

Postgraduate Forum 2010 Report

Endcliffe Village, University of Sheffield

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Those who came to the 2009 PG Forum will recall the wintry conditions that delegates battled through in order to get to York. Here in Sheffield we are used to the perils of snow. Sheffield is a very hilly city and during the winter life can quickly become more akin to the South Pole than South Yorkshire and I had been worried that arctic conditions were to become a tradition of the forum. However, we remained optimistic and were rewarded with a cold but sunny start to the 2010 meeting. We once again experienced an excellent turnout with over 40 delegates attending, with 16 platform presentations and 14 posters covering a diverse range of topics in entomology and even a foray into limacology (the study of slugs).

The PG Forum gives the opportunity for students in all fields of insect science to come together and discuss their work in a friendly atmosphere. The first day consisted of two sessions of student talks and a poster session with the quality of both being exceptional. As the second platform presentation progressed however Sheffield became caught in the grips of a heavy snowstorm and as the day closed we were forced to battle our way back to the accommodation for overnight delegates. With the invited speakers due to arrive the next day I was panicking that they wouldn't make it. However, luckily the snow soon subsided and the second day got off without a hitch.

We were treated to another session of student talks and a poster session before this year's invited speakers arrived. Each year the forum organisers give the event a theme with 2010's being 'Careers Outside of Academia'. We were extremely fortunate to get three great speakers, Myc Riggulsford, Dr Richard Lilley and Dr Richard Handley. Myc works as a science communications consultant. After working as a journalist he now operates his own consultancy 'The Walnut Bureau' and runs highly successful communications workshops with both NERC and University's across the country. Myc gave a very engaging and entertaining talk about the world of science journalism, potential career paths and how best to get your research into the public domain. Dr Richard Lilley is the director of Tracksys Ltd who offer solutions for behavioral research. His captivating talk focused on his career path after university including his navigation of the many opportunities and pitfalls accompanying life after completing a PhD. He finished his talk with an account of life in the private sector and how to make yourself as employable as possible. Rounding off this year's forum Dr Richard Handley from the Environment Agency gave an insight into the inner workings of the public sector and careers open to PhD graduates.

On behalf of everyone I'd again like to thank all those who came this year, especially those who presented and the external speakers. I'd also like to extend my thanks to Dr Archie Murchie and Prof Rod Blackshaw for judging the all the presentation and the RES for providing the prizes. In order to showcase the quality of this years symposium below are extended abstracts from the winners and runners up of both the poster and platform presentations.

Poster

Runner Up

What will stop the invader? Dispersal barriers of the harlequin ladybird (*Harmonia axyridis*)

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Species invasions have wide ranging ecological and economic impacts, and are one of the greatest threats to biodiversity, agriculture and human health. Despite this, we do not understand why only certain species become successful invaders, what determines their success, and what impacts they have on other species. A major aim of invasion biology is to understand invasion success, and dispersal ability is a key life history trait that influences this. Understanding the dispersal ability of a species and what constitutes a barrier to dispersal is critical to predict the spread and potential impacts of an invasive species. Barriers to dispersal can be natural physical features, such as mountain ranges or bodies of water, or the result of anthropogenic change, such as road construction, deforestation or other forms of habitat fragmentation. Traditional methods for estimating dispersal can be difficult. For example, with mark-release-recapture (MRR) the chances of recapturing individuals can be low, extensive time in the field is often required, and it is particularly difficult to do over long distances. MRR also does not help us link dispersal to gene flow between populations, so molecular techniques can be an attractive complementary approach. The recently developed field of landscape genetics¹ combines population genetics and landscape ecology, and allows us to identify migrant individuals and detect genetic discontinuities that arise from barriers to dispersal.

