Postgraduate Forum 2009 Report

University of York, National Science Learning Centre

4th - 5th February

Luke Tilley
Postgraduate Representative
(lant500@york.ac.uk)

If you can cast your mind back to early February this year, you will remember that Britain was covered, almost top to toe, in snow. Despite the wintry conditions, 56 delegates braved the weather, and transport network, to attend the Postgraduate Forum. This year's Forum was held over two days, a new format introduced to allow postgraduates more time to talk to each other. Feedback from attendees suggested that the two-day structure worked well, and the Forum was a success, both enjoyable and productive.

The principal aim of the PG Forum is to allow postgraduates to present talks and posters to their peers to promote the discussion of people's successes and failures in entomological research. There were 15 student talks and 15 student posters on offer, ranging in subject matter from insect behaviour and ecology to molecular biology and genetics. The bringing together of researchers from different fields, united by the stage of their careers, has become a real strength of the PG Forum. This allows those presenting talks and posters to receive feedback and advice from scientists working beyond their own research area.

This year's event included three great invited speaker sessions. Professor Mike Siva-Jothy, University of Sheffield, kicked off proceedings with '*Reproduction and immunity in insects*'. Mike presented an interesting summary of his research, and an outline of his career to date, something that postgraduates are often keen to learn about from senior academics. During the afternoon of the first day, Debbie Wright, Journal Publishing Manager at Wiley-Blackwell, held an '*Early Career Publishing Workshop*' assisted by two of the RES journal editors (Dr Jane Hill, Ecological Entomology; and Dr Keith Walters, Agricultural and Forest Entomology). Debbie and the editors gave delegates advice about scientific writing and publishing, finishing with a Q & A session. The third invited speaker was Dr Calvin Dytham, University of York. Calvin gave a very popular talk titled *'Problems, pitfalls & power: some thoughts on the acquisition and analysis of data'*. He discussed common uses and misuses of data, something that every student needs to consider during their research. This talk stimulated some interesting questions from the sudience, particularly around the topic of appropriate use of statistical tests.

The second day ended with the presentation of prizes for the student talks and posters. Overall, the quality of the presentations was excellent. Particular praise must go to the four winners for their ability to present their research in a clear and interesting manner. The four prize winners have provided extended abstracts for your enjoyment:

Runner-Up Prize: Oral Presentation

Unearthing links within the soil food web: following microbial carbon and nitrogen flow through the trophic levels

Felicity Crotty[1,2], C.D. Clegg[1], R.P. Blackshaw[2], and P.J. Murray[1]
[1]North Wyke Research [2]University of Plymouth
(felicity.crotty@bbsrc.ac.uk)

