Advancing helminth glycomics: structural specificity and immunogenicity of schistosomal and filarial glycans

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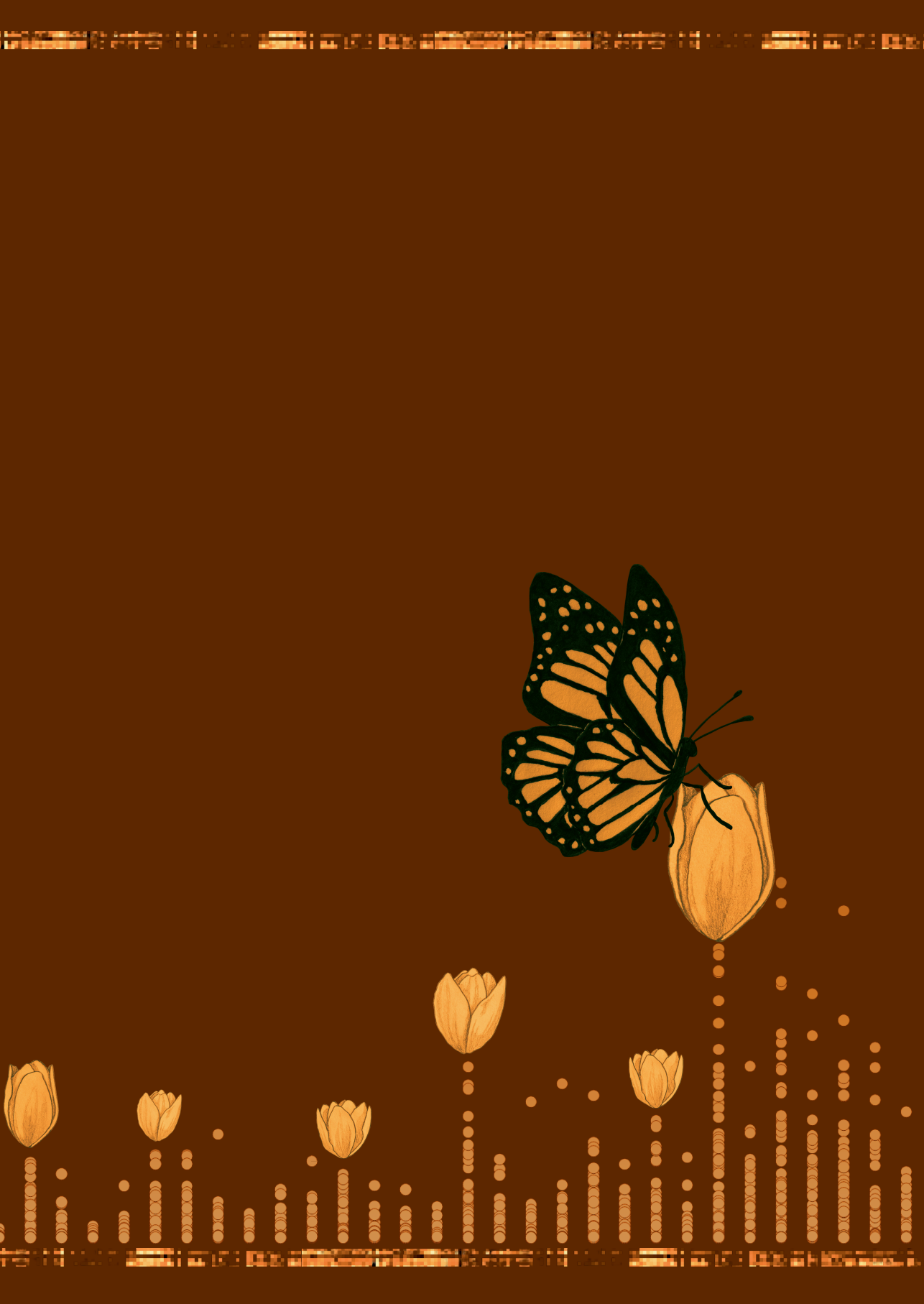
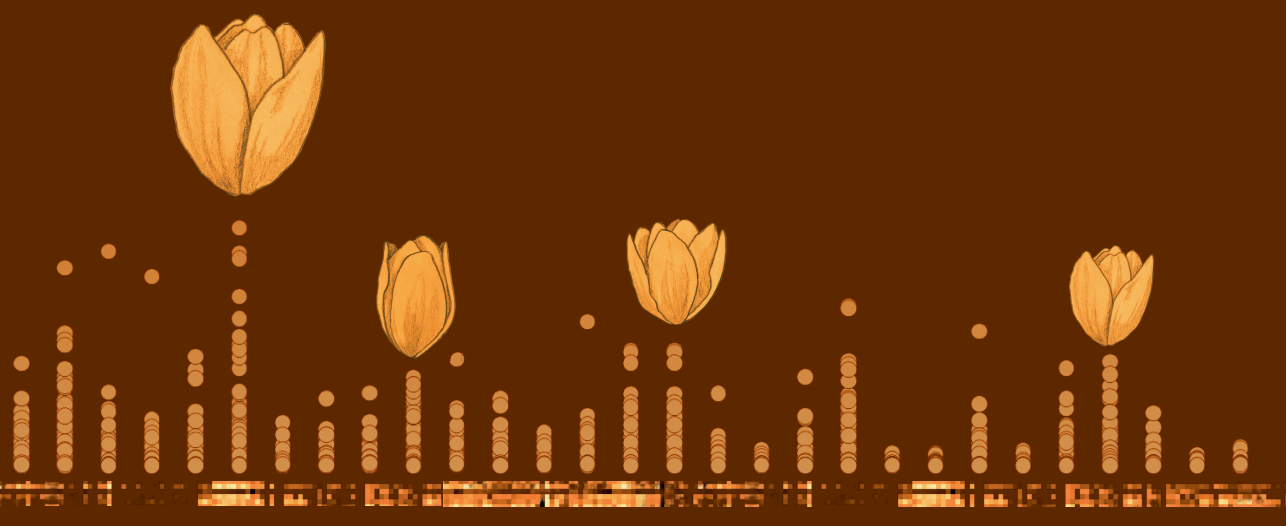


