Schistosomes – *old questions, new technologies*

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Schistosomes are agents of Schistosomiasis, a major Neglected Tropical Disease (NTD) that affects more than 250 million people worldwide. With two separate sexes — a heterogametic female (ZW) and a homogametic male (ZZ) — schistosomes are the exception among flatworms, which are largely hermaphrodites. However, the phenotypic sexual dimorphism of the parasite only becomes apparent by adulthood within the mammalian host, and not during other developmental stages. Male and female worms undergo separate but concurrent sexual differentiation of their gonads and somatic tissues that eventually allows intersexual pairing, a critical step for egg production and life cycle propagation. This is a key but poorly understood process in early intra-mammalian development of schistosomes. To tackle this knowledge gap, we used functional genomics in tandem with cutting-edge molecular tools and focused on two critical developmental transitions: (1) the cercaria–schistosomulum, i.e. from free-living infectious larva to the first intra-mammalian parasitic stage; (2) sexually monomorphic–dimorphic intra-mammalian developmental stages. For transition (1), single cell transcriptomics of female or male cercariae and two-day old (D2) schistosomula revealed sex-biased expression across different cell clusters in both early sexually monomorphic developmental stages. We are currently confirming these findings by droplet digital PCR (ddPCR), that will be followed by spatial validation and functional characterisation of informative genes by *in situ* hybridisation and RNAi, respectively. For transition (2), we accurately determined the timing of sexual dimorphism being established *in vivo*, by morphological analysis and confocal imaging of individual parasites collected from mice infected with either male or female parasites. Preliminary single cell RNA-seq data identified tentative cell populations involved with this sexually monomorphic–dimorphic transition. In parallel, we have been refining culture systems that facilitate the *in vitro* study of schistosome development, including the establishment of dimorphism. We have also been exploring long term gene silencing protocols that include genome editing mediated by CRISPR-Cas, and the long-term preservation of schistosome developmental stages. The latter would positively impact on the 3Rs (i.e. Replacement, Reduction and Refinement) in the use of animals for research. 'Omics' approaches coupled with cutting-edge cellular and molecular technologies, including single cell transcriptomics, *in vitro* long-term culture, and gene perturbation mediated by RNAi and genome editing will shine new light on schistosome biology and help to expose targets for novel control strategies of this major NTD.