A subtle switch

Dr Helen Hesketh details a unique project that is examining the mechanisms of different virus transmission strategies and exploring what triggers the switch between covert and overt forms of a virus

Could you explain the significance of resolving the conflict between horizontal and vertical virus transmission?

We need to better understand how pathogens transmit between their hosts and how they persist when their hosts are absent or in low numbers, which is typical of rare species or those with highly variable patterns in abundance, such as insect pests. For many years, the Indian meal moth and its virus, *Plodia* granulovirus (a baculovirus known as *Pi*GV), have been used as a laboratory model insect-virus system to help answer these questions.

There are two methods of virus transmission in moth populations – horizontal and vertical. A virus can be transmitted horizontally through contact between infected and healthy moth caterpillars in the same generation. As the infected host normally dies, this requires high host population densities to provide the opportunity for the virus to spread. When insect numbers are low, vertical transmission may be favoured – where the virus is passed directly from parent to offspring.

There is a fundamental conflict between these transmission strategies: horizontal transmission relies on the host dying to release infectious particles, whereas vertical transmission relies on the host surviving to produce offspring. In our current project, we have been trying to elucidate the environmental conditions favoured by each method of transmission in order to determine what causes the virus to switch between the two.

The project is a UK partnership between the Centre for Ecology & Hydrology (CEH) and the University of Leeds. What are the key contributions of each partner?

A strong collaborative partnership has been built over 20 years between Professor Rosie Hails, of CEH, and Dr Steve Sait, based at the University of Leeds, through several projects with PhD students based at the two sites. Both teams research the ecology of host-pathogen interactions.

At the University of Leeds, Sait and his colleague Dr Thomas Jones use laboratory microcosms, which are simplified versions of the natural world that capture key characteristics of larger communities of insects and pathogens. This enables long-term studies of the ecology and evolutionary interactions between insect viruses and their hosts to be run under controlled environmental conditions.

Research at CEH, led by myself, is focused on more tightly controlled experiments that tease out how host density and diet affect virus life history traits such as virulence. Alongside Professor Robert Possee, we have developed molecular probe techniques that detect very low levels of active virus in the hosts. These data enable us to develop and apply

Survival of the fittest?

Investigators at the **Centre for Ecology & Hydrology** and the **University of Leeds** seek to determine how environmental factors affect host population density and subsequently influence virus fitness, virulence and persistence. The project is still underway but has already had some surprising results

THE IMPACT OF pathogenic diseases on their host's ecology and evolution is most evident when they reach epidemic proportions, such as the outbreak of foot and mouth disease in the UK. Such epidemics are typically associated with high host population densities, as this makes it easier for the disease to be transmitted from one individual to another.

Naturally occurring insect pathogens are frequently found in above- and below-ground ecosystems. They can cause significant insect mortality and have therefore been exploited as biological control agents for insect pests. However, their role in regulating host populations and the methods by which they are transmitted between insect hosts are still not entirely understood. Baculoviruses are large double-stranded DNA viruses that infect the larvae of insects, primarily moths and butterflies, and are often lethal to the caterpillars. The virus can be transmitted to new hosts when larvae consume infectious virus particles that have been released after the death of the infected host, or in some cases through cannibalism. Such horizontal transmission was thought to be the principal means by which baculoviruses persist and spread in the natural world. However, DNA studies of *Mamestra brassicae* (cabbage moths) at the Natural Environment Research Council Centre for Ecology & Hydrology (CEH), UK, discovered that they could also be infected with non-lethal, asymptomatic *M. brassicae* nucleopolyhedrovirus (MbNPV). This so-called covert infection could be transmitted to their offspring in a process termed 'vertical transmission'.

TRIGGERS OF COVERT AND OVERT INFECTION

Collaborative work between groups from CEH and the University of Leeds, UK, subsequently showed that several populations of moths across the UK are infected covertly with viruses, suggesting that vertical transmission may be fundamental to virus persistence in nature. In addition, it was discovered that covert viruses have the capacity to become lethal, which may be important for their role in regulating pest populations. Following on from these findings, the groups have been working together to determine the ecological conditions that promote either horizontal or vertical virus transmission, using a combination of long-term monitoring of overt virus infection and molecular detection of low levels of covert virus.

