MOAC project 8

MOAC PhD-project proposal

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Project title: Disease resistance in highly social organisms: a study of the innate immune response in the European honey bee.

Supervisor name	Department/email/phone number	Supervisor's advisor*
Dr Dave Chandler	WHRI / dave.chandler@warwick.ac.uk	
Dr James Bull	Biological Sciences / j.c.bull@warwick.ac.uk	Dr Kevin Moffat (biol sci)

Project proposal:

1. Track record of supervisors in research and supervision
2. Background to research project
3. Research project
   - Originality
   - Feasibility
   - Adventure
4. Scientific strategy
5. Technical training
6. Integration of the multi-disciplinary content (this may include a track record of collaboration between supervisors and an indication of how the student and supervisors will interact)
7. Justification of resources required
8. Project management
9. Plan for what the student will do in the first 3–6 months
10. References

1. Track record of supervisors in research and supervision

Dr Dave Chandler has a key research focus on arthropod-microorganism interactions, in particular the ecology, physiology and exploitation of entomopathogens, the biology of mycophagous insects, and arthropod-fungus interactions in sustainable farming systems. His most recent achievements include the identification and development of entomopathogenic fungi as biocontrol agents of the varroa mite (a major invasive pest of honey bees) (Chandler *et al*., 2001; Davidson *et al*., 2003), demonstration of habitat influences on population structure of insect pathogenic fungi, demonstration that the two major clades in *Agaricus* have evolved different responses to specialist fungus-feeding herbivores (Anderson *et al*., 2006) and the development of population based threshold models of pathogen activity. His current and recent projects include collaborations with Rothamsted Research, Keele University, Cardiff University, and ADAS. He is also a principal investigator on an RCUK funded Rural Economy and Land Use initiative grant to investigate the environmental and regulatory sustainability of biopesticides.
Chandler, D. *et al*. (2001) *Biocontrol Science & Technology*, 11, 429-448. Davidson *et al*. (2003) *Journal of Applied Microbiology*, 94, 816-825. Andersen *et al*. (2006) *Environmental Microbiology*, 8, 1625-1634.

Dr Chandler successfully supervised Marion Andersen's PhD.
Andersen, M. *et al*. (2006). *Environmental microbiology*, 8, 1625-1634. Chandler, D., Andersen, M & Magan, N. (2005). *Proceedings of the BCPC International Congress – Crop Science & Technology*, 309-314. Andersen, M., Chandler, D. & Davidson, G. (1998). *Abstract*, British Mycological Society Conference, University of Southampton). Andersen, M., Magan, N, & Chandler, D. (1999). *Abstract*. Proceedings of the 32nd Annual Meeting of the Society for Invertebrate Pathology.

Dr James Bull has developed his career at the interface between empirical data and theoretical ecology. He obtained his PhD in insect host-microparasite population genetics through the Department of Biological Sciences at Imperial College London. Here, he obtained a thorough training in a wide range of microparasite infection bioassays, laboratory microcosm experimental design, statistical analysis and model fitting. In his subsequent post-doctoral work, first at Imperial College's Silwood Park Campus, with Prof. Michael Hassell and Dr Mike Bonsall, and subsequently at the Institute of Zoology, he has gone on to work on a range of quantitative aspects of host-macroparasite interactions. In particular, he has considerable expertise in the design, maintenance and analysis of controlled, replicated ecological experiments using laboratory-based insect populations. He has experience of modelling both host-microparasite (Bull *et al*., 2001; 2003) and host-macroparasite (Bull *et al*., 2006a; 2006b; 2007; 2008) population dynamics. He is currently an independent research fellow in the Biological Sciences department, where his research focuses on statistical and theoretical modelling of the interaction between deterministic host-parasite population dynamic processes and both demographic and environmental stochasticity.

Bull, J. C. *et al*. (2001) *Appl. Environ. Microbiol.* 67 (11), 5204-5209. Bull, J. C. *et al*. (2003) *Appl. Environ. Microbiol.* 69 (4), 2052-2057. Bull, J. C. *et al*. (2006b) *Parasitology* 132, 565-573. Bull, J. C. *et al*. (2006a) *J. Anim. Ecol.* 75 (4), 899-907. Bull, J. C. *et al*. (2007) *Proc. Roy. Soc. Lond. B.* 274, 87-96. Bull, J. C. & Bonsall, M. B. (2008) *J. Anim. Ecol*., in press.