The harlequin ladybird, *Harmonia axyridis*, is rapidly becoming a model organism for studying biological invasions², but very little is known about its dispersal ability. *H. axyridis* is native to Asia (figure 1), but is highly invasive in Europe, North America, and parts of South America and South Africa³. It has been used as a biological control agent of agricultural pests in North America and mainland Europe, but the species is now

established in countries where there is no record of a deliberate introduction, and it is predicted to continue to spread^{2,4}. Data from the UK-based Harlequin Ladybird Survey (www.harlequin-survey.org) indicates a spread of 58 km year⁻¹ northwards and 144.5km year⁻¹ westwards⁴. To gain a full understanding of the natural dispersal ability of the harlequin ladybird we must also investigate dispersal in native populations, where any restrictions to gene flow due to dispersal barriers should be easier to detect.

MRR studies suggest ladybirds frequently move over small distances^{5,6}, however, less is known about long distance dispersal and nothing is known about dispersal barriers. In this study we aim to investigate dispersal in native Japanese *H. axyridis* populations, focusing on the role of natural physical features, geographic distance and climate. Given the spread rate of *H. axyridis* in its invasive range, we hypothesise there will be considerable dispersal in the native range with only major physical features acting as barriers. To test this, we collected over 1000 individuals from 22 sites across Japan, sampling either side of potential dispersal barriers (figure 2). These individuals are currently being genotyped at 18 microsatellite loci, and genetic data will be analysed in a geographical framework to incorporate landscape and environmental features. This will allow us to identify genetic discontinuities and estimate the amount of dispersal between populations. The results of this study will offer a unique insight into the dispersal ability of an invasive species in its native range. Our data will be combined with ecological data being generated by our collaborators, and incorporated into computer models to help predict the spread and impact of the species in its invasive range.

Acknowledgements

We would like to thank everyone who assisted us during fieldwork in Japan, particularly Peter Brown, Hana Akiyama, Dr Satoshi Koyama, Dr Toshiyuki Satoh and Prof Yoshiaki Obara, and students at the Tokyo University of Agriculture and Technology, Japan. Also, the staff and students at the NERC Biomolecular Analysis Facility at the University of Sheffield for assistance with molecular work, and all members of the UK Ladybird Group for their advice and guidance throughout the project. We would also like to thank NERC, the Daiwa Foundation, The Genetics Society and the Royal Entomological Society for the funding that has made this study possible.

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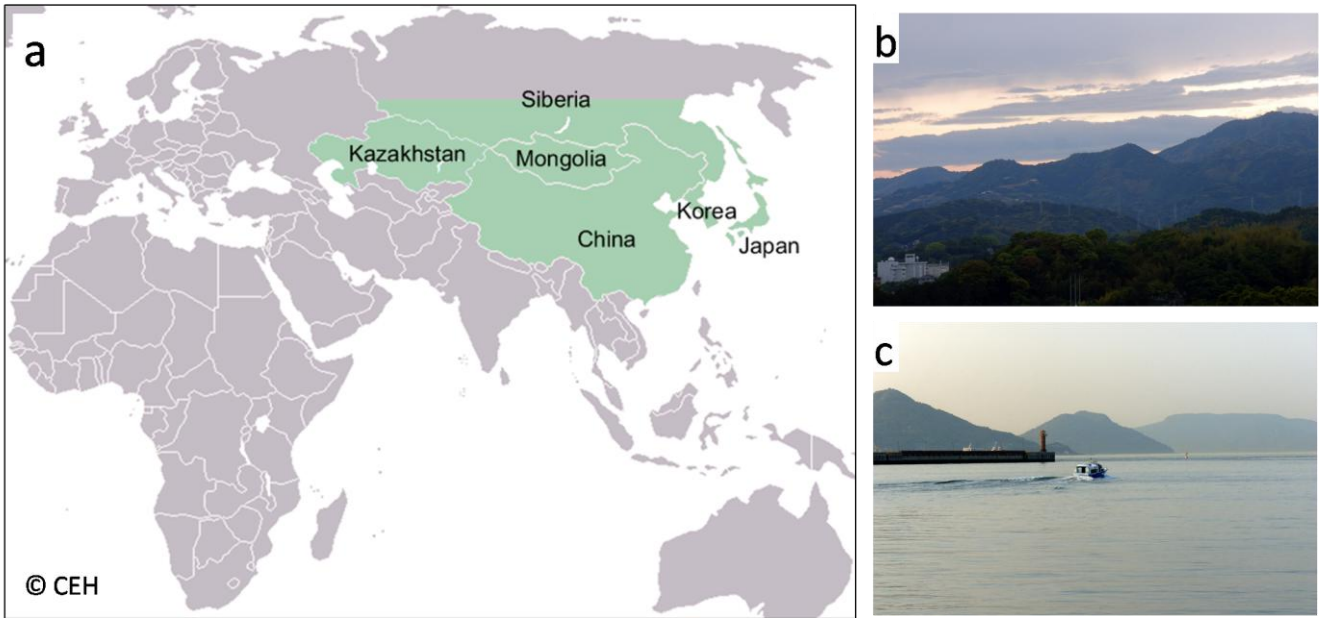


