**BBSRC London Interdisciplinary Biosciences PhD Consortium (LIDo) Project Form**

*Detailed criteria for project approval are listed in the accompanying LIDo Project Vetting Guidelines*

*Projects must be submitted online via the LIDo portal. Paper/Electronic forms will not be accepted under any circumstances. This form is provided strictly as an aide to preparing a proposal ahead of the online submission.*

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|  | Primary Supervisor | Secondary Supervisor |
| Name | Elli Leadbeater | Michael Antoniou |
| Department | Biological Sciences | Medical and Molecular Genetics |
| Institution | Royal Holloway University of London | King’s College London |
| E-mail | Elli.Leadbeater@rhul.ac.uk | michael.antoniou@kcl.ac.uk |
| Website (optional) | <http://ellileadbeater.wixsite.com/insectcognition>; <https://pure.royalholloway.ac.uk/portal/en/persons/elli-leadbeater(a2edc845-5d79-4c83-bab4-6c58102f485f).html> | <https://www.kcl.ac.uk/lsm/research/divisions/gmm/departments/mmg/researchgroups/antonioulab/index> |
| Phone (optional) |  | 020 7848 8501 |
| If you have additional supervisors, please include details here: | | |

**ELIGIBILITY, FUNDING AND RESOURCES (visible to the LIDo Management and Research Committees only)**

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|  | **Primary Supervisor** | **Secondary Supervisor** |
| Title and Duration of Appointment  *(Must hold centrally funded position at lecturer-level or above or a lecturer-level fellowship1 for the entire PhD duration.)* | Professor of Ecology and Evolution | Reader in Molecular Genetics |
| Give details of:  1. RCUK grant funding  2. Other funding.  *Please list:* ***Funding body;******grant type; grant title, start and end dates, and amount*** | ERC Starting Grant: BeeDanceGap, Feb 2016-Jan 2021; €1 422 110  Leverhulme Trust Research Project Grant: Cognition in the Wild, £249 516; Aug 2017- Jul 2020 | Sustainable Food Alliance (USA) project grant: [Beneficial effects of organic food consumption and toxic effects of glyphosate residues: ill-health biomarkers by a multiomics approach](https://kclpure.kcl.ac.uk/portal/en/projects/beneficial-effects-of-organic-food-consumption-and-toxic-effects-of-glyphosate-residues-highlighting-illhealth-biomarkers-using-molecular-signatures-measured-by-a-multiomics-approach(a5128295-1558-45b9-9aa2-0ef1c503f355).html). £694,663; 2016-2020  Breast Cancer UK project grant: [Evaluating the cancer-causing potential of bisphenol combinations in primary mammary epithelial cells](https://kclpure.kcl.ac.uk/portal/en/projects/evaluating-the-cancercausing-potential-of-bisphenol-combinations-in-primary-mammary-epithelial-cells(64f8c4c8-9914-45a4-8733-98dec156d7ab).html). £45,000; 2018-2020  Sustainable Food Alliance (USA) project grant: [Highlighting the health benefits of an organic diet through analysis of the gut microbiome in human subjects](https://kclpure.kcl.ac.uk/portal/en/projects/highlighting-the-health-benefits-of-an-organic-diet-through-analysis-of-the-gut-microbiome-in-human-subjects(ff9bec8f-8d5a-4e7b-9222-27ba782beb38).html). £130,252; 2018-2020  DEBRA UK project grant: Spray-on gene/cell therapy for RDEB. £174,022; 2019-2021  BBSRC LIDo iCASE PhD studentship; industrial partner MilliporeSigma; Defining ubiquitous chromatin opening element (UCOE) molecular mechanisms of action. £185,000; 2020-2024  Sustainable Food Alliance (USA) PhD studentship: Identifying the effects of pesticides on intestinal permeability and gut-bacterial dysbiosis. £163,775; 2020-2023.    Sustainable Food Alliance (USA) project grant: Using nanopore nucleic acid sequencing to deepen understanding of the effects of glyphosate on the gut microbiome of mammals and pollinating insects. $57,000; 2020-2021 |
| Additional Supervisors  *(Indicate the title and funding of any additional supervisors as above.)* |  | |
| Resources required and available for project  *(Briefly describe the consumables, animals, computing and specialist equipment required for the project, including cost estimates. Explain how there will be adequate financial support and appropriate access to resources for the entire PhD project.)* | The project costs will fall within the LIDo support budget of £5000/year. Equipment for ecological experiments is already available within the primary supervisor’s research group. Triple-quad access and training charges for mass spectrometry have been included within the budget. Microbiome sequencing costs are based on inclusion of 60 samples.  Y1:  Bumblebee colonies (60, raised from wild-caught queens): no cost  Bumblee rearing consumables: £500  Insecticides: £300  Nestboxes: £500  **Subtotal: £1300**  Y2:  Mass Spectrometry consumables: £3900  QQQ access charges: £1250/month for 2 months = £2500  **Subtotal: £6400**  Y3:  Illumina Amplicon Sequencing of Gut Microbiome samples (60 samples): £5000  Sampling consumables: £1000  Commercial bumblebee colonies @£60/ colony: £300  **Subtotal: £6300**  **Total: £14000** | |

