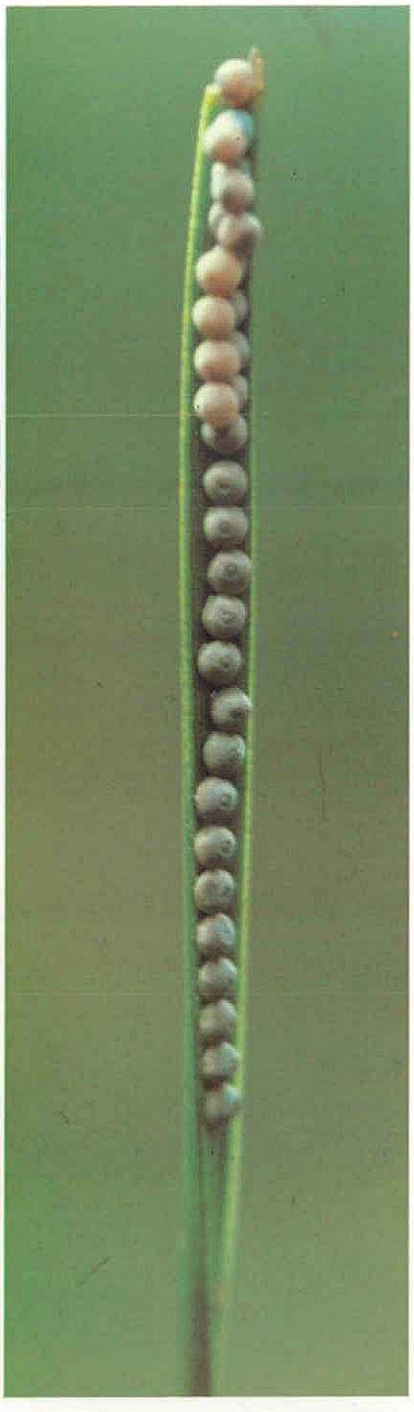
1991 — 1992

REPORT



Institute of Virology and Environmental Microbiology

Natural Environment Research Council

Foreword

The year covered by this report has proved both challenging and fruitful for the Terrestrial and Freshwater Sciences Directorate (TFSD). Implementation of NERC's strategy for the terrestrial and freshwater sciences (*The Green Light*, published in March 1989) is providing a focus for our research in the areas of land use, the maintenance of environmental quality, and the principles which underline environmental management and conservation. Initiatives started in previous years, in particular the Terrestrial Initiative in Global Environmental Research (TIGER), are now well underway. The Environmental Change Network and the Land Ocean Interaction Study are now moving forward as developing programmes, and new ideas are in the planning stage. Increasingly, programmes have been established jointly with other Research Councils so that a much broader approach to scientific problems is possible, and with larger funding.

The Directorate's expertise in the Institute of Freshwater Ecology, the Institute of Hydrology, the Institute of Terrestrial Ecology, the Institute of Virology and Environmental Microbiology, the Centre for Population Biology (Imperial College, London), the Unit of Behavioural Ecology (Oxford University) and the Unit of Comparative Plant Ecology (Sheffield University) provides an unrivalled, interdisciplinary research base with international reputation.

This is the last year in which I shall be writing the Foreword to the Directorates' annual reports. It has been a source of great satisfaction to be associated with the development of the Institute of Virology and Environmental Microbiology (IVEM) as a centre of international repute for research on viruses and other microorganisms. As this report shows, IVEM is at the forefront of research on molecular virology, on the development of microorganisms for applications in agriculture, medicine and veterinary medicine, and on the role of microorganisms in population and community ecology.

Dr P B Tinker
Director of Terrestrial and Freshwater Sciences
Natural Environment Research Council

Front Cover Illustration
Eggs of Panolis flammea *on needles of lodgepole pine.*

Report of the Institute of Virology & Environmental Microbiology 1991/92

Natural Environment Research Council

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Director's Statement

The Institute of Virology and Environmental Microbiology is a component of the Natural Environment Research Council (NERC), an organisation funded by an annual grant from the UK government to support and undertake research in the sciences relating to man's environment.

The mission of the Institute is the study of viruses and other microbes in the natural environment primarily in the following areas: their relationships with vertebrates, invertebrates and plants; their roles in the natural environment including their replication, transmission, vectors, hosts and ecology; the generation and behaviour of genetically modified organisms in the environment, the risks and benefits of their release, together with appropriate aspects of the molecular biology, evolution and genetics of microbes; and the identification, prevention and spread of diseases caused by microbes, primarily in species other than man. The Institute carries out strategic and basic research in these subjects, and also provides a national resource of expertise for sponsored research and training of high quality. It aims to build its sponsored research on the foundation of a first class scientific reputation and to play a major part in the national and international scientific community.

Prior to 1980 the staff were members of Oxford University and known as the Insect Pathology Unit (1963-1971), then the Unit of Invertebrate Virology (1971-1980) when support was provided by NERC. In 1980 the Institute of Virology was founded. Prior to 1984 the research was under the direction of Dr T W Tinsley MA, DSc, Fl Biol, in conjunction with Mr J S Robertson BA. Since 1984 the Director has been Professor D H. L Bishop MA, DSc, F Biol. In 1989 the name and remit of the Institute was expanded to the Institute of Virology and Environmental Microbiology (IVEM) as the expertise of the staff increased to include research into other aspects of environmental microbiology.