Dr Bull is a new independent research fellow in the Department of Biological Sciences. His current contract covers the PhD project period and Dr Kevin Moffat (Biol. Sci.) has agreed to act as academic advisor.

2. Background to research project

Most species on earth are parasites (this includes macroparasites and microparasites (a.k.a. pathogens)). They are highly significant drivers of evolution, population dynamics and ecosystem function. The mechanisms used by animals to combat parasites range from innate and adaptive immune responses of individuals to complex social and behavioural methods of disease management. There are significant challenges in science not only to understand how whole organisms change over time in response to pathogen infection (i.e. dynamic systems biology) but also how responses to pathogens at the level of the individual organism are integrated with responses occurring at the level of populations, e.g. social responses (i.e. integrative systems biology). These knowledge gaps are impairing attempts at a holistic understanding of the co-evolution of pathogens and their hosts. They also stand in the way of developing sustainable systems for managing infectious diseases in humans, livestock and wildlife. In particular, there is a need for tractable systems for studying host-pathogen interactions in large groups of social animals.

This project will address the interactions between disease defence mechanisms operating at an organism level, and social and behavioural disease management strategies using honey bees, *Apis mellifera*. These provide an excellent model organism (Traniello *et al.*, 2002). We have established methods in place for rearing and maintaining colonies of different sizes (from a few individuals to tens of thousands), and controlled experiments are relatively straightforward. Recent advances in the completion of insect genome sequences, together with the development of microarray based approaches for transcriptome analysis, have made it possible to assess changes in global gene expression in response to a range of stimuli and to understand the genetic control of physiological and behavioural processes (Barchuk *et al*., 2007).

Our work on honey bee builds on the expertise that we have built up in earlier work studying insect pathogenic microorganisms as biological control agents of the varroa mite, which is a non-native, highly damaging pest of *A. mellifera* (see Chandler *et al.*, 2000, 2001; Shaw *et al.*, 2002; Davidson *et al*., 2003; Andersen *et al*., 2006). Honey bees are one of a select number of organisms with a complex, highly evolved – but well characterised – social structure. Honey bees function in a largely cooperative and ordered society and carry out different functions according to age, sex and social status. The high density populations in honey bee colonies provide ideal conditions for the spread of microbial diseases, and hence there should be a strong selection pressure on the bee innate immune system. However, the honey bee genome encodes fewer proteins associated with immune pathways than other insects that have been sequenced, including *Drosophila* and *Anopheles* (The Honeybee Genome Sequencing Consortium, 2006; Christophedes *et al*., 2002). It has been postulated that this shortfall is compensated by socially-mediated defences. The role of most of the innate-immunity components remains to be validated, along with understanding how gene expression varies in response to different pathogens, in different bee tissues and according to the life stage, age, caste and social status of the bee.

The genome sequences of *A. mellifera* and some of its pathogens were published at the end of 2006 (The Honeybee Genome Sequencing Consortium, 2006) and the microarray made available shortly thereafter. Our baseline experimental tool for this project will be an *A. mellifera* multipak oligo-array that we have developed with Agilent as part of a Warwick RDF strategic award in 2007/8 and based on the official *A. mellifera* gene set. We had previously validated an array developed by the University of Illinois at Urbana-Champaign (UIAC) (Malik *et al*., in prep). We will also make extensive use of quantitative RT-PCR of selected genes, representing a cost-effective experimental approach. This project will be conducted as a collaboration between Warwick HRI (D. Chandler, E. Ryabov), Biological Sciences (J. Bull) and Rothamsted Research (J. Pell, J. Osborne). Microarray resources have been secured through an RDF strategic award. Further molecular consumables (RT-PCR, RNAi), as well as insect bioassay work, are requested from MOAC, detailed below. Further, J. Bull currently has an equipment grant under consideration by the Royal Society and we are in contact with BBSRC as well as the CB Dennis Trust.

