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Resistance against heterogeneous sequential infections: experimental studies with a tapeworm and its copepod host

J. Kurtz* and K. Hammerschmidt

Department of Evolutionary Ecology, Max Planck Institute of Limnology, August-Thienemann-Str. 2, 24306 Plön, Germany

Abstract

Parasite heterogeneity is thought to be an important factor influencing the likelihood and the dynamics of infection. Previous studies have demonstrated that simultaneous exposure of hosts to a heterogeneous mixture of parasites might increase infection success. Here this view is extended towards the effect of parasite heterogeneity on subsequent infections. Using a system of the tapeworm *Schistocephalus solidus* and its copepod intermediate host, heterogeneity of the tapeworm surface carbohydrates is investigated, i.e. structures that are potentially recognized by the invertebrate host's immune system. With lectin labelling, a significant proportion of variation in surface carbohydrates is related to differences in worm sibships (i.e. families). Tapeworm sibships were used for experimental exposure of copepods to either homogeneous combinations of tapeworm larvae, i.e. worms derived from the same sibship or heterogeneous mixtures of larvae, and copepods were subsequently challenged with an unrelated larva to study re-infection. Contrary to expectation, neither an effect of parasite heterogeneity on the current infection, nor on re-infection were found. The effect of parasitic heterogeneity on host immunity is therefore complex, potentially involving increased cross-protection on the one hand, with higher costs of raising a more heterogeneous immune response on the other.

Introduction

Within the past few years, the fields of immunology and parasitology have seen the fruitful influence of concepts that are derived from ecology and evolutionary biology (Williams & Nesse, 1991; Ebert, 1998; Edwards & Hedrick, 1998; Frank, 2000; Rolff & Siva-Jothy, 2003). Since evolution is based on heritable variation among individuals, the emerging evolutionary view leads to an increasing focus on the relevance of individual genetic and phenotypic variation in the ability of parasites to infect their hosts and of hosts to resist infection (Ebert *et al.*, 1998; Schmid-Hempel, 2003). These concepts have been successful in predicting the likelihood of infection, infection dynamics, and fitness consequences for hosts and parasites.

Evolutionary ecology has revealed that infection critically depends on the particular combination of host and parasite, such as populations or genetic strains (Carius *et al.*, 2001). Such host–parasite interactions put the specificity of defence reactions into focus (Schmid-Hempel & Ebert, 2003). There is now increasing evidence for phenomena of specific recognition in the interaction of invertebrate hosts with their parasites, although the responsible mechanisms remain to be demonstrated (Kurtz, 2005). A specific immunological recognition requires an immunological mechanism of the host consisting of sufficiently polymorphic receptors. Moreover, antigenic characteristics of relevant pathogens need to be sufficiently variable to enable the immune system to discriminate different pathogen species, strains or individuals, depending on the degree of specificity that is achieved.

*Address for correspondence: ETH Zürich Ecology and Evolution, ETH-Zentrum, CHN J12.1, Universitätsstr. 16, CH-8092 Zürich, Switzerland
Fax: +41 44 63 21 271
E-mail: joachim.kurtz@env.ethz.ch

In the first part of this paper, the question of variation in potentially immunological relevant characteristics in a natural population of the tapeworm *Schistocephalus solidus* will be addressed. This parasite uses a copepod such as *Macrocyclops albidus* as its first intermediate host, the three-spined stickleback *Gasterosteus aculeatus* as the second intermediate host, and any species of fish-eating bird as the definitive host (Dubinina, 1957; Wedekind, 1997; Kurtz, 2003). Tapeworms grow in the body cavity of the two intermediate hosts, while reproduction occurs in the bird gut. Eggs are released into the water in the birds' faeces. Copepods prey upon free-swimming larvae (coracidia). Previous studies indicate that the interaction between the tapeworm and its copepod intermediate host is characterized by a high degree of specificity. In sequential exposure experiments, copepods were better protected against subsequent challenge with tapeworms that were derived from a sibship that was previously encountered (Kurtz & Franz, 2003). Studies in other host–parasite systems suggest that carbohydrates on the surface of parasites are likely targets of specific immunological recognition (Jacobson & Doyle, 1996; Nyame *et al.*, 2004). Thus labelling with lectins, i.e. proteins that specifically recognize carbohydrate residues was undertaken, to analyse individual variation in surface carbohydrates of the tapeworm *S. solidus* in its copepod intermediate host. Parasite surfaces were compared in worms derived from different sibships that were bred in the laboratory from parents obtained from a naturally infected population of stickleback hosts. We also analysed whether offspring of the same worm parents (i.e. sibships) shared labelling characteristics.