Appendix.



Summary

General background

Helminths, or parasitic worms, are widely spread infectious organisms that represent a major human, social and economic burden, particularly in developing countries. Schistosomes and filarial nematodes are among the various helminths causing neglected tropical diseases (NTDs), and are most prevalent in sub-Saharan Africa, in south America and in southern Asia. Schistosomiasis is a water-borne disease resulting from infection with *Schistosoma* spp., mainly *S. haematobium* and *S. mansoni* in humans. With an estimate of over 200 million cases worldwide, schistosomiasis ranks second only to malaria as the most devastating parasitic disease. Filarial nematodes, on their hand, are transmitted by insect vectors – mosquitoes, midges and flies – and are responsible for a number of diseases called filariasis. *Wuchereria bancrofti* and *Brugia* spp. cause lymphatic filariasis (LF), also known as elephantiasis, while *Onchocerca volvulus* is the causative agent of onchocerciasis, or river blindness. Together these parasites infect about 70 million people and pathology can result in severe impairments of the lymphatics for LF or of the skin and vision in onchocerciasis.

Parasitic helminths have in common the ability to reside for long periods of time (often over a decade) in their mammalian host. To survive in this hostile environment, these parasites have evolved mechanisms of immune evasion relying on a variety of molecular tricks, allowing them to downregulate their host immune response. As of today, all the players involved in these interactions are far from elucidated. A better understanding of host-parasite biology is however a prerequisite to fight these diseases and develop the currently needed (prophylactic) therapies and as well as the diagnostic tools allowing parasite detection with utmost precision.

Focus and scope of the thesis

Glycans are carbohydrate molecules involved in a variety of biological processes including cellular communication and host-parasite interactions. Consequently, they constitute potential diagnostic or vaccine/drug targets, but their study has been neglected in many regards so that our current knowledge of helminth glycans is still incomplete. The work presented in this thesis aimed to advance this knowledge by investigating **(1)** the structure of the glycans expressed by schistosomes and filarial nematodes (**chapters 2-4**), **(2)** the host antibody response to the parasite glycans

(**chapters 2-4**) and **(3)** how the host glycans are affected by parasitic infection (**chapter 5**).