Figure 1. (a) The native range of *Harmonia axyridis*. (b) Mountain chains or (c) the sea may act as barriers to dispersal.

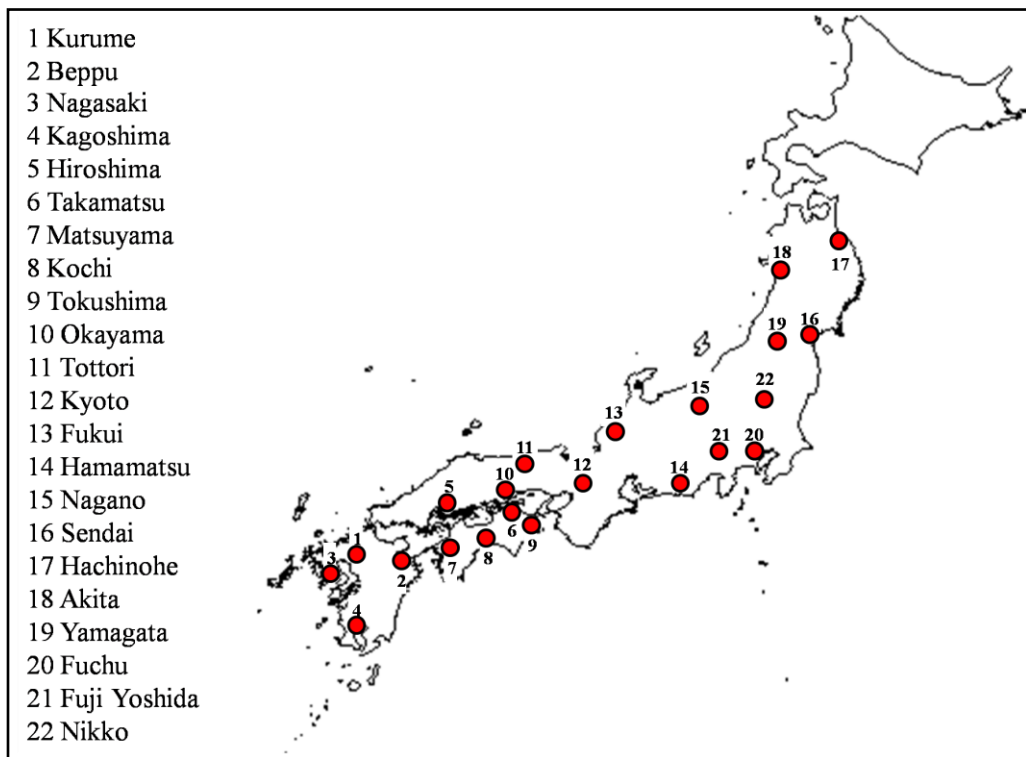


Figure 2. Harlequin ladybirds have been collected from 22 sites across Japan.