The Institute of Virology and Environmental Microbiology is housed in a custom-built facility on a site allocated to NERC *pro tempore* by the University of Oxford. The Institute is in the heart of the University's Science Area. The arrangement allows direct links to be maintained between staff of the Institute and those of several Departments of the University. These include the Biochemistry, Zoology and Plant Sciences Departments, the Sir William Dunn School of Pathology, the Dyson Perrins Laboratory and the Laboratory of Molecular Biophysics. Almost all the project leaders of the Institute, together with their University or Oxford Polytechnic partners, undertake the supervision of graduate students. Staff of the Institute maintain close links with the Oxford University Inter-disciplinary Research Centre (IRC) in Molecular Sciences. 'I'his report, a synopsis of the research areas and achievements in the ten programmes of the Institute, covers research performed over the last year on insects, plant, vertebrate and invertebrate viruses and studies on bacteria associated with plants. Details of the results will be found in the publication record.

David H L Bishop

Biocontrol and Ecology

This group is concerned with investigating the relationship between insects and their pathogens and in the development of insect viruses as pest control agents.

Pest Control

Baculovirus control of cutworms

Cutworms (the larvae of several species of noctuid moth) are pests of a wide range of crops worldwide. They spend much of their time in the soil and can be difficult to control. Baculoviruses (BVs) offer a possible control option. We have been studying their potential using the Common cutworm, *Agrotis segetum*, as a model species. Two types of BV, a nuclear polyhedrosis virus (NPV) and a granulosis virus (GV), have been isolated from *A. segetum*, but until recently only the GV had been investigated in any detail. Our laboratory studies demonstrated that the NPV was highly infectious for *A. segetum* and in the past year we have extended this work to compare the two BVs in field tests. The first trial took place on maize in southern Spain in collaboration with the University of Córdoba and compared each virus in both a spray and a bait form. Results showed that although both BVs caused high levels of cutworm mortality, the NPV acted significantly faster. In the UK a further bait trial on beet-root confirmed these results and also showed that mixing the two viruses gave a speed of kill in between that of either virus used alone. Faster speed of action should result in reduced crop damage and thus the NPV is likely to be the better control agent.

Ecology

Ecology of the Vapourer Moth: nuclear polyhedrosis virus interaction

Many aspects of insect-virus ecology are poorly understood, however, the application of modern molecular techniques offers one possibility for gaining a greater insight into this interaction. This has been attempted in the study of a common UK insect, the Vapourer Moth, *Orgyia antiqua*, and its NPV. The approach has been twofold; first, established, field-based methods have been used to study insect population dynamics, distribution and behaviour and second, a microbiological component has targeted the NPV and its distribution in the host population and environment. This second approach has been made possible by utilizing, for example, restriction enzyme analysis, development of DNA specific probes and the polymerase chain reaction (PCR). PCR is potentially a very powerful tool since it allows the accurate identification of extremely small quantities of virus DNA. Currently, the distribution of virus within the insect's environment is being assessed using PCR on insect, plant and soil samples from several forest sites. Larval behaviour and distribution varies according to instar. This is important epizootiologically since preliminary results indicate that virus distribution also varies with the age and position of foliage and will influence the amount of inoculum available to the larvae.

Risk Assessment

Genetically modified baculoviruses:

Earlier studies emphasized the importance of understanding the ecology of the engineered organism before release. Current studies are looking at the behaviour of the engineered baculoviruses (BVs) in the insects, both singly and in combination with other BVs, in order to assess the potential for survival of the introduced genes within the insect population.

Transgenic poplars:

Poplars are being produced which express the ∂-endotoxin of the bacterium *Bacillus thuringiensis*. This toxin is specific for particular insect groups and thus could be used as a means of controlling the more concealed pest species, such as wood borers and leaf miners. Surveys of the insect fauna on poplar and their sensitivity to *B. thuringiensis* toxins are currently being carried out as a basis for more detailed assessment of the possible consequences of this control strategy.

Iridescent Viruses and Blackflies

Blackflies *(Simulium* spp.) have aquatic larval and pupal stages and are often abundant in fast flowing stretches of rivers, where they filter feed organic matter from the water Following emergence, the adult female flies seek a blood meal before returning to the river to lay their eggs. Several species of blackfly are capable of transmitting diseases (filarial worms) to man and animals during bouts of blood feeding and thus blackflies can attain pest status on medical, economic and social grounds. Work at IVEM has focussed on an iridescent virus (IV) pathogen of blackfliey (designated IV22) from two separate. but complementary, approaches. First, the ecology of the host virus relationship is being studied in blackflies populations in the River Ystwyth, mid Wales, and second, the molecular biology of IV22 is being investigated with the aim of producing a virus containing a small inert genetic marker sequence for use in field studies.

Ecology of blackfly iridescent viruses

Indescent viruses are non-occluded viruses which lack the protective protein coat characteristic of most baculoviruses. As a result, IV infections are often restricted to hosts inhabiting moist environments such as water, soil, vegetation etc. IV infections of blackfly larvae are geographically widespread. Patent infections give the larvae an indescent blue hue and are invariably lethal, but this overt form of the disease appears infrequently in blackfly populations.

A viral infection may not always manifest itself. However. An alternative but equally effective strategy of replication may be to remain in a covert form and exploit vertical transmisson routes via infection of the host offspring. Indeed, several iridovirus isolates from populations of simuliid larvae in mid-Wales have been identified infecting large numbers of apparently healthy larvae without the characteristic symptoms of disease. When patent infections of larvae have been discovered and analysed using restiction enzymes which cleave the viral DNA into characteristic bands, each isolate appears subtly different from any other. The adaptive significance of such variation remains unknown. Consequently. the nature of the host virus relationship and aspects of the ecology of the system are now being investigated using molecular techniques. The polymerase chain reaction should prove valuable in quantifying the frequency and distribution of covert infections with respect to spatial, temporal and age or stage related factors in these populations. In addition, IVs from a number of different host taxa have been shown to be capable of causing patent infections in laboratory-reared simuliid larvae. indicating that they may not be highly host specific.

