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# Surface carbohydrate composition of a tapeworm in its consecutive intermediate hosts: Individual variation and fitness consequences

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**Abstract**

Carbohydrates on parasite surfaces have been shown to play an important role in host–parasite coevolution, mediating host non-self recognition and parasite camouflage. Parasites that switch hosts can change their surface molecules to remain undetected by the diverse immune systems of their different hosts. However, the question of individual variation in surface sugar composition and its relation to infectivity, virulence, immune evasion and growth of a parasite in its different hosts is as yet largely unexplored. We studied such fitness consequences of variation in surface sugars in a sympatric host–parasite system consisting of the cestode *Schistocephalus solidus* and its intermediate hosts, a copepod and the three-spined stickleback. Using lectins to analyse the sugar composition, we show that the tapeworm changes its surface according to the invertebrate or vertebrate host. Importantly, sugar composition seems to be genetically variable, as shown by differences among tapeworm sibships. These differences are related to variation in parasite fitness in its second intermediate host, i.e. infectivity and growth. Surface sugar composition may thus be a proximate correlate of the evolutionarily relevant variability in infectivity and virulence of parasites in different hosts.


*Keywords:* *Schistocephalus solidus*; Carbohydrate; Lectin; Molecular mimicry; Host–parasite coevolution; Fitness; Evolutionary parasitology; Innate immunity

## 1. Introduction

The recognition of molecular patterns that are associated with non-self is a key element for the immunological defence of hosts against pathogens and parasites (Coombe et al., 1984; Janeway et al., 1999; Janeway and Medzhitov, 2002). As a basic principle, it involves immunological receptors that identify targets on the surface of the intruders. Host–parasite interactions thus essentially take place at the interface between the surface of the parasite and its hosts' immune system. It seems that the surface of all parasites contains carbohydrate (i.e. sugar) residues (Jacobson and Doyle, 1996), which are therefore important targets for hosts to distinguish self from non-self. It was shown that carbohydrates, rather than proteins, comprise antigens that dominate the immune response to protozoan and helminth parasites (for review see Gasque, 2004; Nyame et al., 2004). Many of these sugars are specifically recognised by receptors of the host immune system, e.g. lectins (Nyame et al., 2004). Lectins are proteins that specifically bind to carbohydrate residues, such as those occurring in glycoproteins of membranes (White, 1987). They are also useful tools for parasitologists to monitor parasite surface composition. Such 'lectin labelling' showed that many parasites adapt their surface 'coat' to the host (Ham et al., 1988; Horák, 1995; Joachim et al., 1999; Schabussová et al., 2003). This strategy of a parasite to evade its host's immune system became known as 'molecular mimicry' (Damian, 1964). Parasites may try to escape or to reduce immune responses of hosts, either in a passive way by masking themselves with host epitopes or actively by mimicking host immuno-regulatory molecules (reviewed in Damian, 1997).

Many parasites have complex life cycles, which means that they have to pass through several hosts to reach maturity. Presumably, the parasite surfaces needed to cope with the relatively simple immune system of invertebrates, which often serve as intermediate hosts, will be different to those for coping with the elaborate innate and adaptive immune functions of vertebrates, generally functioning as final hosts (Smyth, 1994; Lucius and Loos-Frank, 1997). Changeable surface coating

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may then enable those parasites to evade the immune systems of their different hosts (Damian, 1997).

Parasitologists have been very successful in analysing the physiological and molecular mechanisms that parasites use to exploit their hosts (Smyth, 1994). These studies are essentially based on the assumption that such adaptation is optimal for the parasite. Evolutionary biologists have taken a different approach in assuming that there is considerable variation among individual parasites in the traits that lead to adaptation to their hosts (Carius et al., 2001; Little and Ebert, 2001). Since there is likewise variation in host counter-measures (i.e. immune defence), this may lead to cycles of adaptation and counter-adaptation, culminating in an arms race that became well known among evolutionary biologist as the 'Red Queen' phenomenon (van Valen, 1973; Ebert and Hamilton, 1996).

While parasitologists primarily analysed differences in surface structures between developmental stages of a parasite species, evolutionary biologists focussed on traits of parasites that potentially lead to differences in the fitness of parasites in the different hosts (Ham et al., 1988; Horák, 1995; Joachim et al., 1999; Davies et al., 2001; Schabussová et al., 2003; Gower and Webster, 2004). However, few studies link individual variance in the surface characteristics of a parasite to its ability to infect and exploit its hosts. We bridged that gap by using a multidisciplinary approach and analysed both surface characteristics and fitness of a tapeworm in its two intermediate hosts. To get an understanding of the genetic basis of the traits studied, we further considered variation among genetically related lines by using parasite sibships (i.e. broods).