**Current and Previous PhD Students (visible to the LIDo Management and Research Committees only)**

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|  | **Primary Supervisor** | **Secondary Supervisor** |
| Please provide details of all PhD or PhD candidate students supervised **as primary supervisor** during the last 6 years *(inc. dates of submission and completion).* | Sept 2019- ongoing: Cecylia Watrobska, RHUL (Main supervisor; BBSRC IC-RHUL DTP)  Sept 2017- ongoing: Romain Willemet, RHUL (Sole supervisor; Joan Warrington Studentship)  Aug 2017- ongoing: Gregoire Pasquier, RHUL (Sole supervisor; Leverhulme Trust)  Sept 2016- Aug 2019 (submitted August 2019; completion Oct 2019): Harry Siviter, RHUL (Main supervisor; Reid Scholarship)  Sept 2014- June 2019 (submitted March 2019, completed August 2019): Ash Samuelson, RHUL (Main supervisor; BBSRC DTP) | Saba Hussain; Defining ubiquitous chromatin opening element (UCOE®) molecular mechanisms of action to expedite their biotechnological applications. BBSRC LIDo iCASE Studentship (MilliporeSigma as industrial partner); starts Oct 2020  Scarlett Ferguson; Identifying the effects of pesticides on intestinal permeability and gut-bacterial dysbiosis. Sustainable Food Alliance (USA) PhD studentship; starts Oct 2020  Bethany McCloskey; Advancing the UCOE® gene expression platform; BBSRC iCASE Studentship (MilliporeSigma as industrial partner); submitted Sept 2019, completed June 2020.    Christina Flouri; Gene therapy for Haemoglobinopathies: Enhancing Lentiviral Vector Therapeutic Efficacy; BRC studentship; submitted Sept 2018, completed Mar 2019  Petros Patsali; Advanced personalized gene therapy of β-thalassaemia; Cypriot government funded; submitted Sept 2016, completed Dec 2016    Coralea Stephanou; Advancing lentiviral gene therapy vectors for β-thalassaemia; Cypriot government funded; submitted May 2015, completed Nov 2015    Omar Anakok; Functional dissection of the *HNRPA2B1*-*CBX3* ubiquitous chromatin opening element (A2UCOE); Turkish government funded; submitted Jun 2015, completed Dec 2015 |
| Please explain if any of the above students did not submit their PhD within 4 years of starting, or if any of the students were not awarded a PhD. | N/A | N/A |
| Training and Mentoring  *(Indicate any supervision training courses attended. New PIs yet to supervise a PhD student to completion should indicate additional sources of mentoring and support here.)* | RHUL Mentoring Skills Workshop March 2014  RHUL PhD Supervision Workshop | KCL Research Degree Supervision – Refresher Training Workshop (Webinar)  May 2020 |

**Information for projects website (published on public website)**

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| **Title (limit 20 words)** |
| Bee conservation: Evaluating “inactive” co-formulants as potentially critical amplifiers of threats posed by agricultural insecticides |

