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Abstract

Trypanosoma brucei, a parasitic species which causes human and animal African trypanosomiasis, presents a complex digenic life cycle that requires transmission between mammalian hosts by its insect vector, the tsetse fly. In its mammalian host, the proliferative ‘slender’ bloodstream-form morphs into a transmissible ‘stumpy’ form, which is adapted to survive in the tsetse fly. The slender to stumpy transition occurs in a density dependent quorum sensing (QS) like process for which key molecular regulators have been identified (e.g. Mony *et al*; doi:10.1038/nature12864). Some *T. brucei* subspecies (*T. b. evansi* and *T. b. equiperdum*) that circulate in the field are less able to transition from the slender to stumpy morphotype and so are described as ‘monomorphic’. These subspecies have forgone their tsetse vector and have expanded their geographic range outside of Sub-Saharan Africa. Monomorphic *T. brucei* are transmitted mechanically, between livestock and wildlife, via biting flies (*T. b. evansi*) or during equine coitus (*T. b. equiperdum*). They cause the diseases Surra or Dourine, respectively. Lacking population growth control, they can also display increased virulence compared to pleomorphic *T. brucei*. Using tools developed during the elucidation of the QS pathway, we aim to understand how development has been disrupted in naturally occurring monomorphs. In parallel, we aim to identify additional regulatory mechanisms, through the selection of monomorphic *T. brucei* in the laboratory, which will assist in determining how monomorphism is selected in the field.

Utilising whole genome sequences of 41 naturally occurring monomorphic isolates, we corroborate previous studies in identifying at least four independent monomorphic *T. brucei* clades. We found clear lineage-specific variation in the selection efficacy and heterozygosity of these

lineages, supporting their distinct evolutionary histories. Using genomic variants, we highlighted genes which are under positive selection in monomorphic lineages, but not pleomorphic ones. Variants unique to monomorphic lineages were explored for their contribution to monomorphism. Prioritisation was based on previous identification of the gene as a QS regulator, selection pressure acting on the gene and the position of the variant in relation to predicted domains. Thereafter, monomorphic gene sequences were synthesised for each of the monomorphic lineages and used to replace wild type alleles, via CRISPR-Cas9, in developmentally competent *T. brucei*. We found that two of the mutant genes analysed to date cause reduced responsiveness to the QS signal, as determined using an *in vitro* differentiation assay. Further identified variants are being tested using this analytical pipeline.

In a complementary approach, we selected 40 clonal monomorphic cell lines *in vitro* from a pleomorphic parental cell line via serial passage. Samples were stored periodically across the selection series allowing the tracking of molecular changes associated with the loss of pleomorphism over time. Initial experiments, using a single cell line, highlighted significant differential expression of transcripts from known QS genes during the selection of monomorphism. Once the loss of differentiation has been validated for the 40 clonal monomorphs, transcriptomes and genomes will be analysed to identify common molecular changes which accrue during the progression from pleomorphism to monomorphism.

By combining results from naturally occurring and selected monomorphic *T. brucei*, we have begun to highlight how monomorphism can arise, providing insight into the molecular control of the QS process and diagnostic tools to anticipate increased virulence in the field.