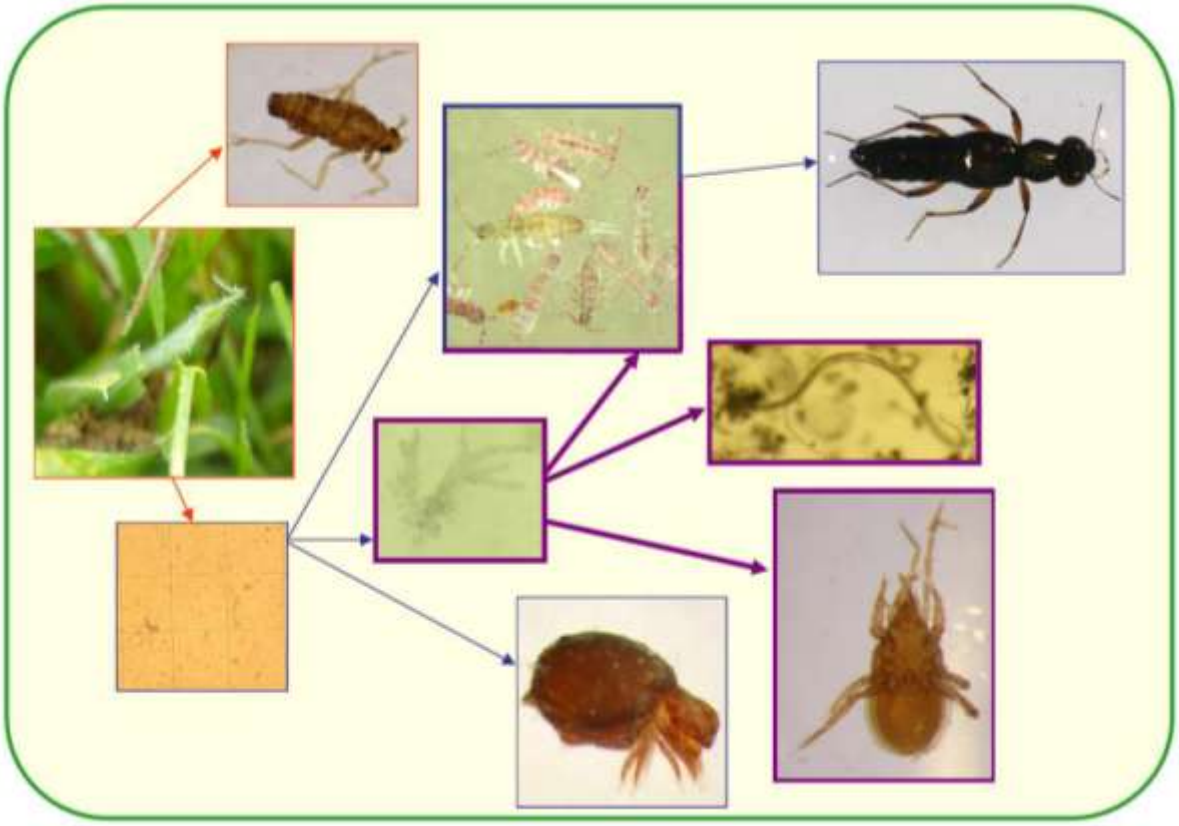
The terrestrial detrital food web is a major component of most terrestrial ecosystems regulating nutrient cycling, litter decomposition and energy flow, but the individual affect of different taxa within the system is unknown. Within all soil food webs there are thought to be two distinct energy channels – bacterial and fungal, which form the basis of the soil decomposer food web. The majority of soil food web diagrams are based on the questionable "one-taxonomic-group-one-diet" hypothesis although taxonomic group rarely represents feeding guilds. There are very few studies which have observed soil food webs directly under field conditions due to the diversity of species, small scale and patchy distribution of invertebrates, although many have inferred food web position from laboratory or mesocosm studies. One technique that can be used to track the feeding preferences of soil organisms in situ without artificially manipulating the interactions that are occurring is the use of stable isotopes. Natural abundance levels of 13C and 15N have been used to infer trophic position in some food web studies, as well as some different enrichment methods, like the direct addition of a labelled substrate or by pulse labelling the plants within the system, but none of these methods distinguish between the bacterial and fungal feeding channels.

To investigate the bacterial feeding pathway *in situ* a fluorescent pseudomonad was cultured in minimal media containing 13C-glucose and 15N-ammonium chloride as the sole C and N source, to enable full isotopic cell labelling (to 99.9 atom% 13C/15N). Labelled bacteria were injected into intact soil cores, and mesofauna were subsequently extracted (Figure 1), identified and separated before analysis by mass-spectrometry. Results showed that some invertebrates were highly labelled particularly root-feeding aphids and Collembola indicating consumption of the added bacteria. These results provide an insight into the movement of C and N through the bacterial energy channel of the soil food web. Protozoa and some other micro-organisms inhabiting the soil are very difficult to extract in enough quantities to obtain stable isotope measurements from. Protozoa are at the next trophic level within the food web and portrayed in food web diagrams to be solely bacterial feeders, predated upon solely by nematodes. To test these assumptions a preliminary experiment was set up to assess whether protozoa could be affectively labelled with stable isotopes and introduced into soil mesocosms to track the soil food web. Protozoa were cultured with 13C-sodium acetate and obtained an enrichment of 13.2 atom% (markedly higher than natural abundance levels). Labelled protozoa were introduced into soil mesocosms using the same introduction and extraction method as for the bacteria. Results from this experiment have shown that protozoa can be labelled with 13C and their consumption traced within higher invertebrates. Combining

the results from the bacterial and protozoa experiments has shown that within the bacterial feeding channel a complicated, inter-connected food web is developing (Figure 2), where the invertebrates can not be compartmentalised as sole feeders of one taxa but are perhaps more polyphagic than is currently thought.