The host–parasite system consists of the cestode *Schistocephalus solidus*, which is confronted with the immune systems of its two intermediate hosts, the copepod *Macrocyclops albidus* and the three-spined stickleback *Gasterosteus aculeatus*. When eaten by a copepod, the parasite, coracidium, penetrates the gut wall and establishes in the body cavity as the procercoid larval stage. After being consumed by a stickleback, it grows as a plerocercoid until it is infective for the definitive host, one of several species of fish-eating birds. Experimentally, we replaced the bird host with an in vitro system for the cultivation of tapeworms (Smyth, 1946; Kurtz, 2003). Tapeworms seriously reduce the fitness of both intermediate hosts (Arme and Owen, 1967; Franz and Kurtz, 2002).

The body surface of cestodes is coated with a layer of mucopolysaccharides and glycoproteins, the glycocalyx (Smyth and McManus, 1989), but detailed knowledge of sugar composition of the glycocalyx of *S. solidus* is not available. To gain insight into a broad range of potentially relevant surface sugars, we used a mixture of three commercially available, plant-derived lectins that bind to distinct sugars for labelling. The sugars identified by these lectins have the potential to be important in host–parasite interactions (Basseri et al., 2002; Obregón-Henao et al., 2003).

We here analysed whether *S. solidus* changes its surface sugar composition when switching from the copepod to the stickleback host. To explore the fitness consequences of surface composition, we related the variation among genotypes to their performance (i.e. infectivity and growth) in the two hosts. We expected tapeworm performance to be mediated by the hosts' immune reactions and so analysed the activation of the innate immune system and the body condition of the stickleback hosts.

## 2. Materials and methods

### 2.1. Parasites

Infected sticklebacks were caught from the brackish 'Neustädter Binnenwasser' (longitude: between 10°40′ and 10°50′ East, latitude: between 54°59′ and 54°60′ North), northern Germany, in autumn 2001 and were kept in large tanks with food ad libitum until *S. solidus* tapeworms were dissected from them in February 2003. Sixteen tapeworms (i.e. plerocercoids) were matched pair-wise with regard to size (body weight) to ensure that the worms reproduced by outcrossing (Lüscher and Milinski, 2003). The worms were bred in pairs for 6 days in an in vitro system replacing the bird's gut (Smyth, 1946; Wedekind, 1997). The eggs from each worm pair were divided into 12 portions and stored at 4 °C in the dark until use. The offspring from one pair will here be referred to as 'parasite sibship', so that in total eight parasite sibships were used in the experiments. We used sibship as a surrogate for different genotypes and are thus able to determine the upper boundary of heritability. In addition to genetic variation this estimate may also contain common environment effects (Falconer, 1981). For 3 weeks before infection, the eggs were kept at 20 °C in the dark. To stimulate the eggs to hatch, they were exposed to light on the day before the infections took place (Dubinina, 1966).

### 2.2. Infection of the first intermediate host: copepods

Copepods of the species *M. albidus* were kept in culture in the laboratory as described in van der Veen and Kurtz (2002). The culture originated from 80 individuals from a small river 'Kremper Au' (Neustadt, Germany), which is connected to the 'Binnenwasser', the source of the parasite population.

Thirty days before the start of the experiments, 10 tanks were set-up with 50 adult females each. Two days before exposure to tapeworm larvae (i.e. coracidia), 1152 adult male copepods were filtered from the culture tanks and randomly assigned to one of the eight parasite sibships. The copepods were singly transferred into a well of a 24-well plate with 2 ml of water and singly exposed to one coracidium each. Thereafter, each copepod was fed ad libitum with five freshly hatched nauplii of *Artemia salina* three times per week. Copepods were kept at 20 °C and 16:8 light:dark cycle.

At 10 days post infection (p.i.), each copepod was screened under the microscope and infection status determined (van der Veen and Kurtz, 2002). Parasite screening itself was done without knowledge of parasite sibship. Two days after checking for parasites, infected copepods were dissected for recovery and measurement of the procercoids. The parasite larvae were killed by adding a drop of 20% formalin in PBS to the water. For measurement of procercoid size (i.e. area), an

image was taken with a video camera and analysed with the image analysis program Image J 1.31v (Wayne Rasband, National Institutes of Health, USA). From each procercoid, two pictures were taken to check for repeatability of the measurements, which was 99.6% (calculated using variance components), and the mean of those two measurements was used.

### 2.3. Infection of the second intermediate host: sticklebacks

Before sticklebacks (second intermediate host) can be exposed to the tapeworm, copepods (first intermediate host) have to be infected. Copepods ($n=960$) were randomly assigned to one of the eight parasite sibships, singly exposed to one coracidium each and screened for procercoids 12 days p.i.

