Ref: 06/JA

Project Title: Identification of germline-specific introns for the spatial and temporal control of Cas9 in *An. stephensi*

Supervisors: Joshua Ang Xin De, Luke Alphey, Michelle Anderson **Research group:** Arthropod Genetics

Project Summary:

Our lab is interested in developing Cas9-dependent gene drive systems which could lead to suppression of local mosquito populations. Gene drive is a genetic engineering technology which increases the likelihood that a gene may be inherited, potentially allowing it to spread rapidly through a population over several generations. One potential use of such drive is to modify mosquitoes so that they can pass on genetic traits that result in infertile female offspring. The success of the system, however, depends heavily on the ability to restrict Cas9 to a precise location and time in the germline of a developing mosquito. Leaky expression of Cas9 outside of that time and place could cause unintended sterility and also result in dissemination of cut-resistant alleles into the population, which can prevent the spread of the gene drive. In fact, both phenomena have been shown to impede the continual propagation of the drive element in *An. gambiae* and *An. stephensi* (Gantz et al., 2015; Hammond et al., 2016). This project will therefore aim to identify *An. stephensi* genes which are differentially spliced in the gonads and determine the intronic sequences. If more time is available after the introns are successfully characterised, they will then be assessed for their abilities to control the expression pattern of Cas9 in a transgenic mosquito.

Details:

Depending on progress, the successful student may undertake the following in the duration of the research project:

- polymerase chain reaction (PCR)
- design of plasmids for mosquito transgenesis
- microinjection of mosquito embryos to generate transgenic mosquitoes
- mosquito rearing and dissection of mosquito gonads
- DNA and RNA extraction
- reverse transcription PCR (RT-PCR)

References for Suggested Reading:

1. Gantz, V. M., Jasinskiene, N., Tatarenkova, O., Fazekas, A., Macias, V. M., Bier, E., & James, A. A. (2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(49), E6736–E6743. https://doi.org/10.1073/pnas.1521077112
2. Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., … Nolan, T. (2016). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. *Nature Biotechnology*, *34*(1), 78–83. https://doi.org/10.1038/nbt.3439

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Please email your CV (no more than two sides of A4) and a covering letter detailing why you would like to undertake the placement and the knowledge and skills that you will bring to the Institute to yvonne.walsh@pirbright.ac.uk.
Closing date to apply: 31.01.20