In the second part of this paper, the potential implications of parasite heterogeneity will be analysed with regard to specific recognition by the host's immune system. The benefits of specific immunological recognition are apparent in experimental situations, where hosts gain in resistance when subsequently challenged with similar pathogens, i.e. where they can benefit from an immunological memory (Kurtz, 2005). However, such benefits might not always apply in natural situations, where encountered pathogens often consist of a mixture of heterogeneous individuals. In such situations, specific recognition might even be disadvantageous, since a more specific immune response will by definition be less cross-reactive, i.e. less effective against a different type or strain of pathogen.

Specificity is thus relevant with regard to infections that involve genetically diverse parasites (Read & Taylor, 2001; Frank, 2002). In general, hosts cope better with a homogeneous insult as compared to a mixture of heterogeneous parasites, which might be harder to control by the immune system (Taylor *et al.*, 1997; Imhoof & Schmid-Hempel, 1998; Wedekind & Ruetschi, 2000; Davies *et al.*, 2002; Taylor *et al.*, 2002; De Roode *et al.*, 2003; Hughes *et al.*, 2004). If the immune defence is specific and can react efficiently against only one type of intruder among a mixture, the remaining parasites might be largely untouched by the host's defence and consequently exploit the host more easily.

However, specificity might not be the only cause of variability in infection. Another important reason is the fact that immune defence itself does not normally come free of cost. There is now good evidence, again mainly from the field of evolutionary ecology, for considerable fitness costs of raising an immune reaction (Sheldon & Verhulst, 1996; Moret & Schmid-Hempel, 2000; Zuk & Stoehr, 2002). Since individuals vary in their ability to cope with such costs, they will also vary in resistance (Schmid-Hempel, 2003). Together with specificity, the costs of defence are also important with regard to an increased infection resulting from heterogeneous exposure. For example, it might be more costly to raise an array of different specific defences against diverse parasites as compared to only one type of specific response that is, however, effective against only one type of parasite, or one type of unspecific response against all heterogeneous parasites (Råberg *et al.*, 2002; Mallon *et al.*, 2003; Moret, 2003; Schmid-Hempel & Ebert, 2003).

The potential advantages and disadvantages of specificity become particularly obvious in the situation of induced immunity. Extended immune reactions are advantageous in cases where a prior parasitic exposure has at least some predictive value with regard to the expectation of subsequent exposures, and if the defence reaction will also be protective towards such subsequent insults (Kurtz, 2004). However, a specific immune response will not be protective against later exposures with a different pathogen, unless there is at least some cross-reactivity (Frank, 2002).

Under natural conditions, hosts will be confronted with both exposure to heterogeneous mixtures of parasites, and with repeated exposures. Inspired by this ecologically relevant situation, we here address the question as to how the heterogeneity of a first insult influences the outcome of a subsequent exposure. Several scenarios are possible, depending on the specificity, induction and costs of the defences involved. If a defence shows specific memory, a homogenous insult will only lead to protective memory against pathogens sharing antigenic characteristics with previously encountered ones. On the other hand, a more heterogeneous prior insult might also induce a more diverse immune response. Subsequent exposure with a parasite, even if not previously encountered, might then result in reduced infection due to cross-protection. However, as immune defence is costly, a more broad-spectrum defence reaction is also likely to be more costly, which could increase rather than decrease later infection.