Stickleback hosts (*G. aculeatus*) were bred in the laboratory from adults, derived from the same 'Binnenwasser' population as the parasites. Offspring from four stickleback pairs, all hatched in July 2002, were raised in family tanks with 15–25 fish each. During the experiment, 176 fish were housed in one of two individual compartments of a tank ($21\times35\times25$ cm), without any contact (visual or olfactory). The tanks were randomly distributed across the shelves in the aquaria room (18 °C and 16:8 light:dark cycle). Fish were fed ad libitum three times per week with frozen chironomids.

Two days before exposure to infected copepods, the sticklebacks were transferred into small tanks with 2 l water and starved to enhance consumption of copepods. On the day of exposure, each of the 176 fish was given one copepod that was infected with one parasite larva. Individuals of the different fish families were randomly assigned to the eight different parasite sibships, and the combination of fish family and parasite sibship was balanced. Two days p.i., the fish were returned to their previous tanks.

One week p.i. half of the fish were dissected and the body cavity screened for tapeworms by rinsing the fish tissues in PBS. At this stage, the worms are still translucent and suitable for lectin labelling. The remaining fish were killed 5 weeks p.i. At that time the tapeworms are large enough to determine their weight to the nearest 0.1 mg. On both dissection dates, a hepatosomatic index ($I_H$) was determined as $100\times$liver weight divided by fish body weight, both measured to the nearest 0.1 mg (Chellappa et al., 1995). $I_H$ is a useful measure of metabolic body condition (i.e. medium term energy reserves) in fish, and was reduced by tapeworm infection in previous studies on sticklebacks (Kurtz et al., 2004).

### 2.4. Immune defence of sticklebacks

To obtain estimates of the activity of the immune system of the sticklebacks, we isolated leucocytes from the head kidneys of freshly dissected fish. We quantified an innate immune reaction that releases reactive oxygen species (ROS) to kill pathogens. This respiratory burst assay measures activity associated with phagocytosis of zymosan particles in vitro in a lucigenin-enhanced chemiluminescence (CL) assay (Scott and Klesius, 1981). For this assay, the cell density was adjusted to $1.25\times10^6$ live cells/ml, corresponding to $2\times10^5$ cells/assay (Kurtz et al., 2004). Unfortunately we did not obtain enough cells for the immune assays from all fish so that data of 153 individuals went into the final analyses.

### 2.5. Lectin labelling of parasites

*Schistocephalus solidus* procercoids and plerocercoids were obtained from experimentally infected copepod and stickleback hosts, respectively, upon dissection. The worms were stored in 250 µl of 4% formalin in 0.01 M PBS, pH 7.4, in a 96-well plate until lectin labelling. This method of preservation did not change the staining patterns compared with fresh material, except for an increase in autofluorescence (Colditz et al., 2002), which is not relevant in our study since we are interested in relative rather than absolute binding intensities of the samples.

To determine the sugar composition on the surface of individual procercoids and plerocercoids, we used the following lectins which were labelled with different fluorescent dyes: fluoroscein isothiocyanate (FITC)-labelled PNA (*Arachis hypogaea*) binds to β-galactose-1,3 *N*-acetylgalactosamine (GalNAc) and D-galactose, tetramethylrhodamine isothiocyanate (TRITC)-labelled WGA (*Triticum vulgaris*) identifies *N*-acetylglucosamine (GlcNAc) and sialic acid residues (PNA and WGA from Sigma-Aldrich) and Alexa Fluor-labelled ConA (*Canavalia ensiformis*) recognises α-mannose and α-D-glucose (Molecular Probes) (Jacobson and Doyle, 1996). Each tapeworm was labelled with all three dyes simultaneously.

All three lectins were dissolved in 0.05 M Tris-buffered saline (TBS), pH 7.4, to a final concentration of 10 µg/ml. For labelling, parasite larvae were singly transferred with 50 µl of 4% formalin in PBS into a well of a 96-well UV-plate (Corning Incorporated). Two hundred microlitres of the lectin mixture was added to each parasite and incubated for 1 h in the dark at room temperature. Parasites were then washed with TBS three times for 10 min. To show that our lectins bind specifically to their ligands we performed controls in which we added a surplus of free sugar with the same binding specificity. Lectins were incubated with their respective inhibitory ligands for 30 min at room temperature in the dark (PNA: 0.2 M α-lactose monohydrate; WGA: 0.02 M *N*,*N*′-diacetylchitobiose; ConA: 0.2 M α-D-methylmannopyranoside and 0.2 M α-D-methylglucopyranoside) and subsequently used for labelling of procercoids. This procedure successfully blocked the labelling of the procercoids.