3. Research project

Honey bees have few macroparasites compared to microparasites, relative to other insects. This could be due to the ability of honey bees to integrate behavioural and innate immune responses. We will use single and co-infection experiments with the macroparasite *Varroa destructor* and the microparasite Deformed Wing Virus (DWV). This experimental host-pathogen assemblage is particularly timely and relevant due to the emergence of varroa as a natural macroparasite of bees, following a host shift from *Apis cerana* (Chandler *et al*., 2001). Varroa infection is typically associated with DWV in natural host populations (Yang & Cox-Foster, 2005) and may be associated with the sudden collapse of the whole bee colony. We hypothesise that host susceptibility to either parasite will be increased when co-infection occurs due the additional pressure on host immune system. This will be quantified using gene expression (qrt-pcr) studies and mortality bioassays.

Our second hypothesis is that host immune response varies between bee castes: in particular workers (nurse vs. forage bees), queens (which may be reared from worker eggs), and drones. We envisage that the integrated host response to parasitic challenge comprises innate immune and behavioural aspects. It is known that the behavioural components of this response are caste specific. It is unknown whether the innate responses are caste specific.

Our RDF strategic award, has allowed us to use microarrays to carry out a genome-wide comparison of the transcriptional responses to fungal pathogens. Microarray analysis will allow us to identify the differences in (immune) gene expression profiles in response to exposure to pathogens in the different groups of honeybees. The data on up- and down-regulation will be used to propose models of the immune response (antimicrobial resistance gene activation). Epigenetic (RNAi) knockouts of the expression of the candidate genes by dsRNA microinjection will be carried out to assess the physiological roles of these genes in the immune response (Nelson *et al*., 2007).

Our ultimate goal is not only to describe statistical associations between observations made at each of three experimental levels (population, individual, genetic) but to develop a mechanistic understanding of the whole system, allowing predictions to be made at the population level, based on information obtained at individual and genetic levels.

Mechanistic population dynamic models of host-parasite interactions are traditionally developed using population level observations on abundance, coupled with individual life history and behavioural characteristics. Further to this, individual responses to interactions with biotic and environmental forces are underpinned by gene expression. Bioinformatic analysis of gene expression data, informed by phenotypic observations at the individual level will be used to link genetic and individual level organisation using forward genetic approaches. This mechanistic understanding across organisational levels will allow us to make reverse genetic predictions about the effects of manipulating gene expression (through RNAi microinjection) on population level responses to disease.

4. Scientific strategy

The system

- This is a multilayer system both in terms of trophic interactions (honeybee, macroparasites, microparasites) and experimental approaches (transcriptional profiling and an ability to study behaviour from the individual to the group, this system is also highly amenable to manipulation in controlled and replicated ecological experiments). Methods are in place for maintaining bees singly and in groups and for studying their behaviour at different spatial scales from bee-bee communication to foraging behaviour in the field.
- Annotated honeybee genome data: the oligonucleotide microarray contains circa 14K genes, comprising selected genes from chalkbrood and DWV plus all annotated genes of *A. mellifera*. This includes identified (putative) genes involved in innate immunity (Toll, IMD pathways) and in RNA antiviral response (RNAi).
- Reverse genetic system: epigenetic RNAi knockout by microinjection of whole insects with dsRNA.
- 'Dry' components of the system include: (a) analysis of transcriptional profiles (honeybee microarray); (b) identification of genes up/down-regulated in the course of infection in different treatments.

Workplan

The project will be done as follows:

1. Bioinformatic analysis. Global expression profiles from current microarray experiments will allow mechanistic hypotheses about the roles on traditional innate and behavioural responses to infection to be generated.
2. Quantifying pathogenesis. The existing method used to infect adult and larval honey bees, which was developed in previous work on varroa-bee-pathogen interactions, will be optimised for this project. In particular, pathogen doses will be established giving the most workable time course for infection for the remainder of the study.
3. Molecular experiments. Quantitative real time PCR experiments will be undertaken to further quantify expression of genes which are differentially expressed following single and co-infection.
4. Data analysis and interpretation. Statistical inference about underlying immune mechanisms based on differentially expressed genes. Development of a model for antimicrobial response in different bee castes and groups vs individuals.
5. Knockouts. Assessment of the effect of epigenetic RNAi knockout of candidate key (hub) genes identified in this project.