We aimed to distinguish between these alternatives, using for experimental repeated exposure the system of *S. solidus* and its copepod intermediate host. For this system, both the effect of heterogeneity on the infection and the induction of specific protection, i.e. memory, have been demonstrated in previous studies (Wedekind & Ruetschi, 2000; Kurtz & Franz, 2003). Individual copepod hosts were initially exposed to either a mix of three tapeworms derived from different sibships, or three worms from only one sibship. Three days later, the same copepod was exposed to one tapeworm derived from an unfamiliar sibship. Infection success resulting from this second exposure was then compared. Primary infection success was also determined to establish whether heterogeneous exposure leads to increased infection, as predicted by Wedekind & Ruetschi (2000).

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Materials and methods

Experimental animals

Worms used in the present experiment were the laboratory-reared offspring of parasites dissected from sticklebacks caught in the lake 'Neustädter Binnenwasser', northern Germany in the autumn of 2001 (for the analysis of surface carbohydrates) and 2003 (for the experimental exposure). Worm pairs matched for weight were placed in gauze tubes submerged in Minimum Essential Medium Eagle (MEM, Sigma M2645) for *in vitro* breeding (Smyth, 1946; van der Veen & Kurtz, 2002). The offspring of worm pairs were used and referred to as 'sibships' (Hammerschmidt & Kurtz, 2005a). Eggs were stored in darkness at 4°C until use and three weeks before infection, eggs were placed in darkness at 20°C. To stimulate hatching, eggs were exposed to light on the day prior to experimental infection (Dubinina, 1966).

Samples of *Macrocyclops albidus* were collected from large, outbred laboratory cultures initiated with specimen from the small river 'Kremper Au', which is connected to the 'Neustädter Binnenwasser'. Copepods were maintained at 20°C, 16 h light per day, individually in 2 ml tap water in the wells of 24-well tissue-culture plates. They were fed twice a week with three and once per week with five freshly hatched larvae of *Artemia salina*. Copepods were starved the day before experimental infection with tapeworms.

Heterogeneity in surface carbohydrates among tapeworm larvae

Variation in surface sugars among individual procercoids, which were obtained from experimentally infected copepod hosts, were analysed and surfaces were compared among eight parasite sibships (i.e. offspring of worm pairs), each contributing to the analysis with eight to ten procercoids. To obtain experimentally infected copepods, adult male copepods were each exposed to one parasite larva and examined 12 days post-infection.

The surface sugar composition of each procercoid was determined by incubation with three lectins simultaneously, each labelled with a different fluorescent dye: Alexa Fluor©-labelled concanavalin A (ConA; purchased from Molecular Probes), fluorescein-isothiocyanate (FITC)-labelled peanut agglutinin (PNA) and tetramethylrhodamine-isothiocyanate (TRITC)-labelled wheat germ agglutinin (WGA) (both purchased from Sigma-Aldrich). These plant-derived lectins bind to distinct sugars: WGA (from *Triticum vulgaris*) identifies N-acetylglucosamine (GlcNAc) and sialic acid residues; PNA (from *Arachis hypogaea*) binds to β-galactose-1,3 N-acetylgalactosamine (GalNAc) and D-galactose; ConA (from *Canavalia ensiformis*) recognizes α-mannose and α-D-glucose (Jacobson & Doyle, 1996). Images were obtained from the procercoids for lectins using an image analysis program IP Lab© 3.6.2 (For Mac OS 9.2.2, Scanalytics, Inc.). The intensity of labelling was quantified on a scale from 0 to 255, with highest values indicating maximum brightness, i.e. labelling (Hammerschmidt & Kurtz, 2005b).

Lectin labelling data presented here have previously been analysed under a different aspect (Hammerschmidt & Kurtz, 2005b). While our earlier focus was on differences between parasite stages in the two intermediate hosts, the present study concentrates on surface heterogeneity among parasite larvae in the copepod host.

Parasite heterogeneity and reinfection

The experiment consisted of first and second parasite exposures (fig. 1). Three tapeworm larvae (coracidia) were provided to each individual copepod for the first exposure whilst the second exposure occurred three days later with one tapeworm larva per copepod.