### 2.6. Fluorescence microscopy

The parasites were examined using an epifluorescence inverted microscope (Leica DM IRB), using filter sets with emission wavelengths of 515–560 nm for the detection of TRITC (absorption/emission maxima of 555/580 nm), 450–490 nm for the detection of FITC (absorption/emission maxima of 494/518 nm) and 340–380 nm for the detection of

Alexa Fluor (absorption/emission maxima of 346/442 nm). With a video camera mounted on the microscope, images were obtained with all three filter settings, digitised and saved in the 8 Bit TIFF-RGB (Red–Green–Blue) mode. To minimise variation in staining due to unequal handling of the parasites, pictures were always taken in the same order of filter settings (TRITC, FITC, Alexa Fluor).

To quantify the intensity of labelling, images were analysed with the image analysis program IP Lab 3.6.2 (For Mac OS 9.2.2, Scanalytics Inc.). We used the thresholding function to identify the outline of the worm, and then obtained mean intensities of this area (scaled from 0 to 255) of the appropriate colour channel (red for TRITC, green for FITC, blue for Alexa Fluor). Additional confocal microscopy was used for a more detailed examination of the localisation of the lectins in procercoids, especially in order to identify relevant sugars, such as those at surfaces that are likely exposed to the immune system of the different hosts. We scanned whole lectin-stained procercoids on a Leica confocal laser-scanning microscope TCS SP1.

### 2.7. Data analysis

To check whether developmental stages and sibships of the tapeworms differed in the intensity of lectin labelling, we calculated an analysis of variance (ANOVA) with labelling intensity of each lectin (PNA, WGA, ConA) as the response variable and parasite stage and sibship as effects. To directly compare the relative intensity with which the different lectins bound to the procercoid and the plerocercoid stage, we also calculated the proportional binding of each lectin in relation to all other lectins, e.g. 100×intensity of WGA/(PNA+WGA+ConA) gives the 'relative WGA-labelling'.

To check for correlations between the intensity of lectin labelling and fitness parameters of parasite and host, we used mean values per tapeworm sibship and calculated Spearman Rank correlations (due to the relatively small sample size of eight sibships and the potential non-normality of the data).

For analyses of fish immune activation, only data from infected animals were considered. Immune and fitness data of three fish with extremely swollen and yellow livers were excluded. These fish also produced extreme values in the respiratory burst reaction and hepatosomatic index, which together suggests that they suffered from another infection besides *S. solidus*. Outlier values (in a boxplot: values greater than 1.5 times the spread outside the closest hinge), for the labelling intensities of three procercoids were also excluded since these extreme values indicated irregularities during the labelling procedure (Quinn and Keough, 2003).

In general, test statistics refer to two-tailed tests, and we considered effects significant at a level of $P<0.05$. Non-significant interactions were removed from the models. Where necessary, we confirmed that the data allowed the use of parametric statistics (e.g. the ANOVAs) by checking that the residuals obtained from the analyses were normally distributed. Analyses were performed with the JMP Version 5.0.1.2 (SAS™) software for Macintosh™.

## 3. Results

### 3.1. Variation of parasite surface sugar composition between the developmental stages and among parasite sibships

Labelling intensity of all three lectins varied and could be significantly explained by parasite stage and sibship for PNA (effect of stage: $F_{1,98}=2253.05$, $P<0.0001$ and sibship: $F_{7,98}=4.08$, $P=0.0006$), by parasite stage for WGA (effect of stage: $F_{1,91}=19.36$, $P<0.0001$ and sibship: $F_{7,91}=2.06$, $P=0.0558$ and the interaction between stage and sibship: $F_{7,91}=3.09$, $P=0.0056$), and for ConA (effect of stage: $F_{1,98}=824.26$, $P<0.0001$ and sibship: $F_{7,98}=1.09$, $P=0.37$). The labelling was generally more intense for procercoids than plerocercoids (Fig. 1). It is thus useful to compare relative, in addition to absolute, intensities of labelling, i.e. to evaluate how strongly the surface of each stage is dominated by one sugar as compared with the others (Fig. 2). The surface of procercoids was dominated by sugars that are recognised by PNA ($F_{1,105}=931.17$, $P<0.0001$), whereas plerocercoids were characterised by WGA-binding sugars ($F_{1,105}=541.62$, $P<0.0001$) and the relative intensity of ConA binding did not differ between the stages ($F_{1,105}=1.12$, $P=0.29$).