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| **Abstract (limit 100 Words):** |
| A wealth of evidence now shows that agricultural insecticides can severely impact social bees at the individual, colony and population levels. Yet we suggest that this research may have underestimated the true impacts of insecticides in the field, because both licensing procedures and academic research focus disproportionately upon active ingredients, almost ignoring the co-formulants that are specifically designed to amplify product efficacy. In this project, we will test the hypothesis that commercial insecticide formulations may present a significantly greater, and yet unrecognized, risk to wild social bees than the isolated AIs upon which ecological risk-assessments are typically based. |

**Information for students (published in password protected area of website)**

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| **PhD project: aims and description (limit 300 words)** |
| *Describe the background and overall goals of the PhD project. Indicate the roles of the primary and secondary supervisors. It must be feasible for students to complete this project and write a thesis in years 2-4 of the LIDo programme.*  In recent decades it has become clear that the insecticides upon which intensive agriculture currently relies can have devastating impacts on non-target insects, and particularly social bees. Abundant evidence now documents a mismatch between the predicted ecological consequences of agrochemical use based on pre-licensing testing, and real-world impacts at the landscape scale. We propose that one source of this disparity is a major knowledge gap regarding the effects of co-formulants in commercial agrochemicals. Co-formulants can be more toxic than the active ingredient (AI; Mesnage, Benbrook, Antoniou, 2019) and are specifically designed to render the product more effective (e.g. by penetration of the insect cuticle or increasing stability of the AI). In this project, we will test the hypothesis that commercial insecticide formulations may present a significantly greater, and yet unrecognized, risk to wild social bees than the isolated AIs upon which ecological risk-assessments are typically based.  The supervisory team combines the expertise of Elli Leadbeater (RHUL; social insect behavior, ecology), and co-supervisor Michael Antoniou (KCL; molecular biology; molecular & cellular toxicology; gut microbiome), with the student spending time in both research groups. At RHUL, we will use a large-scale field protocol (Siviter, *et al,* Leadbeater 2019 Nature) to quantify the real-world effects of insecticide formulations relative toAI alone (Y1). Mass spectrometry (GCMS; LCMS) will be used to compare AI residues in bees that have experienced contact exposure to formulations *vs* AI (Y2). A potential mechanism by which “inert” co-formulants elicit mortality is through unexpected effects on the gut microbiome that render individuals vulnerable to infection (Motta *et al* 2018 PNAS). Thus, in Y3 we will perform metagenomics of the gut microbiota of exposed and control bees. Together, we envisage that these approaches will create a potentially high-impact project that explores a major gap in our understanding of pollinator declines. |
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| **Rotation project: brief description (limit 250 words)** |
| *The rotation should be a self-contained research project that normally forms a part of the bigger PhD project above. Students that continue their PhD in the rotation lab can therefore normally include results from the rotation in their PhD thesis. Include a timeline indicating how this project will be completed in 3 months.* |
| In March 2018, the EU banned the use of most neonicotinoid insecticides for outdoor agricultural use, based on a large body of evidence concerning threats to pollinators. Sulfoximine-based insecticides are rapidly emerging as successors, and are now licensed in over 80 countries globally. We have recently shown that exposure to Sulfoxaflor- the only currently marketed sulfoximine- negatively impacts the reproductive success of bumblebee (*Bombus terrestris*) colonies, using the active ingredient alone. However, in the real world, Sulfoxaflor is delivered in several commercial formulations, including for horticultural use in the UK. Given that these formulations are designed to increase the effectiveness of the active ingredient (see PhD description), we expect that current research may be severely underestimating the risk posed by sulfoximines. Accurate assessment of this risk is nonetheless critical because the EU is currently reviewing the registration status of Sulfoxaflor (as will the UK, upon leaving the EU).  In this rotation project, we will perform an initial assessment of the toxicity of commercial formulations of Sulfoxaflor for bumblebees, using an LD50 assay. LD50 protocols, which estimate the minimum lethal dose of a toxin, are a staple of ecotoxicological testing and thus we are confident that the project can be completed with a very strong sample size within 3 months (1 month pilots; 1 month testing; 1 month analysis). The project will form a stand-alone study with conservation implications that will be of very wide interest, and will provide essential guidance on insecticide choice for the subsequent PhD. |

