Apterygotes, Ephemeroptères, Odontatoptères, Blattopteroides, Orthopteroides, Dermapteroides, Coleoptères, pp. 1–110. (Paris: Masson).

KENNEDY, J.S., (1986). Animal Motivation. 1–19 Landbouwuniversiteit Wageningen.

KERNFELD, K., SAINT, R. BEACHY, P., HARTE, P., PEATTIE, D. and HOGNESS, D. (in press). Alternative splicing generates a family of ultra-thorax mRNA's and proteins in *Drosophila*. Genes & Development.

KLUG, A. and RHODES, D. (1987). 'Zinc Fingers': A novel protein motif for nucleic acid recognition. TIBS 12 – December. pp. 464–469.

KRISTENSEN, N.P. (in press). Phylogeny of extant hexapods, In Insects of Australia.

LEWIN, R. (1988). DNA clock conflict continues. Research News. Science 241: 1756–1759

LUCKLOW, V.A. and SUMMERS, M.D. (1988). Trends in the development of baculovirus expression vectors. Biotechnology. 6: 47–55.

McKEOWN, M., BELOTE, J.M. and BOGGS, R.T. (1988). Ectopic expression of the female transformer gene product leads to female differentiation of chromosomally male *Drosophila*. Cell. 53: 887–895.

MERRITT, D.J. (in press). The morphology of the phallosome and accessory gland material transfer during copulation in the blowfly *Lucilia cuprina* (Insecta, Diptera). Zoomorphology.

MILLER, C.A., and BENZER, S. (1983). Monoclonal antibody cross-reactions between Drosophila and human brain. Proc. Nat. Acad. Sci. USA. 80:7641–5.

MONSMA, S.A. and WOLFNER, M.F. (1988). Structure and expression of a Drosophila male accessory gland gene whose product resembles a peptide pheromone precursor. Genes & Development. 2: 1063–1073.

NAGORCKA B.N., (1988). A pattern formation mechanism to control spatial organisation in the embryo of *Drosophila melanogaster*. J. Theor. Biol. 132:277–306.

NAGORCKA B.N., (in press). Wavelike isomorphic prepatterns in development. J. Theor. Biol.

O'SHEA, M. (1985). Neuropeptides in Insects: possible leads to new control methods, ppo. 133–151. In C.H. von Keyserling, A. Jager and Ch. von Szczepanski (eds.), Approaches to new leads for insecticides. Springer Berlin.

ROUSH, R.T. and McKENZIE, J.A. (1987). Ecological genetics of insecticide and Acaricide resistance. Ann. Rev. Entomol. 32: 361–80.

RUBIN, G.M. and SPRADLING, A.C., (1982). Genetic transformation of *Drosophila* with transposable element vectors. Science. 218: 348–353.

RUSSELL, R.J., DUMANCIC, M.M., FOSTER, G.G., WELLER, G.L., HEALY, M.J. and OAKESHOTT, J.G. (in press). Insecticide resistance as a model system for studying molecular evolution. Evolutionary Biology.

SAINT, R., KALIONIS, B., LOCKETT., T.J., and ELIZUR A. (1988). Pattern formation in the de-veloping eye of *Drosophila melanogaster* is regulated by the homoeo-box gene, rough. Nature. 334(6178): 151–154.

SANDFORD, J.C. and JOHNSTONE, S.A. (1985). The concept of parasite-derived resistance-deriving resistance genes from the parasite's own genome. J. theor. Biol. 113: 395–405.

SCHARRER, B. (1987). Insects as models in neuro-endrocine research. Ann. Rev. Entomol. 32: 1–16.

SCHODDE, R. and CHRISTIDIS, L. (in press). Origin and evolution of the Australian bird fauna – the modern perspective, pp. .. . In D. Shaw The Australian Biota: contemporary studies in evolution, biogeography and systematics. Canberra: Australian Institute of Biology.

SHAW, D.D., COATES, D.J. and ARNOLD, M.L. (1988). Complex patterns of chromosomal variation along a latitudinal cline in S.E. Australia. Chromosoma. 30: 108–117.

SIBLEY, C.G. and AHLQUIST, J.F. (1987). Avian phylogeny reconstructed from comparisons of the genetic material, DNA, pp. 95–122, In C. Patterson (ed.), Molecules and Morphology in Evolution: Conflict or Compromise, (Cambridge: Cambridge Univ. Press).

SIMON, C. (1988). Evolution of 13– and 17–year periodical cicadas (Homoptera: Cicadidae: Magicicidae). Bull. Entomol. Soc. Amer. 34(4): 163–176.

SMITH, P.H., BARTON BROWNE, L. and van GERWEN, A.C.M. (in press). Causes and correlsates of loss and recovery of sexual receptivity in females of *Lucilia cuprina* after their first mating. Journal Insect Behavior.

SNYDER, A.W. (1979). The physics of vision in compound eyes, Vol. 7.6AO, pp. 222–309. In Handbook of Sensory Physiology, Comparative Physiology & Evolution of Vision in Invertebrates.

THOMSON, J.A. (1987). Evolution of gene structure in relation to function, p. 314. In K.S.W. Campbell and M.F. Day (eds), Rates of evolution, (London: Allen & Unwin).

TUBLITZ, N.J., COPENHAUER, P.F., TAGHERT, P.H. and TRUMAN, J.W. (1986). Peptidergic regulation of behaviour: An identified neuron approach. TINS – August. pp. 359–63.

WALKER, A.R., HOWELLS, A.J. and TEARLE, R. (1986). Cloning and characterisation of the vermilion gene of *D. melanogaster*. Mol. Gen. Genet. 202: 102–7.

WALKER, V.K., (in press). Gene transfer in insects, Vol. 7. In K. Maramorosch and G.H. Sato (eds) Advances in Cell Culture.

WHEELER, W.C. (in press). The hierarchy of life, pp. .... In Bo Fernholm, Kare Bremer, Hans Jornvall (eds.), Molecules and morphology in phylogenetic analysis. Proceedings of Novel Symposium 70. Elseviers Science Publisher B.V. Amsterdam.

WHITTEN, M.J. (1985). The conceptual basis for genetic control, pp. 465–528. In G.A. Kerkut and L.I. Gilbert (eds), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol.12. Pergamon Press.

WHITTEN, M.J. and McKENZIE, J.A., (1982). The genetic basis for pesticide resistance, pp. 1–16. In K.E. Lee (ed), Proc. 3rd Australasian Conf. Grassl. Invert. Ecol., Adelaide, SA Gov. Printer, Australia.