### 3.2. Localisation of lectin binding

In the procercoid, PNA stained the surface layer (Fig. 3(a)), which encases the WGA-labelled inner layer (Fig. 3(b)). By contrast ConA labelled the surface layer, but also led to homogenous staining of most interior parts of the body (Fig. 3(c)). Since host immune effectors will have access to the exposed surface of the parasite, PNA might arguably be considered the most relevant label for the procercoid stage within the copepod.

As there is evidence that the procercoid loses its cercomer (head-like appendage at one side of the body with still unknown function; see also in Fig. 3) upon metamorphosis to the plerocercoid in the fish (Dubinina, 1966), it necessarily also loses the outer surface layer (Fig. 3), so that the WGA-binding inner surface will be exposed here. WGA-labelling carbohydrates of the plerocercoid might be the most relevant with regard to exposure to the fish immune system.

### 3.3. Lectin-labelling in relation to fitness parameters of the parasite and its hosts

We did not find a significant relationship between the mean intensity of PNA binding to procercoids and the mean infectivity of a sibship in the copepod ($r_s=0.14$, $n=8$, $P=0.73$), nor did we find any correlation with mean intensity of PNA binding and the mean size of the procercoids ($r_s=-0.11$, $n=8$, $P=0.77$).

For the plerocercoid stage, the mean labelling intensity of WGA of a tapeworm sibship correlated negatively with its infectivity in the fish ($r_s=-0.82$, $n=8$, $P=0.01$). By contrast, the correlation with the plerocercoid body weight was positive ($r_s=0.75$, $n=7$, $P=0.05$) (Fig. 4(a) and (b)).

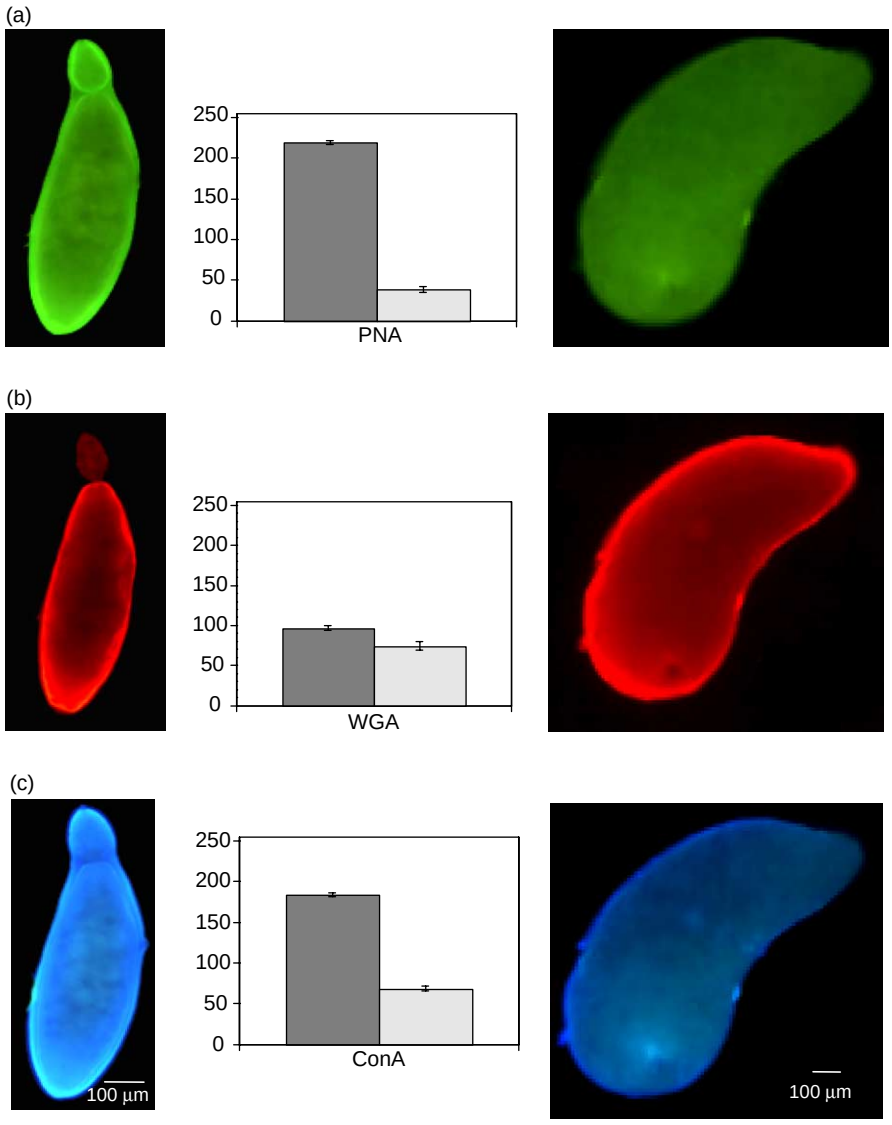


Fig. 1. Epifluorescent micrographs of a procercoid of *Schistocephalus solidus* (left), dissected from an infected copepod and of a plerocercoid (right), dissected from an infected stickleback. The tapeworms were labelled with three fluorescent lectins: (a) PNA (fluoroscein isothiocyanate labelled), (b) WGA (tetramethylrhodamine isothiocyanate labelled), (c) ConA (Alexa Fluor 350 labelled). Mean intensity of labelling with each lectin is shown for both developmental stages in the bar chart in the middle (left bar: procercoid, $n=77$; right bar: plerocercoid, $n=31$).

