Project title: Behavioral Toxicology Fingerprinting: A High-Throughput Framework for Assessing Sub-Lethal Pesticide Effects Across Drosophilidae to Protect Beneficial Insects

Lead Supervisor and Department: Dr. Giorgio Gilestro, Department of Life Sciences (see also **https://lab.gilest.ro**)

Co-Supervisor(s) and Department(s): Dr. Nikolai Windbichler, Department of Life Sciences

Project Description:

Current pesticide regulatory frameworks rely heavily on lethal endpoints and single-species testing, creating critical blind spots. Sub-lethal behavioral disruption—such as impaired foraging, altered sleep-wake cycles, or reduced predator avoidance—can have devastating ecological consequences at field-relevant concentrations that cause no mortality in standard toxicity tests. Furthermore, screening for non-target effects on beneficial insects (pollinators, natural enemies of pests) remains prohibitively slow and expensive, often requiring 12-18 months per compound. This regulatory bottleneck delays the adoption of safer alternatives and allows ecologically harmful compounds to remain in use.

Over the past two decades, our laboratory has established a pioneering track record in developing open-source tools for high-throughput behavioral phenotyping in *Drosophila*. This includes pySolo (Bioinformatics 2009; Nature Protocols 2012), a software suite for automated sleep analysis that became widely adopted as a field standard; ethoscopes (PLoS Biology 2017), a Raspberry Pi-based platform enabling real-time video tracking and closed-loop behavioral manipulation using artificial intelligence; and rethomics and ethoscopy (PLoS One 2019; Bioinformatics Advances 2024), comprehensive R- and Python-based analytical frameworks applicable to sleep and circadian rhythm analysis in any organism. This infrastructure provides a unique foundation for addressing toxicological questions requiring both high throughput and fine behavioral resolution.

Most recently, we introduced *coccinella* (Jones et al., eLife 2023), which combines ethoscopes with highly comparative time-series analysis (HCTSA/Catch22) to extract behavioral fingerprints from simple velocity measurements. Despite its reductionist design, coccinella achieved 71% accuracy in classifying 17 neuroactive compounds and 44.5% accuracy across 40 compounds—outperforming state-of-the-art high-resolution pose-estimation systems (DeepLabCut + B-SOiD) while costing a fraction of the price (£75 per recording unit vs. £10,000+ for industrial camera setups). Crucially, coccinella successfully identified biologically meaningful differences in sleep rebound following different deprivation regimes, independently validating the established 5-minute sleep threshold in *D. melanogaster*—demonstrating that the system captures genuine biological signal rather than technical artifacts (Jones et al., eLife 2023).

This project will leverage our technological infrastructure to address two critical regulatory gaps. **First**, we will systematically characterize behavioral toxicity fingerprints at environmentally relevant concentrations (1-1000 ppb) spanning sub-lethal to lethal ranges. We will compare conventional pesticides against next-generation biological alternatives (RNAi insecticides, antimicrobial peptides, engineered bacteriophages) to identify safer compounds. By analyzing multi-dimensional behavioral features—including circadian rhythm disruption, locomotor patterns, stress-responsive behaviors, and sleep architecture—we will establish sensitive biomarkers that detect neurotoxicity 100-1000× below lethal thresholds. Experiments with resistance-conferring mutants (RdlA301S for dieldrin resistance, ParaL1029F for DDT/pyrethroid resistance) will validate that behavioral changes reflect specific target engagement rather than generic stress responses.

Second, we will implement comparative toxicology across 8-10 *Drosophila* species with documented phylogenetic relationships and varying pesticide sensitivities. By correlating species-specific behavioral fingerprints with phylogenetic distance and known target site sequence divergence, we will develop predictive models estimating effects on beneficial insects—including pollinators (bees, hoverflies) and natural enemies of pests (parasitoid wasps, predatory beetles)—without requiring direct testing on these protected species. This comparative framework will identify compounds with high target:non-target selectivity ratios, accelerating regulatory approval for pest-specific agents while flagging broad-spectrum toxicants that threaten ecosystem services.

We will generate an open-access database linking chemical structures, behavioral toxicity profiles, and phylogenetic predictions—creating a rapid pre-screening tool that regulatory bodies can deploy before expensive multi-generational or non-target organism studies. We will engage directly with UK regulatory agencies (HSE, DEFRA) and European authorities (EFSA) to integrate behavioral biomarkers into pesticide risk assessment frameworks. By establishing sub-lethal behavioral disruption as a regulatory endpoint and providing validated cross-species prediction tools, this project will accelerate the phase-out of ecologically harmful pesticides while de-risking the approval pipeline for safer alternatives—ultimately protecting pollinator populations and agricultural sustainability.

To apply:
Please email g.gilestro@imperial.ac.uk with the following documentation:
▪ Statement of Purpose
▪ Your CV
▪ At least two references must be emailed to *Giorgio Gilestro* (by the referees)