The experimental treatment groups were defined by the first exposure, which consisted of either three coracidia derived from the same worm sibship ('pure'), or three coracidia from three different sibships ('mixed'). Half of the copepods received a mixed exposure, using mixtures of the same three tapeworm sibships to infect 12 copepods on each 24-well plate. On the same plate, four

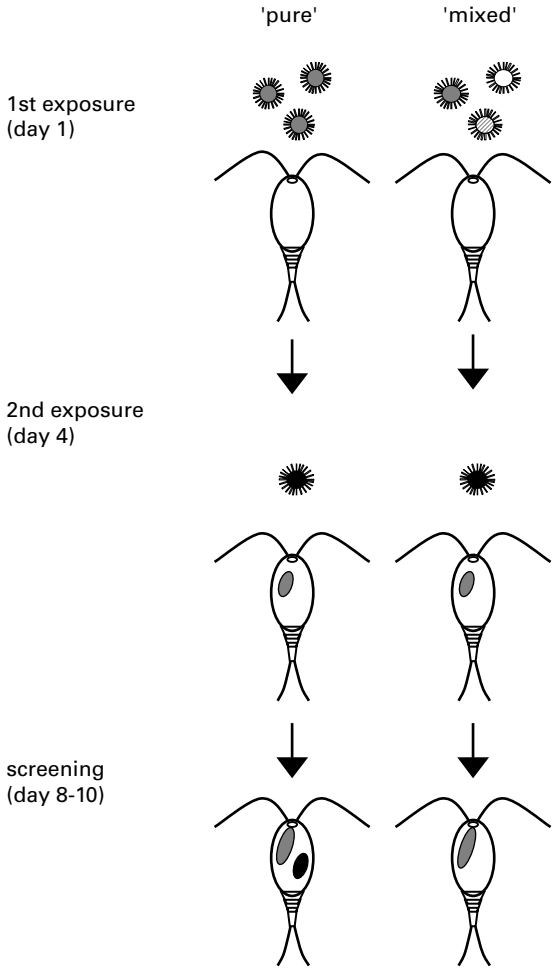

Fig. 1. Experimental design where the consequences of the heterogeneity of a first exposure were tested on the resulting first infection and on reinfection.

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copepods each received a pure infection with either one of these three sibships. In rare cases ($n = 6$) where larvae of a required sibship were not available due to insufficient hatching, another sibship or pure exposure was used instead, leading to a slight imbalance in sample size for the 'pure' and 'mixed' treatment ($n = 181$ and 178, respectively). Positions of the copepods on the plates were random with respect to the treatment, and the experimenter was blind to treatment during the following steps of the experiment.

Coracidia used for the second exposure were from a different sibship than any used for the first exposure of that particular plate, so that all copepods were naïve with regard to the sibship used for re-infection. The same parasite sibship was used for re-infection of all copepods on a plate. All coracidia used for the second exposure were labelled with a green fluorescent vital tracer dye CMFDA (1 μM) to enable later distinction from first infections (Kurtz *et al.*, 2002). Coracidia used for the first exposure were not labelled, except for three initial plates, where they were labelled with a blue fluorescent dye CMAC (20 μM). Worms from the first exposure were large enough for easy identification, which was why additional labelling was omitted later on.

After exposure, copepods were kept in the dark to prevent the dye from bleaching. Between four and six days after the second exposure, copepods were individually transferred to microscope slides and screened for tapeworm larvae (Kurtz *et al.*, 2002). In the copepods, labelled worms were easy to distinguish from both unlabelled worms and worms that were labelled with the other dye. Based on a previous study (Kurtz *et al.*, 2002), no effect was expected of the dyes on parasites or hosts with the dye concentrations used here. However, even if there was an effect, this would not influence our conclusions, since we only compared those copepods infected with worms that were labelled similarly.