The observed associations between the labelling characteristics and infectivity of a worm sibship could not significantly be explained by differential activation of the stickleback immune system, as estimated from the respiratory burst reaction (correlation of the respiratory burst with procercoid-PNA: $r_s=-0.14$, $n=8$, $P=0.73$; with plerocercoid-WGA: $r_s=0.30$, $n=8$, $P=0.45$) (Fig. 4(c)). Likewise, there was no detectable link between these tapeworm surface characteristics and the metabolic condition of the infected sticklebacks (correlation of the hepatosomatic index $I_H$ with procercoid-PNA: $r_s=0.09$, $n=8$, $P=0.82$; with plerocercoid-WGA: $r_s=-0.33$, $n=8$, $P=0.41$).

## 4. Discussion

Using lectin labelling to analyse carbohydrates, we found the surface of the tapeworm *S. solidus* to be highly glycosylated. The relative proportion of surface sugars varied between the two developmental stages, the pro- and plerocercoid. Importantly, sugar composition seems to be genetically variable, as shown by differences between tapeworm sibships. These differences relate to variation in parasite fitness, i.e. infectivity and growth in the second intermediate host, the stickleback. We thus conclude that the differing ability of individual parasites to adapt their surface to their consecutive intermediate hosts leads to evolutionarily relevant variation in fitness. However, with the rather simple measures of host immune activity that were analysed here, we could not identify the immunological basis for such variation.

How did the surface sugar composition vary between the two developmental stages of the parasite? In the procercoid stage, which has to deal with the immune system of an invertebrate (a copepod), relatively more PNA-binding sugars (GalNAc, D-galactose) were found, whereas in the plerocercoid

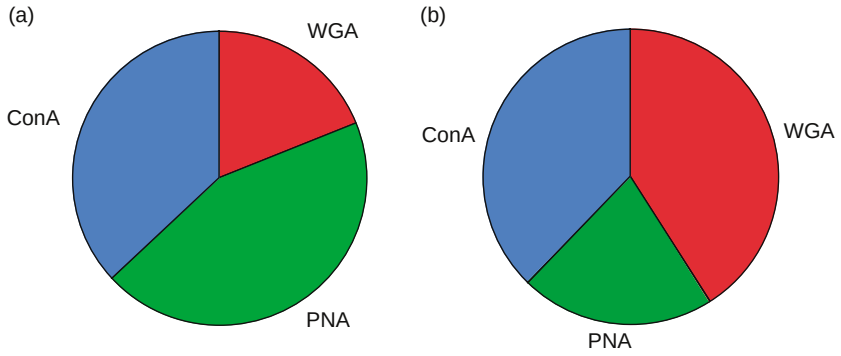


Fig. 2. Relative lectin binding of PNA (green), WGA (red) and ConA (blue) for the (a) procercoid ($n=77$) and the (b) plerocercoid stage ($n=31$).

stage, which has to evade the immune system of a vertebrate (the stickleback), relatively more WGA-binding sites (GlcNAc, sialic acid residues) were identified.

In our study, lectin labelling thus shows the surface of the procercoid to be dominated by GalNAc and D-galactose. This is in line with several reports in the literature, where such sugars were mainly found on the surface of parasite larvae in invertebrate hosts. It was reported that these sugars are important in interactions between insect vector tissues and their parasites (Knowles et al., 1991; Burton et al., 1999). In the case of the nematode *Onchocerca lienalis*, the parasite is thought to mimic host sugars not be recognised as foreign by the invertebrate host's immune system (Jacobson and Doyle, 1996). However, PNA-binding sugars were also hypothesised to protect underlying tissues of parasites from enzymatic digestion in the intestines of their vertebrate hosts (Sandeman and Williams, 1984; Ingold et al., 2000; Obregón-Henao et al., 2003). Procercoids of *S. solidus* also face the harsh environment of the stomach and gut of the stickleback before penetrating through the gut wall into the body cavity. The high