First Prize

Transgenesis of the diamondback moth using the *piggyBac* transposable element

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The diamondback moth, *Plutella xylostella* L. (Lepidoptera:Plutellidae), is a widely distributed and damaging pest of wild and cultivated cruciferous plants, such as cabbage, broccoli and cauliflower. It is difficult to control, mainly due to widespread resistance to insecticides, so the development of new control measures is needed. Genetics-based control may provide new alternatives, for example the release of insects carrying a dominant lethal (RIDL[®]); in effect, genetic sterilisation to achieve the same result as the sterile insect technique (in which radiation-sterilised insects are mass-released to suppress the wild population). Genetic transformation (or transgenesis) – the insertion of exogenous DNA into the insect genome, is a key underpinning technology for any such approach.

Insect transgenesis has been successfully achieved by using transposable elements. These genetic elements, also called transposons, are sequences of DNA that move from place to

place within the genome of a single cell and allow material to be inserted or relocated. One of the most commonly used transposons in insect transgenesis is *piggyBac*.

The transposable element *piggyBac* was originally isolated from baculovirus reared on cultured cells of the cabbage looper moth, *Trichoplusia ni* Hübner (Lepidoptera:Noctuidae). The *piggyBac* transposon is flanked by short inverted repeats that initiate non-replicative insertion into the DNA, mediated by an enzyme called transposase. Wild type ‘autonomous’ *piggyBac* elements encode their own transposase, but for insect genetic engineering defective ‘non-autonomous’ elements are used from which the transposase gene has been deleted and which therefore cannot catalyse their own transposition. The transposase binds to the *piggyBac* ends and catalyses the insertion of the transgene into a target sequence (TTAA) in the genome. Transposon-bearing plasmids can be injected into embryos and transiently exposed to transposase. If, as a consequence, the transgene is inserted into the genome of the germ-cells, it can be passed to subsequent generations, leading to the production of a new, stable, transgenic line. The *piggyBac* transposon has been shown to mediate germline transformation of the silkworm (*Bombyx mori*), the pink bollworm (*Pectinophora gossypiella*), the red flour beetle (*Tribolium castaneum*), the sawfly (*Athalia rosae*), and several dipteran species.

In order to determine if the diamondback moth could be transformed, pre-blastoderm embryos were micro-injected with one or the other of two different DNA constructs (Table 1) containing different promoters and fluorescent marker genes: one construct (OX3441) has the *Hr5ie1* promoter driving the expression of a red fluorescent protein (DsRed) and the other (OX4389) has the *Opie2* promoter driving the expression of a green fluorescent protein (ZsGreen). Both DNA constructs were flanked by *piggyBac* sequence and were co-injected with a source of transposase.

Table 1. Transformation summary of OX3441 and OX4389 in diamondback moth		
	OX3441	OX4389
Number of embryos injected	1925	1344
Number of injection survivors	1462	603
Number of transgenic lines	7	1
Transformation efficiency (%)	0.48	0.17

Transgenic individuals were found among the progeny of injected individuals, and showed expression of the fluorescent protein, which was easily detected under a fluorescent stereomicroscope (Figure 1).