Data analyses

Statistical analyses were performed using JMP 5.01 for Macintosh (© SAS, 1989–2002). To partition the variance in the intensity of lectin labelling and to check whether sibships of tapeworms differed therein, analyses of variance were calculated with the intensity of each lectin (WGA, PNA, ConA) as the response and the parasite sibship as a random factor. In the experiment that focused on the effect of heterogeneous exposure, the prevalence and intensity of primary infections between the treatments 'pure' and 'mixed', and the prevalence of secondary infections in relation to these treatments, were compared using likelihood ratio Chi square (LR χ^2) test statistics. Power details were obtained from the freeware program G Power © (Buchner *et al.*, 1997). All test statistics referred to two-sided tests, and with significant differences considered at a level of $P < 0.05$.

Results

Heterogeneity in surface carbohydrates among tapeworm larvae

Labelling was performed with three fluorescent lectins to analyse carbohydrate surfaces of tapeworm larvae, obtained from their copepod intermediate host. The intensity of labelling with all three lectins varied considerably among procercoids (figs 2, 3). Parasite

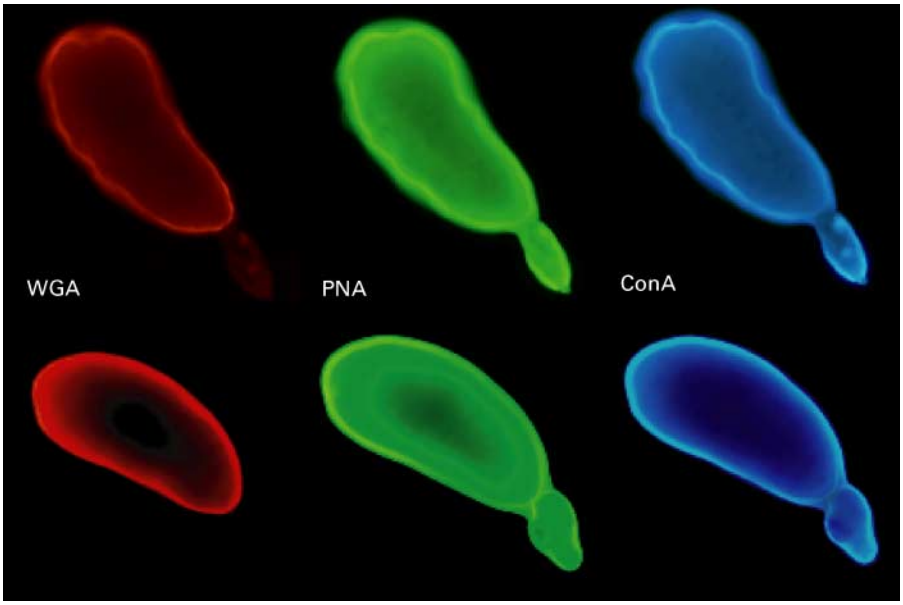

Fig. 2. Epifluorescent micrographs of procercoids of the tapeworm *Schistocephalus solidus*, dissected from the copepod intermediate host. Tapeworms were labelled with three fluorescent lectins: WGA (TRITC labelled), PNA (FITC labelled), ConA (Alexa Fluor 350 labelled); two worms are shown (top, bottom), which differ in the binding intensity of the three lectins.

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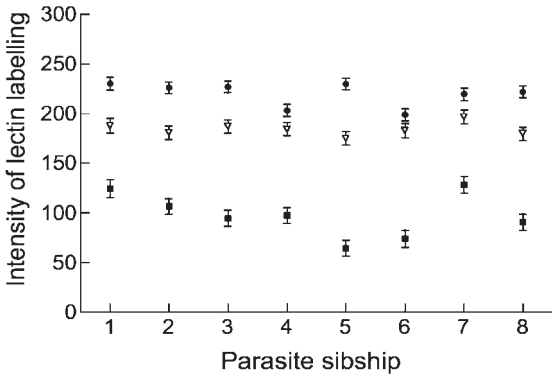