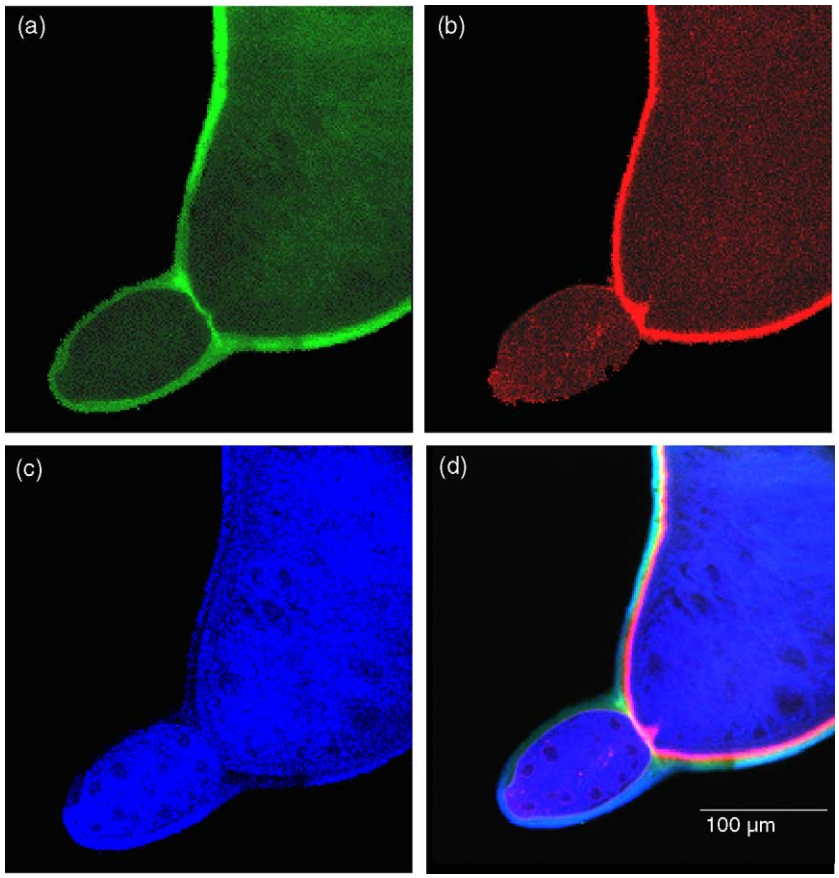


Fig. 3. Micrographs (confocal microscopy) of a procercoid of *Schistocephalus solidus*, dissected from an infected copepod and labelled with different fluorescent lectins: (a) PNA (fluoroscein isothiocyanate labelled), (b) WGA (tetramethylrhodamine isothiocyanate labelled), (c) ConA (Alexa Fluor 350 labelled) and (d) composite image. Generally, the micrographs were taken as single plane images in the same plane and then merged to the composite image for better visualisation.