Molecular biology of IV22

Molecular studies have built on previous work carried out at IVEM locating and sequencing the gene coding for the major structural protein (MSP) of IV22. About 40% of the virus particle consists of the MSP and this protein can be expected to have a major influence on the transmission and infection characterisics of the virus. The promoter region for the MSP gene is now being characterized using a reporter gene (chloramphenicol transferase gene) in a cell culture based transient expression system. The product of this gene has the ability to cause colour changes in a specific substrate, allowing quantitative analysis of gene promoter function. By successive deletion of DNA sequences at either end of the promoter, the essential regions for promoter function can be identified. Preliminary results have indicated that the promoter region is considerably larger than in related viruses and that it may only function if present in its entirety. When the functioning of this gene and its promoter are fully understood, an area in the genome will be defined in which an inert marker sequence can be safely inserted without altering the function or stability of the virus. A marked virus such as this would be of great value in field studies, allowing elucidation of the replicative, persistence and transmission strategies of IV22 within blackfly populations.

Molecular Biology of Baculoviruses

Baculoviruses are being studied, using genetic engineering techniques, for use as insect control agents.

Field trials with genetically engineered baculovirus insecticides

A gene encoding an insect-specific scorpion toxin was inserted into the genome of *Autographa californica* nuclear polyhedrosis virus (AcNPV). In laboratory studies it has been shown that production of the toxin in virus-infected caterpillars reduces feeding damage by the insect on cabbage plants in comparisons with the unmodified AcNPV. A formal application to the UK Health and Safety Executive for permission to test this virus in a small scale field trial has been completed. This trial will be conducted in the summer of 1992.

Selection of recombinant baculoviruses with 100% efficiency

The baculovirus expression vector system is a powerful tool for the production of foreign proteins in insect cells. The selection of baculovirus recombinants containing a foreign gene in lieu of a region of the virus genome is a difficult process. In a method reported previously we described how linearization of circular AcNPV DNA with a restriction enzyme improved the recovery of recombinants to an efficiency of 25-30%. Recent developments have now raised the efficiency to nearly 100%. This has been achieved by inserting other unique restriction enzyme sites within a virus gene essential for replication in insect cells. Digestion of the modified virus DNA removes the vital function and prevents replication. The crippled virus may be rescued, however, by recombination with a plasmid vector containing the foreign gene of interest ***and*** a complete copy of the essential virus gene. The selection system is so effective that it has been brought to the market by a biotechnology company and is now marketed worldwide very successfully.

Baculovirus molecular genetics

Underpinning the molecular genetic studies to modify baculovirus insecticides and develop novel expression vectors is a detailed analysis of the primary genetic structure of AcNPV. This has involved sequencing the complete 130,000 nucleotides of the virus genome and using the data to identify virus genes via comparisons with existing DNA sequence libraries (GENBANK and EMBL). The information now available has produced some exciting results. For example, a chitinase gene has been identified within the virus DNA. This gene produces high levels of functional enzyme. Its role in virus pathogenicity is unclear, but it may aid the virus in its passage across the gut barrier. Virus deletions mutants are being constructed to investigate its function. This gene also has very high similarity with a bacterial chitinase gene; the match is so close that it suggests that AcNPV acquired the gene from *Serratia marcescens* (sometimes part of the caterpillar gut flora) in a recent evolutionary event. This poses many new questions concerning the flow of genetic information between different species.

Latent baculovirus infections

Baculovirus infections normally result in the death of the insect larvae at the end of a very aggressive virus replication cycle. There have been some examples, however, where viruses have been found to grow in insect cells in culture but do not result in cell death. The virus maintains itself at a low level, or in a 'latent' state, which apparently does not harm the host cell. This phenomenon is not well documented in the insect. Recent studies in Oxford have identified an insect population *(Mamestra brassicae)* which contains a baculovirus in the latent state. Control insect populations of the same species do not have the virus infection. The virus DNA is detectable in the infected population using the sensitive polymerase chain reaction method to amplify virus DNA sequences. The virus may be activated by a super-infecting virus which presumably provides a 'helper' function to trigger replication. This system offers the chance to study how.

Flavivirus Research

The tick-borne flaviviruses are found in the United Kingdom, most of Europe, the CIS (formerly USSR), India, Japan, the USA and Australasia. They may produce encephalitis, haemorrhagic fever and/or an incapacitating febrile illness in humans and many species of vertebrate wildlife. In view of the predicted changing climatic patterns worldwide, there is a possibility that these viruses could be redistributed either because the tick populations establish in new regions or because of changing patterns of land usage resulting from the altered climate. Such redistribution might lead to epidemic/epizootic outbreaks of disease in areas that previously did not experience significant problems with these viruses. Analysis of their genetic and antigenic relationships using molecular probes will provide a database for identification of gene pool movements/ inter-changes etc. This technology is also being used to develop vaccines and diagnostic reagents.