Questions addressed in this thesis	Parasite (species) Parasitic disease (in host, if applicable)	Study type	Chapter
(1) Which glycans are expressed by the parasite?	*S. haematobium* & *S. mansoni* Schistosomiasis	Mass spectrometry (MS)-based structural glycomics	**2**
	B. malayi Lymphatic filariasis		**3**
	O. volvulus Onchocerciasis		**4**
(2) Are antibodies to parasite glycans elicited in the infected host?	*S. haematobium* & *S. mansoni* Schistosomiasis (in humans)	Glycan microarray-assisted study	**2**
	B. malayi Lymphatic filariasis (in rhesus macaques and humans)		**3**
	B. malayi, L. loa, M. perstans, O. volvulus, W. bancrofti Filariasis (in humans)		**4**
(3) Does parasitic infection alter the host glycans?	*B. malayi* Lymphatic filariasis (in rhesus macaques)	MS-based structural glycomics combined with UPLC profiling	**5**

Study and finding overview

At the outset of the present research, while knowledge of filarial nematode glycomics was rather limited, decades of work on *S. mansoni* had provided a substantial amount of data. This parasite is known to express complex, sex and stage-specific, structurally unique glycans that have immunogenic and immunomodulatory properties. Despite marked biological differences (*e.g.* organ of residence, pathology, host compatibility) no comparable work had been undertaken on the highly prevalent *S. haematobium*. In **chapter 2**, three major classes of glycans expressed by this species – the protein-linked *N*-glycans and *O*-glycans and the glycosphingolipid (GSL) glycans – were characterized using a mass spectrometry (MS)-based workflow in combination with glycan sequencing techniques. Three developmental stages of *S. haematobium* were examined, spanning a significant part of the parasite life cycle. Glycan expression was highly stage-specific and encompassed features already described in *S. mansoni*. Marked differences in the relative amount of various glycan motifs were however observed when comparing the same life-stages from each species, and major structural differences were reported in the GSL glycans, that are built on different core-structures. In addition, previously undescribed glucuronic acid (GlcA)-containing GSL

glycans were characterized in *S. haematobium* worms and eggs. Upon investigation, acidic GSL glycans were also detected in *S. mansoni* eggs, but their abundance was much higher in the eggs of *S. haematobium*. Importantly, the high-resolution power of porous graphitized carbon nano-liquid chromatography (PGC-nano-LC)-MS/MS analysis revealed strikingly different fucosylation patterns of the egg GSL glycans between the two species. Specifically, fucoses in *S. haematobium* eggs were found as single residues attached in proximity to the GSL glycan core contrasting with the heavy fucosylation of *S. mansoni* egg GSL glycans forming multifucosylated motifs located terminally and adjacent to the subterminal GlcA in acidic structures. Antibody responses to schistosome glycans were assessed in *S. haematobium* and *S. mansoni* infections using glycan microarrays containing a broad coverage of each parasite glycome with a particular focus on the differential structures. Immunoglobulin (Ig) G and IgM were observed in sera from infected individuals to a variety of glycans, many of them being immunogenic in both infection groups. Multifucosylated glycans and egg GSL glycans containing the newly identified acidic epitope were found to be highly antigenic. Interestingly, *S. haematobium*-infection sera contained significantly higher IgG to the acidic GSL glycans than the *S. mansoni*-infection sera, indicating that the observed structural differences yielded a differential IgG response in infected individuals. This species-specific glycobiology could potentially be exploited for diagnostic purposes, to help detect *S. haematobium* infections and discriminate them from infections with *S. mansoni* in a serological test.

Our exploration of filarial nematode glycans was initiated in **chapter 3**, with the study of the *N*-linked and GSL glycans of *Brugia malayi*, one of the causative agents for LF. Many glycans – both *N*-linked and GSL – carried the filarial nematode immunomodulatory substituent phosphorylcholine (PC). PC-substitution of N-acetylhexosamine (HexNAc) residues, as previously reported in filarial nematodes, was observed in abundance, and PC was also detected attached to mannose in the *N*-glycans. Terminal GlcA, fucosylated HexNAc as well as (fucosylated) α-linked galactose (αGal) were other major motifs expressed in *B. malayi* glycans. In the infected host, IgG and IgM are elicited to these non-mammalian features, as revealed by screening of a microarray composed of glycans isolated from *B. malayi*. A subset of anionic and zwiterrionic GSL glycans appeared to be particularly antigenic both in *B. malayi*-infected human and rhesus macaques. The rhesus macaques were part of a longitudinal cohort that was monitored during establishment of infection with *B. malayi* and was therefore informative regarding the dynamics of emergence of anti-glycan antibody responses in LF. Serum IgM appeared early but decreased in the course of the infection while serum IgG responses increased gradually from 5 weeks post-infection and remained high thereafter. Interestingly, in humans chronically infected with *B. malayi*, anti-glycan IgG decreased sharply in sera from the same individuals after

anthelminthic treatment, which constitutes a promising feature for diagnostic applications.