Figure 1: Example of mesofauna extraction

Figure 2: Food web diagram developed from ^{13}C results from bacterial and protozoa experiments.



First Prize: Oral Presentation

Could Mosquitoes Become 'Resistant' to Repellents?

Nina M Stanczyk[1,2], John FY Brookfield[2], James G Logan[1], Linda M Field[1]
[1]Rothamsted Research [2]University of Nottingham
(nina.stanczyk@bbsrc.ac.uk)

Repellents are utilised worldwide as personal protection against many biting insects including mosquitoes, which are vectors of pathogens that cause disease. An important vector species is *Aedes aegypti* which transmits both dengue fever and yellow fever. Yellow fever and dengue fever have severe impacts on the health and economic state of the human population, with 500 million people living in areas at risk from these diseases. There is no cure for yellow fever or dengue fever, and it can be difficult to control the mosquito population, so often repellents are the last line of defence.

N-N-Diethyl-*m*-toluamide (DEET) is one of the most effective and commonly used repellents worldwide. However, during laboratory repellency trials a small proportion of

mosquitoes still respond to human odours and attempt to bite despite the presence of DEET. This could lead to repellents being unreliable when used in the field, and may increase the transmission of the disease.

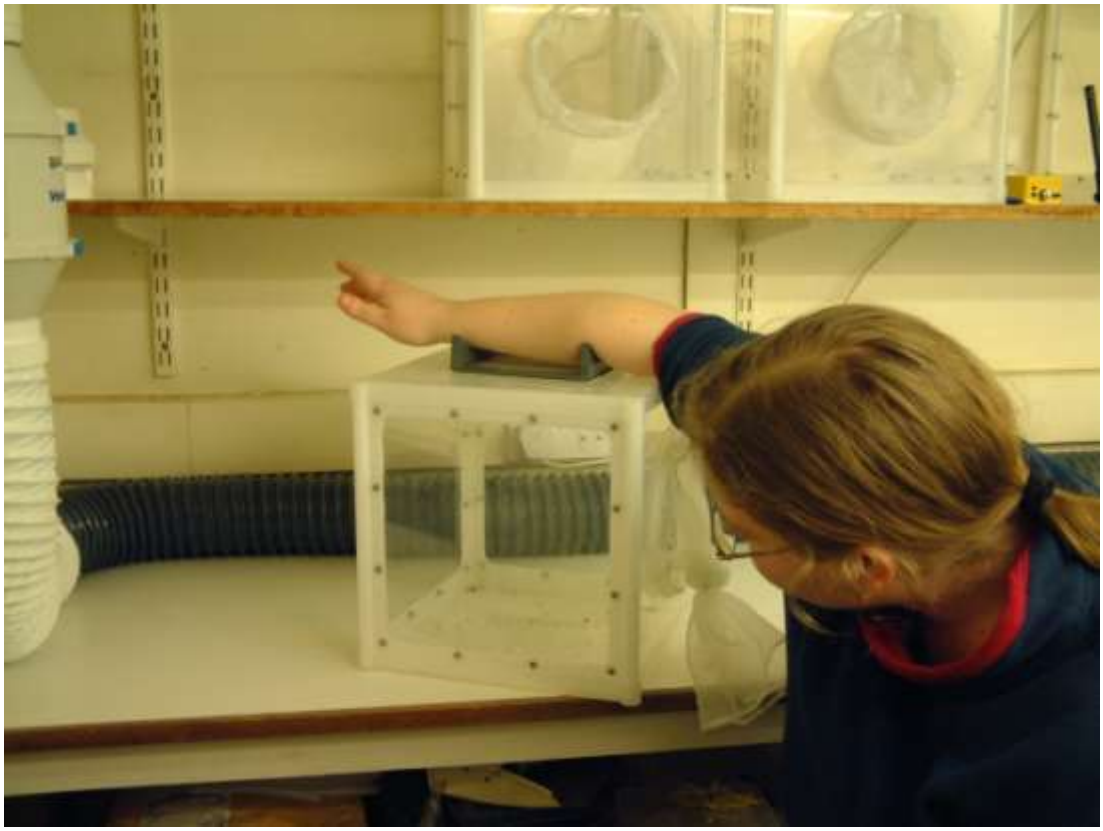
In this study behavioural assays identified *Ae. aegypti* females that were insensitive to DEET by counting mosquitoes that landed on and attempted to probe a DEET-covered arm. These insensitive mosquitoes were selected and bred as a separate line. Over several generations there was an increase in the proportion of the offspring that were insensitive. This shows the trait of insensitivity to deet is heritable, and will increase in the population if there is selection pressure.