Migratory birds may cause the spread of pathogenic viruses

Monoclonal antibodies have been prepared and characterised at the Institute. They have been used to show that a tick-borne flavivirus associated with seabirds (Meaban virus - off the North West Coast of France) is very closely related to louping ill virus, a disease of sheep, grouse and rodents that inhabit upland grazing areas in the UK. The envelope gene of each virus was sequenced. They were identical in the two viruses implying that they have exchanged genetic material at some time during their evolution. This observation opens up a completely new dimension in our concepts of these viruses. Similar procedures were then used to analyse a pathogenic flavivirus that fatally infected two children in Japan. The monoclonal anti-bodies and the genetic sequencing techniques demonstrated that this virus was absolutely identical to louping ill virus. It is most unlikely that the Japanese virus evolved completely independently of louping ill virus. It is therefore suggested that louping ill virus was carried to Japan by a bird which migrated from the United Kingdom.

The development of anti-idiotypic antibodies

The preparation and purification of some virus-coded antigens (proteins) can be difficult and in many cases the methods employed may damage the required protein. One method of overcoming this problem is to prepare anti-idiotypic antibodies (Anti-ids), which are the mirror image of antibodies (Abs) and represent the original antigen. Thus anti-ids can be used to mimic the antigens. Monoclonal anti-ids have been prepared against a neutralising monoclonal antibody that identifies tick-borne encephalitis virus. One of these anti-ids behaves functionally in the same way as the antigen that it mimics and is therefore likely to be useful as a diagnostic agent and a potential vaccine

Development of infectious clones of tick-borne flaviviruses

The entire genomes of two tick-borne flaviviruses are being sequenced in order to prepare infectious clones. These clones will be used to study virus-vector relationships at the molecular level. The definition of virus virulence and the development of safe, effective live virus vaccines will become realisable objectives.

Long term survival of louping ill virus in the environment

Louping ill virus is maintained in an epidemic cycle involving sheep and ticks but it is known that other animals, particularly feral birds and rodents become infected with louping ill virus. How the virus survives when amplification hosts such as sheep are not available needs to be investigated. It is possible that the virus can pass from generation to generation in the tick population. Birds and rodents may develop low level persistent infections. These concepts are being analysed.

Tick-borne Pathogens

Saliva-activated transmission of tick-borne encephalitis virus Despite a century of research on diseases transmitted by arthropod vectors, the role of vector saliva in disease transmission has only recently been studied. Using Thogoto virus, a tick-transmitted virus related to the influenza viruses, we demonstrated that the saliva of a competent tick vector enhances virus transmission from infected to uninfected ticks as they feed together. This phenomenon was named 'saliva-activated transmission' or SAT. To determine if SAT is widespread, studies were undertaken with tick-borne encephalitis (TBE) virus, the most important arthopod-borne virus in Europe. Again, the feeding behaviour of ticks, related to salivation, enhanced virus transfer. Interestingly, SAT of TBE virus was also observed with *Ixodes ricinus*, the common sheep tick and competent vector of TBE virus; this tick species did not demonstrate SAT with Thogoto virus, for which it is not a vector. Thus, not only is there an intriguing correlation belween vector competence and SAT, but there also appears to be more than one SAT factor.

Evolutionary relationships between tick- and insect-transmitted orbiviruses

Previous research revealed significant differences in the composition of the outer surface layer of tick-transmitted orbiviruses that circulate in UK seabird colonies, and the gnat-transmitted orbiviruses represented by bluetongue virus (BTV). Extension of this comparison to the inner core structure confirmed that there is considerable evolutionary divergence between these two groups. In particular, the amino terminus of VP7, the major core protein, differed markedly from the equivalent region in VP7 of BTV and African horse sickness virus. This region is thought to interact with the surface layer of the virus particle. Such differences are consistent with the hypothesis that arthropod vectors play a significant role in the evolution of the viruses that they transmit.

Evolutionary relationships between tick-transmitted nairoviruses

The nairovirus group of tick-transmitted viruses contains the important human pathogen, Crimean-Congo haemorrhagic fever (CCHF) virus. To determine the relationships between CCHF virus and related viruses, the small RNA segment, encoding the nucleoprotein, was sequenced in collaboration with CDC Atlanta. The S segment of Hazara virus, the only other member of the CCHF virus serogroup but non-pathogenic for humans, was also examined. Comparison of the sequence data with those previously published for Dugbe nairovirus revealed a common evolutionary pathway, although Dugbe virus possesses a truncated nucleoprotein gene. These results are being used to develop reagents for the specific diagnosis of CCHF virus infections.

Distribution of Lyme disease in the UK

In the UK the zoonotic borreliosis, Lyme disease, is transmitted between wildlife species by the sheep tick, *Ixodes ricinus*. The aetiological agent, *Borrelia burgdorferi*, can be detected in infected ticks using the polymerase chain reaction (PCR). In collaboration with ITE Banchory, our distribution map of *B. burgdorferi*- infected ticks now covers 15 PCR-positive sites: 7 in Scotland, 3 in northern England, and 5 in the south. The Lyme disease spirochaete appears to occur wherever its tick vector is common.

Bluetongue Orbivirus Research

Three different orbiviruses are under study at Oxford, Bluetongue virus (BTV) of sheep, epizootic haemorrhagic disease (EHD) virus of deer and African horse sickness (AHS) virus.

EHDV-I and AHSV-4 capsid structures

The complete nucleotide sequence and predicted amino acid sequences of four major capsid proteins of African horse sickness virus (serotype 4, AHSV-4) and epizootic haemorrhagic disease virus of deer have been determined and compared with those of Bluetongue virus of sheep, and their phylogenetic relationships established. The comparison revealed that of the four capsid proteins the innermost protein, VP3 is the most conserved (57-58%), and the outermost protein, VP2, is the most variable (74-79%), although in tertiary structures all VP2 proteins appear to be similar. Both VP7 and VP5 proteins are reasonably (44-50%) homologous.

