Older, wiser and less infectious? Exploring the impact of mosquito age on *Plasmodium* transmission

Identifying mechanisms to block *Plasmodium* transmission requires a deeper understanding of the factors that affect the vector competence of the *Anopheles* mosquito. One such factor is mosquito age. Senescence has been well described in *Drosophila*, characterised by increasing dysbiosis, loss of barrier integrity, oxidative stress, and changes to immune competency, which are underpinned by shifts in gene expression. The midgut is particularly susceptible to age-related disruption and serves as the epicentre of mosquito-*Plasmodium* interactions, exposing *Plasmodium* to a dysregulated environment. However, knowledge of both mosquito ageing and the capacity of *Plasmodium* to respond to vector age is limited. We have therefore investigated the changes associated with mosquito ageing and the effect they have on the parasite's development.

Initially, we investigated the gene expression changes that occur in the midguts of mosquitoes as they age. Uninfected *An. stephensi* midguts were pooled at emerged, young, middle-aged, and old timepoints (1, 7, 14, and 21 days post-emergence) and underwent RNA sequencing. Transcriptome analysis revealed age-related clustering and shifts in gene expression profiles with increasing age. Older mosquitoes expressed fewer genes associated with membrane transport and carbohydrate metabolism and exhibited increased numbers of oxidative stress, aberrant protein folding, innate immune response, and cellular proliferation-associated genes. To examine whether these impacts affect the parasite's phenotype, *An*. *stephensi* females were infected with *P. berghei* at young, middle-aged, and old timepoints (7, 14, and 21 days post-emergence). Metrics of parasite development (from ookinete to sporozoite stages) were measured over the 21-day extrinsic incubation period using fluorescent microscopy. The capacity to transmit to a naïve host was also explored. As expected, parasite phenotype differed significantly with vector age. Younger mosquitoes exhibited increased vector competence, characterised by higher infection burdens, increased oocyst size and faster maturation rates, which declined with increasing age. Interestingly, old mosquitoes were unable to transmit to a naïve host, despite the presence of sporozoites in dissected salivary glands. This data provides preliminary evidence of midgut senescence as well as plastic parasite development in response to vector age at the time of infection.