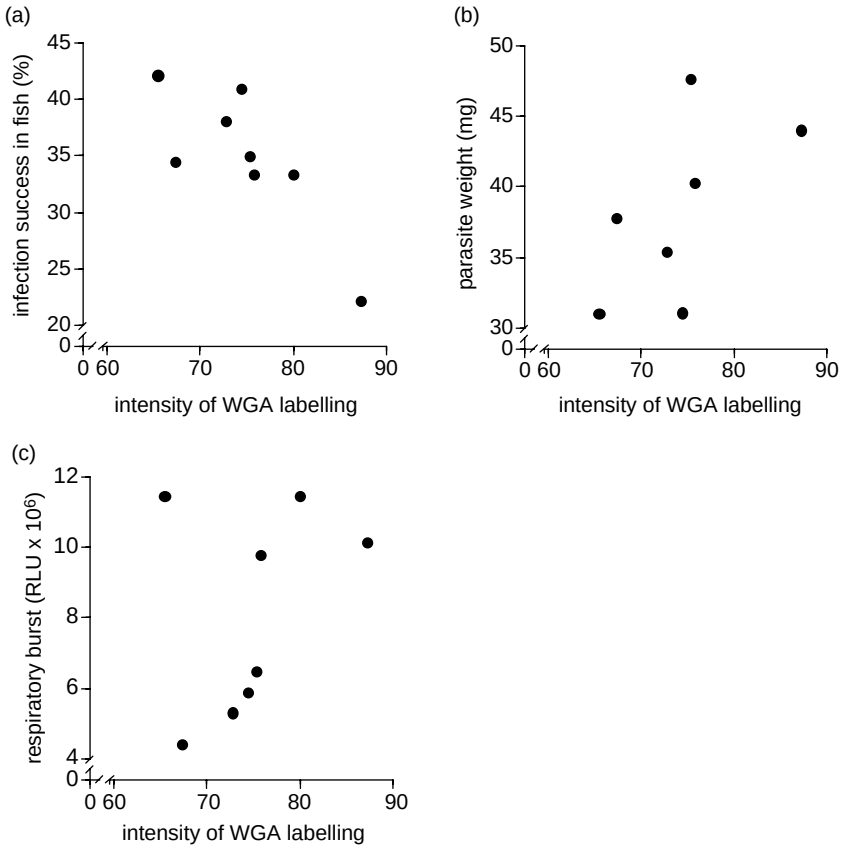


Fig. 4. Relationship between the mean WGA-labelling intensity of plerocercoids of the tapeworm *Schistocephalus solidus* and (a) infectivity (%) in the fish, (b) plerocercoid weight (35 days p.i.) and (c) stickleback innate immune response (respiratory burst 7 days p.i.). Data points show the mean of a parasite sibship (2–6 individuals).

intensity of PNA-binding sugars on procercoids of *S. solidus* could therefore function as a protection in both the invertebrate and vertebrate host.

Within each stage of the parasite, we analysed the variance of surface sugar composition among tapeworm sibships, which we correlated to their average fitness. For the procercoid stage in the copepod host, we did not find any correlations of lectin binding and fitness. A possible explanation could be that the surface of the oncosphere, which is exposed to the copepod immune system shortly after infection, rather than the procercoid, is mostly relevant for the interaction with the copepod immune system (Dubinina, 1966; van der Veen and Kurtz, 2002). Due to technical problems in removing the outer layer of the coracidia, we could not label oncospheres.

On the surface of the plerocercoid stage in the fish host, we mainly found WGA-binding sugars, which were previously reported for other parasites (Jacobson and Doyle, 1996; Fuchs et al., 1999; Obregón-Henao et al., 2003). WGA recognises both GlcNAc and sialic acid residues, but a more detailed analysis would be needed to distinguish them. Sialic acids have so far rarely been reported for helminth parasites (but see Maizels et al., 2004). Arguably, sialic acid residues on the surface of *S. solidus* could help in evading the immune system of the stickleback, since all vertebrate cells are covered with terminal sialic acids, whereas the absence of sialic acids, e.g. on parasite surfaces, is known to act as a non-self signal (Maizels et al., 2004; Schauer, 2004).

In the stickleback, we found that parasite sibships that bound more WGA were inferior in infecting their host, but superior when it came to increasing their body size. Size is important for worm fitness, since it directly relates to egg production (Wedekind, 1997). Plerocercoids seem to face different parts of the immune system at different time-points of infection: in a recent study, tapeworm growth was for example found to be regulated by components of the adaptive immune system (Kurtz et al., 2004). Tapeworms may cope best with either the innate part of the defence system (thus being superior in infecting the stickleback) or with adaptive immunity (thus growing bigger later during an established infection) (Hammerschmidt and Kurtz, in press). WGA-binding sugars, which may protect from adaptive immunity but seem more prone to detection by the innate defence system, could mediate such a trade-off. Parasite surface sugars can even play a more active role, such as immuno-suppression or immuno-avoidance (Tomaska and Parish, 1981; Ma et al., 2002; Plows et al., 2005). There are indications for suppression of the stickleback immune system by *S. solidus* during later stages of the infection (Scharsack et al., 2004).

We here made use of plant lectins, which have been used in other studies to specifically detect parasite surface

carbohydrates (Jacobson and Doyle, 1996; Joachim et al., 1999; Schabussová et al., 2003). Lectins also occur in animals, where they fulfil an important role in the innate immune system (Epstein et al., 1996; Turner, 1996; Fujita, 2002). Since the interaction of lectins with their sugars is specific, lectins may enable specific recognition of pathogens within the innate immune system. Especially among invertebrates, there are indications that multiple lectins might mediate non-self recognition (Wilson et al., 1999; Marques and Barracco, 2000; Zhang et al., 2004). In our host–parasite system, it was shown that the copepod is capable of specific immunological memory (Kurtz and Franz, 2003). The current study points towards variation in tapeworm surface sugar signatures as potential targets for such specific recognition (Kurtz, 2005).

In summary, we found that surface glycoconjugates appear to play an important role in the interaction of the cestode *S. solidus* with its intermediate hosts. Our results match findings in other host–parasite systems, where parasites also change their surface coat depending on the host and so evade the hosts' immune systems. Most importantly, we found that stage-specific differences in the surface sugars among individuals in the second intermediate host are associated with important aspects of the fitness of these tapeworms. Surface sugar composition may thus be a proximate correlate explaining variance in infectivity and performance of that parasite in its different hosts. It seems that parasites may use different strategies within their host species to evade diverse immune systems.

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