Assembly of seven structural proteins of BTV and heterologous orbivirus proteins in insect cells

The process of virus assembly is critical to the success of an infection. Therefore, we have constructed baculovirus vectors which have the capability of expressing up to four proteins simultaneously. Using combinations of multiple protein expression vectors, complete virus like particles(VLPs) consisting of seven viral structural proteins have been assembled into virus-like particles in the absence of viral dsRNA genome.

Using similar methods it has been demonstrated that the capsid proteins representing BTV and EHDV can be interchanged to produce heterologous particles. The results indicate that the functional regions of EHDV and BTV VP3 proteins, involved in interacting with each of the minor proteins are conserved. The efficacy of assembled VLPs as vaccines has been tested in sheep in collaboration with scientists in Onderstepoort (South Africa) and Townsville (Australia). The data conclusively demonstrate that VLPs are highly immunogenic even when given at low concentrations (l0µg).

Presentation of foreign epitopes on BTV CLPs

The high level production of CLPs and VLPs from baculovirus vectors has been adopted as a carrier system for the insertion of foreign antigens. Chimeras based on BTV VLPs containing immunogenic protein sequences from other pathogenic viruses (e.g., rabies virus glycoproteins (G), hepatitis B virus pre-S2 region, HV and SIV *gag* and *env* proteins etc) have been successfully constructed. Preliminary studies indicate that these chimeric CLPs and VLPs are highly immunogenic. The data demonstrate that these particles will be efficient multiple vaccine delivery systems.

The data obtained from the molecular studies on BTV and 3-dimensional structural studies could now permit manipulative experiments to be undertaken on the role of BTV proteins in virus replication and morphogenesis. The studies should lead to a more detailed molecular understanding of the various protein-protein interactions that contribute to the BTV virion self-assembly process. The fact that morphological structures can be identified when the BTV structural proteins are co-expressed from recombinant baculoviruses opens up new possibilities for research into the determinants of intermolecular (protein-protein) interactions.

Cryo-electron Microscopy

Electron microscopy (EM) is a useful tool for determination of the structure of viruses. The most commonly used specimen preparation technique has been negative staining. Unfortunately, this procedure may cause alteration of the specimen by dehydration and chemical modification. Such artefacts make structural analysis and use of three dimensional reconstruction techniques impossible, and interpretation of the images generally difficult.

Recent developments in cryo-electron microscopic techniques allow ultra rapid freezing of biological macromolecules in a thin layer of vitreous ice. From this, an accurate three-dimensional reconstruction can be made using computer-aided image processing. Cryo-EM is also ideal for investigating interactions between macro-molecules over a short time scale, as the specimen is trapped rapidly and can then be observed directly. Such structures are beyond the capabilities of a crystallographic approach due to intrinsic technical problems.

Structure of Bluetongue virus (BTV)

A collaboration involving IVEM, the Department of Molecular Biophysics, Oxford University and the Institut de Biologie Structurale, Grenoble, has been recently initiated to apply the techniques of cryo-electron microscopy and image processing to the study of virus structures. These studies aim to answer the question of how architecturally complex viruses are synthesized and how they function during a virus infection. The procedure by which encapsidation of the 10 segments of double stranded RNA within the two shells of proteins in BTV is achieved, and how the minor proteins function and are physically associated is being investigated. We have determined the three dimensional structure of double shelled and single shelled virus particles to 35 Å resolution. We have also compared the structure of authentic particles, made in BTV infected mammalian cells, with synthetic virus-like structures made by infection of insect cells, using different combinations of recombinant baculoviruses expressing BTV proteins.

Structure of Bluetongue virus tubules

The NS1 protein forms tubules of about 52 nm in diameter. Cryo-EM and image processing has shown that the tubule structure consists of a one-start helical ribbon of NS1 dimers. A variation in tubule diameter was encountered: the two different surface lattice conformations were shown to be pH dependent.

Our results thus far have revealed small but significant anomolies in the core structure of the synthetic particles. We have also explained how the subcore structures are assembled and have identified the structure and positioning of the outer shell haemagglutinin protein on the BTV virus particle. Future developments await the installation of cryo-EM facilities on site in Oxford.

Plant Virology

The Comoviridae is a family of plant damaging viruses which includes genera that are transmitted by beetles (comoviruses), by nematodes (nepoviruses) or by aphids (fabaviruses). The molecular properties of three species of nepovirus and one fabavirus are being studied in an attempt to understand the determinants of virus-vector specificity and disease capacity.

Some nepoviruses encapsidate extragenomic RNA species known as satellite RNA which have been analysed to elucidate features of their replication. The nucleotide sequence in the satellite RNA from a strawberry isolate of strawberry leaf roll virus (SLRV) contained one long open reading frame encoding a polypeptide of 36K molecular weight but comparisons with other satellite RNA sequences showed virtually no homologies: a satellite RNA of similar size and structure (from a lilac isolate of arabis mosaic virus; ArMV) contained an ORF with a theoretical coding capacity of 38849. Biologically active transcripts of the ArMV satellite RNA were produced from a DNA clone and function was analysed by reverse genetics. These tests showed that the protein is essential for replication (provided an appropriate genome-coded function is also available).