When considering antibody responses from a diagnostic perspective, cross-reactivity with other infections is an important aspect. Because of prior reports of shared glycan features throughout the filarial nematode phylum, the work performed in **chapter 4** aimed to compare the antibody responses to *B. malayi* glycan antigens in five major filarial infections of humans. In addition to *B. malayi* infection sera, the *B. malayi* glycan microarrays were incubated with sera from individuals infected with either *O. volvulus*, *Mansonella perstans*, *Loa loa* or *W. bancrofti*. IgG binding was observed in all infection sera to a subset of αGal-terminated and PC-containing GSL glycans, indicating exposure to these glycan motifs in all five filarial infections. Individuals infected with *B. malayi*, *O. volvulus* or *M. perstans* showed very similar IgG responses with binding to many additional *B. malayi* glycans compared to non-endemic control sera and to *L. loa* or *W. bancrofti* infection sera. The glycomic study of *O. volvulus* performed in this chapter could largely explain the marked IgG cross-reactivity with *B. malayi* infections, as both species express highly similar *N*-linked and GSL glycans. Because of their variable properties, and consequently variable potential for diagnostic applications, a glycan microarray-assisted study was conducted to address the role of specific IgG subclasses involved in the strong IgG responses reported in infections with *B. malayi*, *O. volvulus* and *M. perstans*. The main drivers of glycan recognition by IgG were IgG1 and IgG2. Importantly, IgG2 was the subclass showing the strongest decrease in all *B. malayi*-infected individuals examined after anthelmintic treatment. The findings in this chapter show the complexity of anti-glycan antibody responses in filarial nematode-infections and untangle broadly cross-reactive glycan motifs of *B. malayi* from infection-specific features holding diagnostic potential.

Aberrant host glycosylation is known to be a possible outcome of infectious diseases, notably due to dysregulation of glycosyltransferase and glycosidase activities by the infectious agent. With most serum proteins being glycosylated in mammals, serum is an interface of choice to observe such alterations. In **chapter 5**, the longitudinal cohort of rhesus macaque sera provided the opportunity to investigate this angle in filariasis. The serum and IgG *N*-glycans of uninfected macaques were characterized by combining a validated hydrophilic interaction-ultra performance liquid chromatography (HILIC-UPLC) workflow with MS. Besides extending the current glycomic knowledge on a key animal model, this healthy baseline allowed monitoring of alterations of the *N*-glycome during establishment of infection with *B. malayi* using HILIC-UPLC profiling. Changes affecting many different serum *N*-glycans in the total profile were observed early post-infection, prior to microfilaremia. Most of them were monodirectional – *i. e.* specific *N*-glycans were either increased or decreased as a result of the infection but did not fluctuate over time. This resulted in an overall decrease in sialylation of the serum *N*-glycans compensated by increased

galactosylation and mannosylation. Interestingly, these findings were not identical to observations made previously in the serum of dogs infected with the filarial nematode *Dirofilaria immitis*, suggesting that the reported alterations could be infection or host species specific. This work shows the strong impact of parasitic infection on the host glycobiology. In the future, complementary research using targeted glycoproteomics to identify the altered glycoprotein(s) and glycosylation site(s) contributing to the global changes observed in the serum *N*-glycome could provide effective clinical biomarkers of infection.

Conclusion

The work presented in this thesis covers significant ground regarding various aspects of glycans in schistosomiasis and filariasis. By structurally defining parasite glycans unresolved so far, the present studies substantially extend our current knowledge of helminth glycomics. This research also provides insights into the host antibody responses to the characterized glycans and into infection-induced alterations of the host serum *N*-glycome. This constitutes valuable information to tackle the current challenges associated with the major parasitic diseases that are schistosomiasis and filariasis.