The Indian meal moth, *Plodia interpunctella*, and its granulovirus *Pi*GV are being used as the model system for the project, as both forms of virus transmission are present in the moth, and it is possible to study them over multiple generations in the laboratory.

At the University of Leeds, populations of host and pathogen have been raised in plastic boxes for many generations, under controlled conditions. A key environmental factor – diet quality – has been varied so that the moths are fed either a high quality, nutritionally rich food that supports high

Experimental microcosm containing baculovirus-infected moth larvae in selection experiments.

mathematical models to determine viral growth rates within individual insects. This can improve our understanding of pathogen evolution by allowing us to identify which traits are selected under certain environmental conditions.

Could you outline the project's activities to date?

The project has two key parts. The long-term studies allow us to investigate the virus as it transmits in insect populations that fluctuate in density. Shorter controlled experiments enable us to tease out the responses of the virus or the host independently of each other. The virus is then allowed to co-evolve alongside the insect hosts, with two different quality diets providing an environmental selection pressure on both host and pathogen. We have found that reducing diet quality affects several life history traits in the moths. For example, those reared on very poor quality diets take 40 per cent longer to develop to adulthood than those with high quality diets. This is significant, because it means that, where diets are poor, the susceptible larval stage will be exposed to the virus for longer.

What are the next steps in achieving your objectives?

In order to identify the potential differences in virus traits, we are running a series of experiments concurrently at the University of Leeds and CEH using viruses sampled from both the long-term populations and selection experiments. This is the final exciting step after three years of allowing the virus to evolve under different diet selection pressures. By comparing viruses from the beginning and end of experiments (and, hopefully, other times in between) we can determine how the virus has evolved.

Are you participating in any upcoming events relating to the project?

An exciting outcome from a presentation made by Jones at the 2013 International Congress of Ecology was that a group of parasitologists gathered to discuss their different views on covert pathogen infections in diverse host-pathogen systems. We plan to discuss further research needs and opportunities to collaborate in this area.

We are currently analysing our data and writing a number of articles which will present our results to the scientific community. I will showcase our findings at the Society for Invertebrate Pathology's annual meeting in Mainz, Germany, in August 2014, and we are hoping to update readers with the final results in a follow-up article in *International Innovation* later this year.

population densities, or a poor quality, nutritionally poor food which fewer moths can survive on. At certain life cycle stages, the moths and their virus are sampled for molecular testing to determine the prevalence of covert or overt virus forms and to discover any changes in traits like pathogenicity.

SURPRISING RESULTS

The group's initial prediction was that horizontal transmission would be favoured in good quality environments as a response to high population density and that prevalence would increase because of frequent interactions between infected and susceptible moths. Furthermore, this would have an effect on the key virus traits of pathogenicity and virulence, potentially making the virus more virulent to the moths. Interim results have surprised the project team as Dr Helen Hesketh, an insect pathologist from CEH, observes: "The interactions that take place within what at first appear relatively simple laboratory systems are in fact highly complex. Contrary to our initial hypothesis that the virus would become more virulent and prevalent in the populations on a high quality diet, the overt virus is no longer detected. Whether this is because the virus has switched to largely covert infections remains to be seen". The project is therefore investigating which viral traits favour the selection of a particular transmission tactic, how this is timed and what mechanisms facilitate virus persistence in the covert state. Identifying the conditions that favour one or other mode of transmission may have important implications for understanding host and disease ecology and how we manage insect pests.

Moth larvae lethally infected with baculovirus. The host biomass has been liquified as it is converted to infectious viral particles.

INTELLIGENCE

RESOLVING THE CONFLICT: THE ECOLOGY AND EVOLUTION OF HORIZONTAL VERSUS VERTICAL TRANSMISSION IN A MODEL INSECT-VIRUS INTERACTION PROJECT

OBJECTIVES

To understand the ecological conditions that favour horizontally or vertically transmitted viruses; in particular, the effect of covert infections on host population dynamics and the impact of contrasting transmission strategies on host and pathogen life history traits.

KEY COLLABORATORS

Dr Steven M Sait; **Dr Thomas Jones**, University of Leeds, UK

Professor Rosemary S Hails; **Professor Robert D Possee**, Centre for Ecology & Hydrology, Wallingford, UK

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