Genomic RNA from viruses in the Comoviridae is translated into a polyprotein that is subsequently cleaved by virus-coded proteases; the capsid protein is coded in the RNA-2. ArMV and CLRV have one capsid protein (55K or 52K respectively) whereas SLRV resembles fabaviruses in having two capsid proteins totalling 72K. The complete sequence of bases in the RNA-2 of SLRV was determined and Edman degradation was used to identify the N-terminal amino acid sequences in both capsid proteins. Thereafter, each capsid coding region was modified to create methionine initiation codons and each construct was introduced into baculovirus transfected insect cells (to facilitate the production of sequence-specific antisera). In parallel, the separate capsid components were expressed in *Agrobacterium*-transformed *Nicotiana tabacum*. The appropriate proteins were synthesised and transgenic plants expressing either the small or the large capsid subunit resisted mechanical challenge by SLRV virions. Hitherto, capsid-derived transgenic resistance in plants has been reported only when the complete capsid proteins have been expressed.

The virus species, CLRV, comprises many serological variants which naturally infect trees. Because CLRV spreads to a significant extent in pollen to infect seed, epiphytolics are extremely difficult to manage - except by use of genetically resistant stock. To assess options for using virus-derived resistance against CLRV, the genomic RNA of two serologically distinct isolates (from birch or rhubarb) have now been cloned and sequenced. Sequence homologies have been identified and evidence for genetic recombination between these viruses was obtained.

Currently, poplars in the UK yield £600/ha and there are 13,500 ha of poplars in England and Wales; the planting of poplars is targeted to expand at a rate of 1000 ha/year. Poplar mosaic virus (PMV), which is prevalent in poplar germplasm and ubiquitous in many commercial clones, diminishes growth by 30-40%. The virus spreads by unknown means and no source of 'traditional' resistance is conveniently available to breeders. Consequently, we are assessing options for using virus-derived defence proteins in transgenic plants.

Molecular Virology

The research undertaken by the molecular virology group concerns understanding the molecular events involved in the infection courses of viruses. The particular questions that are under investigation concern defining the coding and, or morphogenetic strategies of a representative positive sense RNA virus (poliovirus, a member of the Picornaviridae), a negative sense RNA virus (rabies, a member of the Rhabdoviridae) and an ambisense RNA virus (Rift valley fever virus, a member of the Bunyaviridae).

Completion of the sequence of the tripartite RNA genome of a candidate vaccine strain (MP12) of Rift valley fever virus (RVFV)

The entire sequence of the RNA genome of the MP12 derivative of RVFV has been obtained. The RVFV small (S) RNA species has an ambisense coding strategy. It is 1690 nucleotides long and codes for the 27.4 kDa viral nucleoprotein in its 3' half and a 29.9 kDa non-structural protein in its 5' half. The intergenic region of the viral S RNA is rich in polyguanylic tracks, 4 to 6 residues in length. The middle (M) RNA is 3885 nucleotides long, negative sense and codes for a 130.8 kDa precursor to the viral glycoproteins (G1: 65 kDa, G2: 56 kDa) and other non-structural proteins. The large (L) RNA is 6606 nucleotides long, also negative sense and codes for a 243.6 kDa protein that exhibits sequence similarities to the RNA polymerases of other viruses.

Morphogenetic studies of a positive sense RNA virus

Previous studies demonstrated that expression in insect cells of the entire coding region of poliovirus type 3 cDNA in a baculovirus vector led to the recovery of immunogenic, but non-infectious, poliovirus-like particles that were devoid of nucleic acids. The data indicated that the P1 coding region of poliovirus was processed in insect cells by the viral coded proteases to yield the VP0, VP2 and VP3 proteins. These self-assembled into virus particles. To complete the studies, the individual VP0, VP1 and VP3 genes of poliovirus have been expressed using single and multiple gene baculovirus expression vectors. The results demonstrate that the separate proteins assembled into virions, supporting the hypothesis that assembly does not require P1 intermediate.

Morphogenetic analyses of a negative strand virus, rabies

The genes coding for the four major structural proteins of the CVS strain of rabies virus, namely N, M1, M2 and G, have been cloned, sequenced and expressed using baculovirus vectors. As shown previously, G elicits protection against virulent virus challenge and is a candidate subunit vaccine. N expression resulted in the formation of doughnut-shaped particles. However, when expressed together with M1, N was recovered in complexes of N and M1, indicating that the proteins interact in the absence of viral RNA and other viral-coded components. Crystallographic analyses of M1, free or complexed with N, are underway.

Virus Replication

Individual proteins of the human immunodeficiency virus (HIV) and the simian immunodeficiency virus (SIV) are being expressed in the recombinant baculovirus and the biological activity of the expressed proteins is being characterised. There are three areas of research activity.

Structure and function of the major core antigen of HIV, p55gag

In the study for p55 core antigen, the amino acid sequences required for the assembly of the HIV particle have been localised to 8 amino acids at the junction of the p24 and p15 domains of p55. Lack of this sequence in the expressed protein results in extensive surface distortion in the infected cell, and no HIV particles which would be present in cells infected with the wild type virus sequence. Inclusion of the sequence at the terminal boundary of the gag expressed protein restores particle formation. The sequences responsible for RNA capture by the forming particle, long thought to be intimately linked with particle assembly, have been shown not to be formally necessary although it is possible they act as co-factors in the process. Particles formed in the absence of the RNA capture signals are less uniform in size and exhibit altered sedimentation properties when compared to the unmutated sequence. HIV particles formed in this system are non-infectious and may be as non-replicating vaccine candidates.