Fig. 3. The intensity of binding of three fluorescent lectins to *Schistocephalus solidus* procercoids (WGA (■), PNA (•), ConA (▽)) to indicate that sibships differ significantly in the intensity of labelling with WGA and PNA; means and standard errors are shown for each parasite sibship.

sibships contributed significantly to such variation for the two lectins WGA and PNA (WGA: $F_{7,68} = 6.044$, $P < 0.0001$; PNA: $F_{7,68} = 2.946$, $P = 0.009$), explaining 39.7% and 23.9% of the total variation, respectively, whereas the labelling intensity with ConA did not differ significantly among the sibships ($F_{7,68} = 0.880$; $P = 0.528$). Labelling with the three different lectins was not significantly correlated across sibships (WGA with PNA: $r_S = -0.26$, $n = 8$, $P = 0.53$; WGA with ConA: $r_S = 0.52$, $n = 8$, $P = 0.18$; PNA with ConA: $r_S = -0.42$, $n = 8$, $P = 0.28$).

Parasite heterogeneity and reinfection

A total of 359 copepods, distributed over fifteen 24-well plates, were included in the experiment (one well accidentally containing two copepods was excluded). Forty copepods (11.1%) died during the experiment, which was independent of the treatment ('pure' first exposure: 9.9%, 'mixed': 12.4%; LR $\chi^2_{1,357} = 0.529$, $P = 0.467$). Six copepods were accidentally lost or killed during screening for parasites, leaving 313 copepods for final analyses.

Primary infections, i.e. infections resulting from the first exposure, were analysed in the first place with a mean prevalence of 77.3%, which was independent of the treatment. 77.6% of the copepods were infected with tapeworms resulting from a primary exposure with larvae derived from one worm sibship ('pure'), compared to an almost equal prevalence of 77.0% after a 'mixed' exposure to larvae from three different sibships (LR $\chi^2_{1,311} = 0.020$, $P = 0.889$). The intensity of the primary infection was also largely independent of this treatment, mounting to a mean of 1.74 tapeworms per infected copepod in the 'pure' treatment and 1.62 worms after a 'mixed' exposure ($F_{1,240} = 1.759$, $P = 0.186$). Although there seemed to be a slight shift towards multiple infections after 'pure' exposure, this trend was also not significant; 57.7% of infected copepods harboured more than one parasite after a 'pure' exposure, compared to 49.6% after a 'mixed' exposure (LR $\chi^2_{1,240} = 1.568$, $P = 0.211$). Tapeworm sibships and mixtures, respectively, seemed to differ in their infection success, although the design of the experiment did not allow separating such worm line effects (which were not the focus of the current experiment) from potential differences among the experimental plates (prevalence: LR $\chi^2_{8,304} = 19.245$, $P = 0.014$; intensity: $F_{8,233} = 2.970$, $P = 0.004$).

The main focus of the current experiment was the effect of the diversity of parasites used for a primary pathogenic insult on the resistance of copepods against secondary infection. The likelihood of a secondary infection was not affected by the diversity of a primary exposure. 34.8% of copepods that had received a 'pure' primary exposure were infected with a single, unfamiliar tapeworm larva encountered during the second exposure, compared to 36.8% of copepods with a history of 'mixed' prior insult (LR $\chi^2_{1,311} = 0.144$, $P = 0.704$). As in the first infection, tapeworm lines differed in their infection success (LR $\chi^2_{6,306} = 15.471$, $P = 0.017$). There were also indications for differences in susceptibility among copepods. Individuals that were infected after the first exposure were also more likely to be infected during the second exposure (41.3% compared to 16.9%; LR $\chi^2_{1,311} = 15.581$, $P = 0.0001$), which shows that individual copepods sustainably differ in their resistance to tapeworm infections. In a nominal logistic model with all three factors, worm sibship and prior infection were significantly associated with a secondary infection success (table 1), while prior exposure had no effect.