Olfactory cues are one of the primary stimuli mosquitoes use to locate their hosts. If mosquitoes detect DEET via their olfactory system it is likely that the selected and unselected mosquitoes will differ in their response. In order to test this hypothesis, electroantennograms were carried out recording antennal responses to different olfactory stimuli including DEET. They demonstrated a difference in the response to DEET between the selected insensitive line and unselected mosquitoes, with the selected mosquitoes responding less. This suggests that the behavioural alteration is caused by a change in the olfactory system that detects DEET. Single-sensillum recordings will be carried out to identify which sensilla on the antennae have an altered response in the selected line.

Once heritability of the insensitive trait is maximized in the selected line, genetic crosses will be carried out to determine the mode of inheritance. Molecular techniques will also be used to track changes in expression in the selected line and determine the gene(s) involved, which is likely to be in the olfactory pathway.

This research provides an insight into how insensitivity to repellents can arise and spread throughout a population when the trait is selected for. This information could aid control programmes that seek to use repellents. A more detailed understanding of the gene(s) responsible for insensitivity may lead to novel control methods.

Nina suffering for her research



Runner-up prize: Poster

The effect of industrial particulate cryolite on terrestrial invertebrates

Kate Brougham, Gordon Port
Newcastle University
(k.m.brougham@ncl.ac.uk)

There has been impressive economic growth in may countries recently, China and India are two examples, but what impacts might there be on the environment? With a boom in construction and engineering there has been an expansion of the aluminium industry. A predicted knock-on effect of this is a crisis involving a world-wide surplus of sodium hexafluoroaluminate or cryolite (NaF AlF_6).

Through its use in the process to reduce alumina to aluminium, particulate cryolite released from aluminium smelters is a contributing factor to elevated environmental fluoride concentrations associated with the 2km fall out zone surrounding smelter sites.

This may appear to be an unusual subject for an entomologist, however interestingly since 1933 cryolite has also been used as an active ingredient in several insecticide products in the US. Cryolite products have gained fame amongst pesticide companies as after 76 years of use, there is no record of insects developing resistance to its toxic effects.

This begs the questions: how toxic is industrial cryolite pollution, how does it differ to insecticidal cryolite and what other physiological effects does it have on invertebrates?

Preliminary research has been conducted using the Diamondback moth (*Plutella xylostella* (L.), (Lepidoptera: Yponomeutidae)), one of the most important pests of brassica crops in the world. This species is of interest due to its high genetic flexibility which ha allowed it to develop resistance to a wide range of insecticides relatively quickly.

Whereas LD_{50} bioassays showed cryolite to produce a significantly greater mortality in 3^{rd} instar larvae in comparison to 1^{st} instars (Binary logistic regression $P < 0.001$), on the flip side, weight gain was significantly reduced with dose in 1st instars ($P < 0.001$) where change was not affected in 3^{rd} instars. Through Kaplin Meier Survival Analysis, initial investigations suggest that this weight decrease may severely affect survival after exposure, in comparison to 3^{rd} instar larvae where resulting health seemed to be unaffected.

Another important observation to note is that for both instars when concentrations of cryolite on leaf surface reached $200\mu g/cm^2$, the previously increasing mortality peaked and mortality decreased at higher concentrations.

Possible explanations are being considered for these observations, including the possibility that starvation due to particle and mouthpart size could be the cause, investigated using SEM imaging.