Structure and function of the major surface glycoprotein gp120 of HIV

For studies on the viral surface glycoprotein, many mutants of gp120 have been made which abolish bioactivity (binding to the cell surface marker CD4). We have observed that CD4 binding can be rescued by the co-expression of a number of mutant pairs in the same cell showing that gp120 function can be contemplated *in trans*. By aligning the sequences shown to be active *in trans* complementation, a minimum region of gp120 required for CD4 binding can be derived. Such structural studies on isolated domains in gp120, although not representative of the full length active molecule, may provide data that can be usefully employed in understanding the role of the protein during virus infection.

Production and characterisation of the major surface glycoprotein of simian immunodeficiency virus (SIV) as a candidate vaccine for SIV, the animal model of AIDS

SIV gp120 has been expressed and characterised by bioactivity and monoclonal antibody binding. The molecular has been produced in bulk and purified using an immune affinity column. Small animal immunogenicity trials have begun prior to testing this gp120 protein for its capacity to immunise macaques.

Molecular Microbial Ecology

The Molecular Microbial Ecology group at IVEM is engaged in a detailed analysis of the temporal and spatial distribution of the microbial community on the plant surface with a view to understanding the factors that drive colonisation, survival, dispersal, succession and adaptation of microorganisms to this environment. A major objective is to be able to predict the consequence of inoculating genetically modified microorganisms (GMOs) into the habitat. Knowledge of how component microbial populations of root and leaf habitats can be influenced by and adapt to changes in environmental conditions (competition, climatic, tissue type, physiology of the plants etc) will allow the design of vectors with potential for targeted crop protection or bioremediation.

Phytosphere Microbiology

Detailed compositional surveys of the bacterial, filamentous fungi and yeast communities colonising the leaf (phyllosphere) and root (rhizosphere) of field grown sugar beet have been undertaken.

Phyllosphere: the distribution of microorganisms on the plant surface has been investigated over two consecutive seasons. *Cladisporium* and *Cryptococcus* were the most commonly isolated fungi. *Pseudomonads sp.* were the most common bacteria. The comparative analysis of the fatty acid content of bacterial cells identified *P. aureofaciens* to be the most successful group.

Rhizosphere: the quantitative and qualitative changes in the microbial community resident on the rhizoplane of field and glasshouse grown sugar beet have been closely monitored over an entire season. *Pseudomanas sp.* are predominant (< 70%) on fibrous roots. As the plants mature and tubers develop, the diversity of the bacterial community increases.

Bacterial growth and spatial distribution of microorganisms was also assessed with a root overlay model developed in the laboratory.

Molecular biology

Genetically stable recombinant *P. aureofaciens* were genetically engineered by marking their chromosome at two non-essential, well separated loci. The recombinant *P. aureofaciens* SBW25 is indistinguishable from wild type in competition assays performed *in vitro* or *in planta*. A physical map of SBW25 is currently being constructed from data collected by pulse field gel electrophoresis of restriction enzyme digested chromosomal DNA. This map will precisely locate the site of insertion of the marker genes and allow any occurrence of genetic reassortment in the plant grown GMO to be determined.

Survival Mechanisms

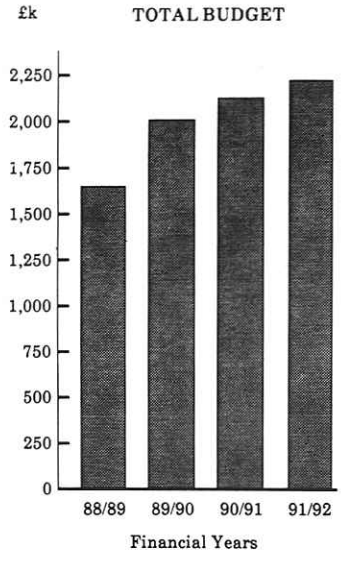
In response to changing environmental conditions, fluorescent pseudomanads produce a range of different colony forms. Bacteria belonging to each class of colony show different behavioural characteristics and display preference for particular niches. The plasticity of phenotype observed in these bacteria in response to environmental change constitutes a sophisticated survival stratagem. Initial work has documented the adaptive evolution of behavioural variants in a simple laboratory environment. Future work will include investigations on the molecular mechanisms of this phenomenon.

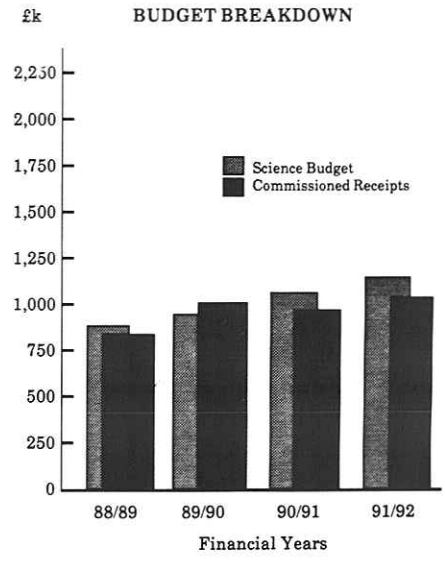
Plasmid Biology

Investigations to describe the genetic environment of the phytosphere are being undertaken so that valid predictions concerning the potential for genetic exchange by natural plasmid populations can be made. Over 18% of the bacterial isolates so far screened, by direct physical extraction or by DNA-hybridisation contained plasmids.

Appendix 1

Financial Support





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British Biotechnology Ltd; Commission of the European Communities; Department of the Environment; Health and Safety Executive; Medical Research Council; Ministry of Agriculture, Fisheries and Food; Ministry of Defence; Virbac Laboratories, France; The Wellcome Trust; Wellcome International Trading.