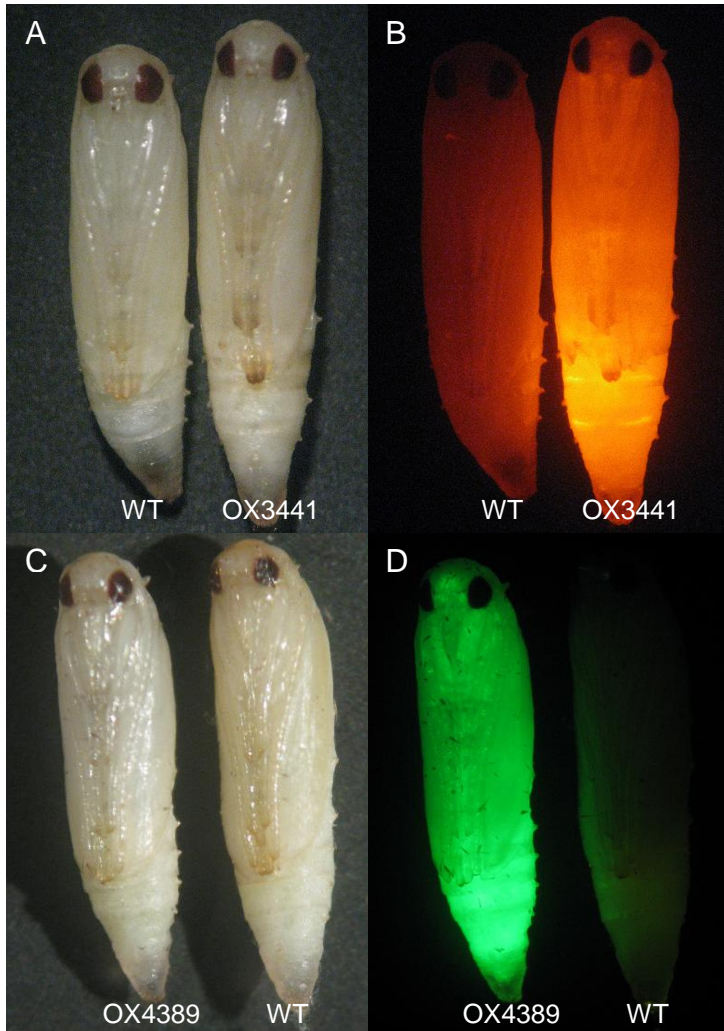


Figure 1. Expression of the red fluorescent protein DsRed in wild-type (WT) and OX3441 pupae under (A) white light and (B) DsRed-excitation wavelength light and expression of the green fluorescent protein ZsGreen in wild-type and OX4389 pupae under (C) white light and (D) ZsGreen-excitation wavelength light.

These results represent the first germline transformation of diamondback moth. We are now attempting to transform the diamondback moth by ϕ C31-mediated site-specific integration, a system allowing repeated integration of transgenes into a known genomic location. This would allow for direct comparison of transgenes in the same genomic location, thereby minimising the variations in expression caused by position effects that occur with *piggyBac*.

Presentations

Runner Up

The effect of slugs on seedling recruitment and community composition in upland hay meadow plant communities

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Species-rich upland hay meadows are of high biodiversity importance and are internationally rare. They are categorised by the National Vegetation Classification (NVC) as the neutral grassland type MG3 (*Anthoxanthum odoratum*-*Geranium sylvaticum* grassland) and are predominantly found in upland valleys in northern England where traditional hay-making practices still persist. Agricultural improvement since the 1940s has greatly reduced the area of MG3 meadows (<1000ha in England and <100ha in Scotland) resulting in vast areas of species-poor improved or semi-improved meadows. There is increasing interest in restoring botanically diverse meadows, but little is known about the effect of invertebrates on community composition.

So why slugs? In this project a number of invertebrate-plant interactions are being investigated, including pollination and herbivory. Although there are insect herbivores present in the meadows, the slugs have some unique features, such as large population size and breadth of diet, which means they are likely to have more impact on plant community structure than any insect herbivores. Slugs are most commonly thought of as pests of arable and horticultural crops and little attention has been given to their role in semi-natural grasslands.

In March 2008, a three year mesocosm experiment was set-up at Close House Biology Field Station, Newcastle upon Tyne (NZ128659), to investigate three key questions: (i) How does slug grazing affect seedling recruitment and community composition? (ii) Which plant species are selectively grazed by slugs? (iii) Does the effect of slug grazing change over time? Plastic mesocosms (1 m x 1 m x 0.39 m) were sown with either a species mix typical of a semi-improved or unimproved upland hay meadow plant community, with or without the addition of slugs. Soil was sourced from a local mid-secondary successional grassland in order to replicate low-medium fertility field conditions. Mesocosms to which slugs were added received 50 slugs per m² (of the grey field slug, *Deroceras reticulatum* Müller) in April 2008 and again in October 2009 (to represent a high population density as found in field investigations). Mesocosms in which no slugs were added contained a low residual slug population; regular monitoring and removal maintained this low background population. To control slug migration, copper-impregnated matting (Spinout) was used as ground cover and attached to the perimeter of all mesocosms (Fig. 1). An appropriate cutting regime was used to simulate vertebrate grazing in autumn and spring, and the annual late-July hay-cut found in the meadows. Plant species richness and abundance cover (%) were recorded in mid-

summer 2008 and 2009 and analysed using, the multivariate technique, Canonical Correspondence Analysis (CCA).