Preliminary comparisons of toxicity between industrial and insecticide cryolite recorded in the literature, have suggested that industrial pollution is up to ten times more toxic. Work is now focused on confirming this as well quantifying cryolite output from an aluminium smelter in Northumberland, and investigating the potential of using X Ray Diffraction as part of a monitoring program.

SEM of third instar of the diamondback moth (*Plutella xylostella*)

First Prize: Poster

The Genetic Identification of Forensically Important Blowfly Species in the UK

Helen Godfrey, Judith A. Smith
University of Central Lancashire
(HGodfrey@uclan.ac.uk)

The examination of entomological specimens from a crime scene can often provide criminal investigators with estimations for concerning the time, manner and place of death. As different species are known to be attracted to a corpse at particular stages of decomposition, the identification of the species present and of their lifecycle stage (their age) can help investigators to estimate a time since death. Estimation for a post mortem interval (PMI) relies upon the application of species specific lifecycle data, therefore the correct identification of the species present is critical for an accurate PMI to be established.

Currently based on morphological characteristics, species identification is often problematic for closely related species (Figure 1), fragmented specimens and immature stages. In order to overcome these difficulties, molecular techniques including DNA sequencing have been applied in order to differentiate between samples down to the species level.

Figure 1. *L.illustris* or *L.caesar*? Due to the similar morphological characteristics exhibited by closely related species, identification down to the species level is often very difficult.

Preliminary studies at the University of Central Lancashire using previously published sequence data has demonstrated the usefulness of a range of genetic markers to distinguish between various UK blowfly species of forensic importance. mtDNA and rRNA genes have been used as their conserved functions make them particularly useful tools for studying evolutionary differences. Mutations accumulate slowly over time resulting in a higher degree of between (inter) compared to within (intra) species variation that can be exploited for more accurate species identification.

Published sequence data for 16s rRNA, cytochrome oxidase I (COI) and II (COII), 5.8s and 28s rRNA and the internal transcribed spacer 2 region (ITS2) has been examined in

order to find species specific differences which can be used to differentiate between species (Table 1).

Table 1. The differentiation of various UK blowfly species based on a range of genetic markers. '✓' indicates differentiation based on sequence data, '×' indicates no differentiation. '-' indicates no sequence data available.

Genetic marker and length of sequence data	5.8s rRNA (167 bp)	16s rRNA (550 bp)	28s rRNA (2200 bp)	COI (488 bp)	COII (616 bp)	ITS2 (460 bp)
C.loewi	-	-	-	✓	-	-
C.vicina	✓	✓	✓	✓	✓	✓
C.vomitoria	✓	✓	✓	✓	-	✓
C.mortuorum	-		✓	-	-	-
L.ampullacae	-	-	-	✓	-	-
L.caesar	×	✓	✓	✓	✓	×
L.illustris	×	✓	✓	✓	✓	×
L.richardsi	-	-	✓	-	-	-
L.sericata	×	✓	✓	✓	✓	✓
L.silvarum	-	-	✓	-	-	-
P.rudis	-	-	✓	-	-	-
P.regina	-	-	-	✓	✓	-
P.terraenovae	-	-	-	✓	✓	-
P.azurea	-	-	✓	-	-	-
Average sequence I.D	0.99 (n = 20)	0.97 (n = 36)	0.98 (n = 14)	0.89 (n = 55)	0.92 (n = 34)	0.95 (n = 29)

An examination of the data revealed that the COI and 28s rRNA genes could provide sufficient information to distinguish between 14 UK blowfly species. This demonstrates the potential for genetic identification of entomological specimens. However, as only 14 of the 32 known UK blowfly species are currently represented in GenBank® (with 6 of these being represented by only a single sequence), the lack of sequence data for UK species needs addressed. The research being undertaken at UCLan aims to generate sequence data for a range of UK blowfly species, to assess the most appropriate genetic markers for identification through phylogenetic analysis and to develop a molecular tool kit for blowfly identification.

I would like to thank all the speakers and poster presenters for an interesting meeting. The RES Postgraduate Forum 2010 will be held 3rd- 4th February at the University of Sheffield, so please look out for it.