5. Technical training

The student will receive a wide range of training in cutting edge molecular techniques based around quantitative real time PCR and microarrays, as well as specialist Honeybee bioassay and infection skills in the laboratory of Dr Dave Chandler. In addition, Dr James Bull will supervise the student in the development of statistical modelling techniques and inference.

6. Integration of the multi-disciplinary content (this may include a track record of collaboration between supervisors and an indication of how the student and supervisors will interact)

This project forms a logical progression of an established collaboration between Drs Dave Chandler (WHRI), James Bull and Kevin Moffat (both Biol. Sci.). In September 2007, Drs Chandler and Bull were awarded a Warwick Research Development Strategic Award in order to develop microarray analysis of Honeybee genome expression in response to disease. This in now part of an on-going collaboration with Dr Judith Pell at Rothamsted Research. Further, Drs Chandler and Bull have recently been joined by Dr Moffat in order to extend their investigation into global expression in response to pathogens, in *Drosophila* hosts. They are currently co-supervising a Warwick Systems Biology mini-project with the aim of translating their established Honeybee system into *Drosophila*.

7. Justification of resources required

Resources fall into three categories: Bioassay, molecular and computational.

Bioassay costs are nominal and fall within the existing budgets of Drs Chandler and Bull. Microarray costs have been met by a Warwick Research Developments Fund Strategic Award. This project will develop quantitative real time PCR techniques and the majority of requested funds are for Qiagen kits and associated general molecular consumables. We request a standard desktop pc, with associated consumables, for dedicated use by the student, in order to carry out data analysis and preparation of results for publication. The only specialist software required is the statistical programming platform, R. This is open source and no specific funds are requested for this.

We request 2K per annum for each of the three years for continuing molecular and computer consumables.

8. Project management

The majority of this project is practical in nature, and will be carried out in the laboratory of, and under the supervision of, Dr Chandler, at WHRI. Further assistance in specific molecular techniques will be provided by Dr Eugene Ryabov (WHRI). The success of this integrated project relies strongly on close coupling of mechanistic hypotheses and empirical data. This will be an iterative process and will require the student to spend some time working with Drs Bull and Moffat in the Department of Biological Sciences. The exact distribution of time between theory and practice will depend somewhat on the interests and aptitudes of the student.

9. Plan for what the student will do in the first 3–6 months

The aim for the first 6 months will be to take existing whole genome (microarray) data from infected Honeybees and develop mechanistic hypotheses about the role of behavioural and classical innate immune responses to pathogen infection.

1. Get to grips with existing Honeybee bioassay techniques.

2. Assist in the analysis of existing microarray data from global expression of the Honeybee genome.

3. Develop specific mechanistic hypotheses (through analysis of microarray data and background reading), identifying specific genes for qrt-PCR investigation.

10. References

Andersen *et al*. (2006) *Environmental Microbiology*, 8, 1625-1634. Barchuk, A.R. *et al*. (2007) *BMC Developmental Biology*, 7, 70. Chandler, D. *et al*. (2001) *Biocontrol Science & Technology*, 11, 429-448. Chandler, D. *et al*. (2000) *Biocontrol Science & Technology*, 10, 357-384. Christophides, G.K. *et al*. (2002) *Science*, 298, 159-165. Davidson *et al*. (2003) *Journal of Applied Microbiology*, 94, 816-825. Malik, N., Chandler, D. & Ryabov, E. *Journal of Invertebrate Pathology*, in prep. Nelson C.M. *et al*. (2007) *PLoS Biol*ogy, 5:e62 Shaw *et al*. (2002) *Biological Control* 24, 266-276. The Honeybee Genome Sequencing Consortium. (2006) *Nature*, 443, 931-949. Traniello, J.F.A. *et al*. (2002) *PNAS*, 99, 6838-6842. Yang, X. & Cox-Foster, D. L. (2005) *PNAS*, 102, 7470-7475.