Appendix 2

Staff list April 1991 - March 1992

Director

D H Bishop, DSc, FIBiol	1984-present	DCSO

Assist Director

E A Could, Ph.D	1988-present	SPSO

Project Leaders

P F Entwistle	1964 1991	SPSO
J I Cooper, PhD	1972-present	PSO
P A Nuttall, PhD	1980-present	PSO
P Roy, PhD	1986-present	Univ
I M Jones, PhD	1987-present	PSO
R D Possee, PhD	1981 -present	PSO
M J Bailey, PhD	1987-present	SSO
J S Cory, DPhil	1984-present	SSO

Scientific Staff

M D Ayres	1975-present	HSO
A Basak, PhD	1990-1992	HSO
A Belyaev, PhD	1991-present	HSO
C Blachere	1989-1991	SO
T F Booth, PhD	1987-present	HSO
T C Bourner	1989-present	SO
A Buckley, PhD	1988-present	HSO
D Carey	1972-present	HSO
T M Carty	1980-present	SO
K B Chaloner	1982 present	SO
A Davies, PhD	1989-present	HSO
C B Doyle	1990-present	SO
C J Doyle	1985-present	HSO
M L Edwards, PhD	1974-present	SSO
R Ellis	1992-present	SO
A Fleetwood	1991-present	SO
M Gibbs	1991-present	HSO
B M Green	1978-present	SO
T Gritsun, Ph D	1990-present	Fellow
I.C Hauxwell	1989-present	SO
S Higgs, PhD	1988-1991	HSO
M L Hirst	1987-present	HSO
E Hodgson	1987-present	SO
S C Howard	1978-present	HSO
L Hongmei	1990-1991	HSO
L D Jones, Ph D	1981-present	HSO
J Jowett	1989-present	SO
H J Killick	1967-present	PSO
P A Kitts, PhD	1989-1991	SSO
N Kobayashi, PhD	1991-present	HSO
M Labuda, Ph.D	1991-present	Fellow
A Livesley, PhD	1991-present	HSO
M Lopez-Ferber, PhD	1989-present	HSO
P T Loudon, PhD	1989-1991	EISO
A C Marriott, Ph D	1987-present	HSO
D McCall	1976-present	HSO
P McCormack, PhD	1991-present	HSO
A Merryweather, PhD	1988-present	HSO
S R Moss	1978-present	HSO
M Nakura, PhD	1992-present	HSO
T Nakura	1992-present	HSO
B J Powell, PhD	1989-present	HSO
C Prehaud, PhD	1991-1992	SSO
K Purdy	1990-1991	SO
P Rainey, PhD	1991-present	HSO
E Snezhkov, PhD	1992-present	Fellow
L Stewart, PhD	1988-1991	HSO
I P Thompson, PhD	1989-present	SSO
C Tiley	1991-present	SO
K N Venugopal, PhD	1989-present	HSO
C Vroon	1990-1991	HSO
L Ward	1990-1991	HSO
C Watkins	1989-present	HSO
J Wenrong	1990-present	HSO
T Williams, PhD	1990-present	HSO

Administrative & Support Staff

L M Atkinson	1988-present	EO
M M T Berwick	1987-1991	SG2
R J Broadbent	1983-present	PTO
S Clarke	1989-present	Typ
A C Forkner	1989-present	TOI
C D Hatton	1977-present	S.Ph
P Henbest	1988-present	IB18
J C Jeacock	1990-present	AO
R M Mackenzie	1984-present	PTO
R Pinniger	1992-present	IB6
A A Primarolo	1981-present	PTO
E Robins	1990-present	Typ
J Sloley	1991-present	PS
A Sloper	1985-present	SG2
D W Stone	1987-1991	IB6

Students

D Bertioli	1988-1991
N Craine	1990-present
C Fu	1991-present
C Harrold	1991 present
R Hawtin	1990-present
S Kreiah	1988-present
A Lilley	1990-present
K Monastyrskaya	1990-present
M A Morse	1988-present
R Paul	1991-present
I Polkinghorne	1991-present
J Pullen	1988-present
A Richards	1989-present
S Shiu	1988-1991
S Thompson	1988-1991
S Thompson	1991 present
V Urquidi	1988-1992
I Van Batal	1990-1991
S Vasconcelos	1991-present
A Walley	1988-1991
H Wang	1991 present
A Whitehouse	1991-present
L Yuanyi	1988 present
P Zanotto	1991-present

Contractual & Short Term Workers

G F Bamford	1988-present
F Fry	1989-present
E Hemmings	1981-present
J Kurtz, MD	1988-present
C J Lawrence	1989 present
S Morton	1986-present
G Pinnuger	1986-present
M Tinson	1989-present

Visiting Workers

C Argentini, PhD	1990-1992
S Brautigam, PhD	1990-1992
V Fulop, PhD	1989-1991
L Hongmei	1991-present
J Kopecky, PhD	1991 1991
J Kurzio, PhD	1992-present
H LeBlois, PhD	1989-1992
P Loudon PhD	1991-present
L Lu	1991 present
R Pinto, PhD	1991-1992
T Polyzoni, PhD	1991-1992
N W Scott, PhD	1988 1992
S Tanaka, PhD	1991-present
J Zhao	1991-present

Appendix 3

Publications 1989 - 1990

Atkins D, Koberts K, Hull R, Prehaud C, Bishop D H L.. 1991 Expression of the tobacco mosaic virus movement protein using a baculovirus expression vector *The Journal of Virology,* **72**: 2831-2835

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