

Single cell phenotyping to understand sexual dimorphism in schistosomes

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Schistosomes are parasitic flatworms that cause Schistosomiasis, a major Neglected Tropical Disease (NTD) that affects more than 250 million people worldwide. With distinct sexes, a heterogametic female (ZW) and a homogametic male (ZZ), schistosomes are an exception among flatworms, which are largely hermaphroditic. Sexual dimorphism in schistosomes becomes most apparent by adulthood within the mammalian host. The cellular and molecular mechanisms underlying the sexual differentiation of these parasites are poorly understood, partly due to intrinsic challenges in assessing their development *in vivo*. Therefore, robust and reproducible approaches for maintaining and developing parasites *in vitro* are needed to overcome these difficulties. However, to date, only a few studies have focused on protocols that allow cultured parasites to reach sexually dimorphic stages, and none have been reproduced, limiting the ability to understand their unique sexual biology. Here, we refine a protocol for long-term culture of newly transformed cercariae that developed into sexually dimorphic forms *in vitro*. We assessed the effect of two different sera, Foetal Bovine Serum (FBS) and Human Serum (HS), added to the culture medium supplemented with human red blood cells. We found that in contrast to FBS-cultured worms, HS-cultured parasites digested red blood cells, a crucial step for long term parasite development. Additionally, while most FBS-cultured parasites did not progress beyond an early liver stage, sexual dimorphism was clearly established in the HS-cultured worms, albeit delayed compared to *in vivo* development. Moreover, EdU pulse-chase experiments revealed a continuous proliferation of cells over time in HS-cultured parasites, while a significantly lower number of proliferating cells were detected in FBS-cultured worms. Phenotypic differences between worms in cultured in the two sera were mirrored by significant changes at the single-cell transcriptomic level. Whereas all predicted cell clusters were present in HS-developed parasites, the worms cultured in FBS were strikingly depleted of key stem cell populations required for somatic and germ line development in the mammalian host. By coupling phenotype analysis of cultured parasites with single cell transcriptome profiling, this study represents a *proof-of-principle* for *single cell phenotyping* in schistosomes. Moreover, the protocol described in this study paves the way to study parasite development *in vitro*, positively impacting the principles of the 3Rs (Replacement, Reduction and Refinement) for animal research, and allowing for in-depth studies of sexual dimorphism establishment as well as *in vitro* screening for novel control strategies across the life cycle of these major human parasites.

Acknowledgements. The authors thank Julie Hirst for technical assistance with the *Schistosoma mansoni* life cycle maintenance at Aberystwyth University (AU), and Anais Bordes for technical support with the *Schistosoma mansoni* life cycle maintenance at University of Oxford. The authors also gratefully acknowledge Hefin Jones and Amanda Gibson from the Centre of Excellence for Bovine TB (CBTB), AU, for providing access to the CBTB facilities, the Cell Counter and hosting the 10X Chromium Controller, and the Biology Dept. Imaging Suite, University of Oxford for their support & assistance in this work (Funder: EPA Cephalosporin Fund and Department of Biology, Project Number CBR00830/REF CF 401). The authors acknowledge NHS Blood and Transplant (NHSBT), Non-Clinical Issue (NCI) for providing human blood products. GR is supported by UKRI Future Leaders Fellowships [MR/W013568/2].