Discussion

Using lectin labelling, considerable variation in surface carbohydrates among sibships of the tapeworm *S. solidus* in its copepod intermediate host was observed. Immunological recognition could be based on such heterogenic parasite characteristics. However, the heterogeneity of a prior parasitic exposure did not significantly influence the likelihood of a subsequent infection with an unfamiliar genotype of the same parasite species. As to the present experimental host–parasite system, there is no indication that a more broad-spectrum immune response that may result from heterogeneous prior experience, is more protective against a later infection. We could also not confirm the result of a previous experiment in the same system showing a higher prevalence, but a lower intensity resulting from heterogeneous as compared to homogenous parasitic exposures (Wedekind & Ruetschi, 2000).

Table 1. Nominal logistic fit for the effect of prior exposure treatment ('pure' vs. 'mixed'), worm sibship and prior infection on secondary infection success (whole model LR $\chi^2_8 = 31.945$, $n = 313$, $P < 0.0001$).

	df	Wald χ^2	P
Treatment	1	0.1601	0.6891
Worm sibship	6	14.0060	0.0296
Prior infection	1	13.8451	0.0002

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Variation in carbohydrate surfaces of tapeworms within their invertebrate intermediate host is relevant especially with reference to an unexpectedly high degree of specificity in the interaction between invertebrates and their parasites (Kurtz, 2005). Specific immunological recognition not only requires immunological receptors that enable the detection of small differences among parasites but also sufficiently variable structures of parasites as targets for detection. The invertebrate immune system is devoid of antibodies that detect primarily peptide antigens in the vertebrate immune system. The few examples for specific recognition within invertebrate immune systems indicate that parasite carbohydrates rather than peptides may be the main targets for detection. The snail *Biomphalaria glabrata* seems to make use of somatically diversified fibrinogen related proteins (FREPs), which are lectin-like proteins and receptors for carbohydrate residues (Adema *et al.*, 1997; Zhang *et al.*, 2004). In the present tapeworm–copepod system, the parasite surfaces are sufficiently variable to enable specific recognition, which confirms our previous observations of specific immunological memory in this system (Kurtz & Franz, 2003) and puts carbohydrate recognition into focus for further studies on potential mechanisms that may underlie specific recognition in invertebrates.

From the parasites' perspective, it would be beneficial to possess a surface that is hard to detect by the hosts' immune system. The large variation in surface sugars observed among sibships alludes to potential genetic variation and might also indicate that surface sugars change quickly during co-evolution with their hosts' detection system, as predicted by the 'Red Queen' hypothesis (van Valen, 1973). Heterogeneous surface carbohydrates may thus constitute a moving target for the hosts' immune system.

Moreover, parasites with more than one host might be forced to change their surface according to their different hosts' immune systems. In a previous study with the same system of *S. solidus* and its consecutive intermediate hosts (copepods and sticklebacks), we found a strong shift in the tapeworms' surface upon host switch (Hammerschmidt & Kurtz, 2005b). Parasite surface characteristics were furthermore associated with fitness parameters such as infectivity and growth in the second intermediate host (Hammerschmidt & Kurtz, 2005a).

Although the results from lectin labelling indicate that there is sufficient antigenic variability among tapeworm larvae in their copepod host, previous findings that more heterogeneous mixtures of tapeworms are more likely to infect their copepod host (Wedekind & Ruetschi, 2000) were not confirmed. No precise conclusions can be drawn about this unexpected discrepancy between the current and this previous experiment. Insufficient statistical power is a possibility, although unlikely. Although the sample size in the present study was slightly lower (313 compared to 413 copepods), the infection rate was far higher (77.3% compared to 17.9%), so that the power with regard to the intensity of infection was higher rather than lower. Such power would have been sufficient to detect the rather large effect sizes observed in the previous study (Wedekind & Ruetschi, 2000), namely 58.6% higher prevalence and 22.0% reduced intensity after heterogeneous exposures. The statistical power for detection of such effects in the present study would have been 0.991 for prevalence and 0.987 for intensity.