In 2008, slugs had no significant effect, but in 2009 were a significant driving factor in shaping community composition. A particularly interesting finding was the association of *Rhinanthus minor* L. (Hay rattle) (Orobanchaceae) with a low slug density environment. *R. minor* was first sown in autumn 2008, into mesocosms with an unimproved community only, and germinated in spring 2009 following the mandatory vernalization period.

The results indicate the selective grazing of *R. minor* seedlings by slugs; this is of particular interest for several reasons. *R. minor* is a hemi-parasitic annual, making its contribution to the sward on a yearly seedling recruitment cycle. Plants form parasitic root connections (haustoria) with host plants, particularly grasses and legumes, enabling a wider diversity of less competitive wildflower species to develop. As such, *R. minor* is a keystone species and is used as a management tool in the restoration of species-poor grasslands. A critical early step in the restoration process is to sow *R. minor* into the sward with the specific goal of reducing the grass dominance. This research is the first to show how the selective removal of *R. minor* by slugs may be relevant to the restoration management of species-rich meadows.



Fig. 1. Mesocosms in June 2009.

First Prize

Face Flies and Faecal Fumes – Oviposition of the trachoma vector *Musca sorbens*

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Trachoma is a painful, debilitating disease estimated to be responsible for almost 4% of blindness cases worldwide. The bacteria responsible are transmitted by the Bazaar Fly *Musca sorbens*, native to Africa and Asia. This fly feeds on human nasal and ocular secretions and lays its eggs on faeces lying exposed on the ground, in which its larvae develop. Control of flies has been shown to reduce the incidence of trachoma, but at present little is known about the chemical ecology of this species, and species-specific attractants to bait monitoring or control traps do not yet exist.

Musca sorbens have been found to emerge from a greater proportion of sampled human faeces than from the faeces of any other animal tested. This suggests that human faeces are an optimal breeding medium for *M. sorbens* and hence, in environments lacking basic sanitation, human faeces may be a major source of these flies.

This study aims to identify compounds used by *M. sorbens* to locate a suitable oviposition medium. In trapping experiments carried out in The Gambia in Wali Kunda (a rural fishing village) and Farafenni (a bustling market town s), significantly more female *M. sorbens* were caught from traps baited with human faeces than from those baited with the faeces of any other animal (calf, cow, dog, donkey, horse, sheep), suggesting that human faeces are its preferred oviposition medium. This raises the possibility that compounds produced by human faeces could be oviposition attractants for *M. sorbens*.

These potential attractants were collected using air entrainment, a technique in which charcoal-filtered air is blown over a sample and the odours collected on a polymer matrix from which they can be extracted to produce liquid samples. Analysis of these samples by gas chromatography (GC) and coupled GC-mass spectrometry revealed quantitative and qualitative differences in the volatile chemicals emitted by human faeces and those produced by the faeces of domestic animals used in the study, which also litter the environment in areas where trachoma is prevalent.

Using coupled GC-electroantennography a number of compounds from human faeces have been found to stimulate receptors on the antennae of *M. sorbens* females. It is likely that these are semiochemicals associated with the location of a suitable oviposition site. Further behavioural work will be carried out in the field and using laboratory bioassays to test this hypothesis.

Control of *M. sorbens* by the provision of latrines, reducing the availability of human faeces in the environment, would be desirable as a solution to many other hygiene-related

problems as well as trachoma. There is however a need to accurately monitor fly numbers to determine the success of control interventions. Efficient trapping mechanisms based on semiochemicals such as those sought in this study would be useful for such surveillance and may even be useful a control tool.



Sheep forage in a Farafenni street littered with refuse and excreta, an ideal breeding ground for *M. sorbens* (my own picture).



A faeces-baited fly trap showing a typical afternoon's catch (my own picture).