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| References related to the project (limit 5): | |
| 1 | Siviter H, Brown MJF and Leadbeater E (2018) Sulfoxaflor exposure reduces bumblebee reproductive success. Nature 561:109-112, doi:10.1038/s41586-018-0430-6 |
| 2 | Mesnage R, Benbrook C and Antoniou MN (2019) Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. Food and Chemical Toxicology 128: 37-145 |
| 3 | Mullin CA, Chen J, Fine JD, Frazier MT and Frazier JL (2015) The formulation makes the honey bee poison. Pesticide Biochemistry and Physiology 120 (2015) 27–35 |
| 4 | Siviter H, Horner J, Brown MJF and Leadbeater E (2019) Sulfoxaflor exposure reduces egg-laying in bumblebees (*Bombus terrestris*). Journal of Applied Ecology, 00:1–10. https ://doi.org/10.1111/1365‐2664.13519 |
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| **Techniques / Approaches:**  **Describe the specifics next to the area(s), which are appropriate. (limit 50 words per box)** | |
| Molecular Biology | 16S rRNA sequencing of gut microbiome. The student will analyse faecal bacterial composition by amplifying hypervariable regions of 16S rRNA. We (KCL) currently use the MiSeq platform (illumina sequencing), but may transition to nanopore-based sequencing. The most cost-effective approach will be used (sequencing in collaboration with Charles A Mein; QMUL). |
| Genetics |  |
| Biochemistry / Biophysics | Mass spectrometry is required to identify insecticide residues. The student will develop a QuECheRs method to identify residues in whole bees that have been exposed to various insecticide formulations and will receive training in the analysis of MS-QQQ data from RHUL’s MS technical support (Francesca Mealor) |
| Chemistry |  |
| Microscopy / Electrophysiology |  |
| Image Processing |  |
| Simulation / Modelling |  |
| Bioinformatics | Compositional data analyses of gut microbiome samples will be undertaken by the student at KCL. For this purpose, the student will be trained in using the statistical programming language R, the general purpose programming language Python, and the Bash command language. |
| Mathematics / Statistics | Generalised linear modelling and Generalized additive modelling approaches to ecological data using the R programming language; training provided at RHUL. |
| Engineering |  |

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| **Explain how this project is interdisciplinary (50 words):** |
| The project relies upon tools external to the field of ecology (microbiology, and specifically, molecular profiling of microbiological communities) to understand the drivers of potentially catastrophic pollinator decline. To this end, the student must receive training in molecular (KCL), microbiological (KCL) and ecological (RHUL) techniques, and in mass spectrometry (RHUL). |

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| **Area of Biology:**  **(select up to 2 best fits)** | | **BBSRC Research Committee Area:**  **(select one only)** | | |
|  | Cell Biology |  | | Animal disease, health and welfare |
|  | Neurobiology | X | | Plants, microbes, food and sustainability |
|  | Development |  | | Genes, development and STEM\* approaches to biology |
| x | Physiology |  | | Molecules, cells and industrial biotechnology |
|  | Structural Biology |  | |  |
|  | Chemical Biology | **BBSRC New Ways of Working: (select best fit(s))** | | |
|  | Biotechnology |  | State-of-the-art Imaging | |
|  | Microbiology | x | Large-scale (bioinformatics) data analysis or resource development | |
|  | Genetics |  | Computational or mathematical modelling | |
|  | Ageing |  | New approaches to biomolecular characterization | |
| x | Evolution |  | Synthetic biology | |
|  | Immunology |  | Integrative and Systems biology | |

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| **BBSRC Strategic Priority: (select best fit(s))** | |
|  | World Class Basic Bioscience |
| x | Agriculture & Food Security (including animal pathogens) |
|  | Industrial Biotechnology and Bioenergy |
|  | Bioscience Underpinning Health (including ageing) |
| **BBSRC Responsive Mode Priority: (if applicable)** | |
| x | Animal health |
|  | Bioenergy: generating new replacement fuels for a greener, sustainable future |
|  | Combatting antimicrobial resistance |
|  | Data driven biology |
| x | Food, nutrition and health |
|  | Healthy ageing across the life course |
|  | New strategic approaches to industrial biotechnology |
|  | Reducing waste in the food chain |
|  | The replacement, refinement and reduction (3Rs) in research using animals |
|  | Sustainably enhancing agricultural production |
|  | Synthetic biology |
|  | Systems approaches to the biosciences |
|  | Technology development for the biosciences |
|  | Welfare of managed animals |