Wedekind & Ruetschi (2000) indicated that tapeworms were mostly obtained from self-fertilized worms, while in the present study outcrossed offspring were produced by sexually reproducing worm pairs. Thus heterogeneity of offspring produced by an outcrossing worm pair is already high enough to obscure any effects of increased heterogeneity in worm mixtures of diverse sibships. In line with this argument, the enhanced infection success of larvae derived from outcrossing tapeworm parents has been reported by Christen *et al.* (2002) and Christen & Milinski (2003). On the other hand, we also found that antigenic relatedness within worm sibships is sufficient to lead to line-specific immunological memory in the copepod host even against outcrossed worm clutches (Kurtz & Franz, 2003).

However, most likely the observed discrepancy between current and previous outcomes results from differences between the host and parasite populations, which were used in the experiments. In the present study, hosts and copepods were studied from large outbreeding, potentially co-evolving natural populations, whereas Wedekind & Ruetschi (2000) used tapeworms from a small population in combination with copepods from a geographically remote habitat. Distinct differences between these populations have also been observed for several other aspects of the interaction between this parasite and its host (Schärer & Wedekind, 1999; Christen *et al.*, 2002; Schjørring, 2004). In particular, the tapeworm population used in previous studies might have had a history of inbreeding that could result in reduced heterogeneity (Schjørring, 2004). Such divergent results obtained from different populations of the same species of parasite and host suggest caution when transferring results obtained for a particular population, or even a laboratory-adapted host–parasite model, to the whole species of the parasite or beyond.

The present study thus relates to two different types of costs of immunity. Firstly, there are energetic or immunopathological costs of raising and/or maintaining immune reactions (Moret & Schmid-Hempel, 2000) and secondly, costs might arise from an inability to react against many different types of parasites. Raising an immune reaction against one type of pathogen might impede the reaction against a different pathogen and such a cost, known as an 'option cost' (Zuk & Stoehr, 2002), only applies to reactions that are specific.

Arguably, the evolution of induced immune defences might be regarded as a means of dealing with the first type of costs of defence (Armitage *et al.*, 2003; Schmid-Hempel & Ebert, 2003). Even in invertebrate hosts, some induced defences remain active for some time after an infection has been cleared (Faulhaber & Karp, 1992; Janeway *et al.*, 1999; Little & Kraaijeveld, 2004; Jacot *et al.*, 2005). The fact that many immune reactions are inducible could be an argument for some sort of cost, because otherwise constitutive defences might often be the optimal strategy, since there is no delay between parasitic exposure and execution of the defence reaction.

The most elaborate form of induced defence is the acquired immune response of vertebrate hosts (Janeway *et al.*, 1999). Here, immunological memory is highly specific, leading to strong protection against similar parasites that are encountered later (Ahmed & Gray, 1996; Zinkernagel *et al.*, 1996). Vertebrates are able to deal with 'option costs' as a large number of different antigens can be recognized. This is achieved through a virtually unlimited number of T and B cell clones responsible for specific memory, which are set aside for specific defence against diverse parasites (Janeway *et al.*, 1999). On the other hand, the amount of different parasites that an invertebrate can specifically combat is likely to be much more limited (Klein, 1989; George, 2002) to such an extent that an individual may be able to defend itself efficiently against one type of parasite, but this may compromise its defence against a later insult with a different parasite.

In the present study, neither reduced nor enhanced protection against re-infection after a heterogeneous as compared to a homogeneous previous exposure was found and it is possible that the two types of costs are equally strong in this particular system. With regard to protection against later infection, a potential benefit resulting from more broad-spectrum induced immunity may be compensated by the higher cost of raising such a broader response. Such costs could be energetic as more immune effector molecules may be needed or immunopathological where a higher degree of cross-reactivity may bear the risk of reaction with self tissue.

However, a more realistic explanation is based on the fact that no effect of the heterogeneity of exposure on the primary infection itself was found. If in this specific tapeworm population, homogeneous exposure was already too heterogeneous to find any differences in infection as compared with a heterogeneous exposure, then differences in cross-protection against re-infection may also be unexpected. In conclusion, more studies are needed to gain insight into an ecologically relevant situation where hosts are confronted with repeated exposures to heterogeneous mixtures of pathogens.

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