Zoology **106** (2003): 327–339

http://www.urbanfischer.de/journals/zoology

ZOOLOGY

REVIEW

Sex, parasites and resistance – an evolutionary approach**

Joachim Kurtz*

Max Planck Institute of Limnology, Department of Evolutionary Ecology, Plön, Germany

Summary

Immune systems are among the most diverse biological systems. An evolutionary arms race between hosts and rapidly evolving pathogens is supposed to be a reason for this diversity, and might explain why most eukaryotic hosts and parasites reproduce sexually. In this review, I will focus on possible benefits of sexual reproduction in hosts and parasites, using a model system consisting of a tapeworm and its two intermediate hosts, copepods and sticklebacks. We found that the hermaphroditic tapeworms can increase their infection success by reproducing sexually with a partner (outcrossing), instead of reproducing alone. The defence system of the copepods provides highly specific discrimination of antigenic characteristics of the tapeworms. This supports the finding that tapeworms benefit from outcrossing, but contradicts the conventional notion that the immune system of invertebrates, in contrast to vertebrates, is not able to react with specificity. Finally, sticklebacks seem to benefit from optimal diversity in their specific immune system. Previous studies showed that female sticklebacks prefer mates, which sire offspring with an optimal diversity in the MHC (genes involved in antigen presentation). We now found that these individuals suffer less from tapeworm infection. Furthermore, they are able to reduce the expression of an unspecific immune trait, thereby possibly avoiding harmful side effects of a highly activated, unspecific immune system.

Key words: host-parasite co-evolution, sexual selection, immunity, MHC, inbreeding

Introduction

Most plants and animals reproduce sexually. Only very few species are asexual over extended evolutionary periods (Barton and Charlesworth, 1998; Chaplin et al., 1994; Darwin, 1871; Judson and Normark, 1996). Why are there hardly any organisms that live completely asexual? The widespread occurrence of sex is puzzling, since there is a built-in cost to sexual reproduction. Consider a population of sexual males and females, where a mutation arises that causes females to reproduce asexually, i.e. to produce only asexual daughters. If these asexual females have the same family size as sexuals, their numbers relative to sexual females will double each generation, because they produce only asexual daughters. This situation has been described as the 'two-fold cost of sex' (Barton and Charlesworth, 1998; Maynard Smith, 1978; Williams, 1975).

In essence, sex is the exchange of genetic information between individuals (Barton and Charlesworth, 1998; West et al., 1999). This recombination of genetic information should be the relevant point when seeking for advantages of sex outweighing its costs (Otto and Michalakis, 1998). Sex and recombination should be most advantageous when the environment is changing quickly. The environment the parent generation was optimally adapted to will then differ from the environment the offspring will encounter.

*Corresponding author: Joachim Kurtz, Max-Planck-Institute of Limnology, Department of Evolutionary Ecology, August-Thienemann-Straße 2, 24306 Plön, Germany; phone: +49 4522 763256; fax: +49 4522 763310; e-mail: kurtz@mpil-ploen.mpg.de

** Presented at the 96th Annual Meeting of the Deutsche Zoologische Gesellschaft, in association with the Deutsche Gesellschaft für Parasitologie, in Berlin, June 9–13, 2003

0944-2006/03/106/04-327 $ 15.00/0

Parasites are arguably the most rapidly changing component of the environment. Parasites (the term is used here in a broad sense, including all types of pathogens, from viruses and bacteria to macroparasites) are ubiquitous, and there is perhaps no organism without any parasites (Poulin, 1996; Windsor, 1998). In contrast to the abiotic environment, parasites constantly adapt to their hosts. An initially rare parasite genotype, which maximally exploits the host, will increase in abundance. As a consequence, the next host generation will be confronted with large numbers of this highly virulent parasite.

To keep pace, hosts have to react with counter-adaptations, such as an improved immune defence system, against this type of parasite. In such a co-evolutionary arms race, standing still actually means falling behind, a process referred to as 'Red Queen' co-evolution (van Valen, 1973). However, often parasites seem to stay in front in this race, because they have shorter generation times and appear in larger numbers. In such a situation, sexual reproduction could be helpful, bringing together beneficial traits in one individual more easily. Furthermore, offspring will differ from their parents (whom the parasites have adapted to) and among each other (thereby increasing the chance that at least one of them might escape from the most virulent parasite genotypes). All in all, sex could speed up host adaptation to co-evolving parasites (Ebert and Hamilton, 1996; Hamilton et al., 1990).

A similar kind of argument might be applicable to advantages of sexual reproduction for parasites, in particular when their capacity to evolve quickly does not exceed that of their hosts. Especially macroparasites with often rather long generation times might benefit from sexual reproduction, enabling them to keep pace with a quickly adapting host immune system.

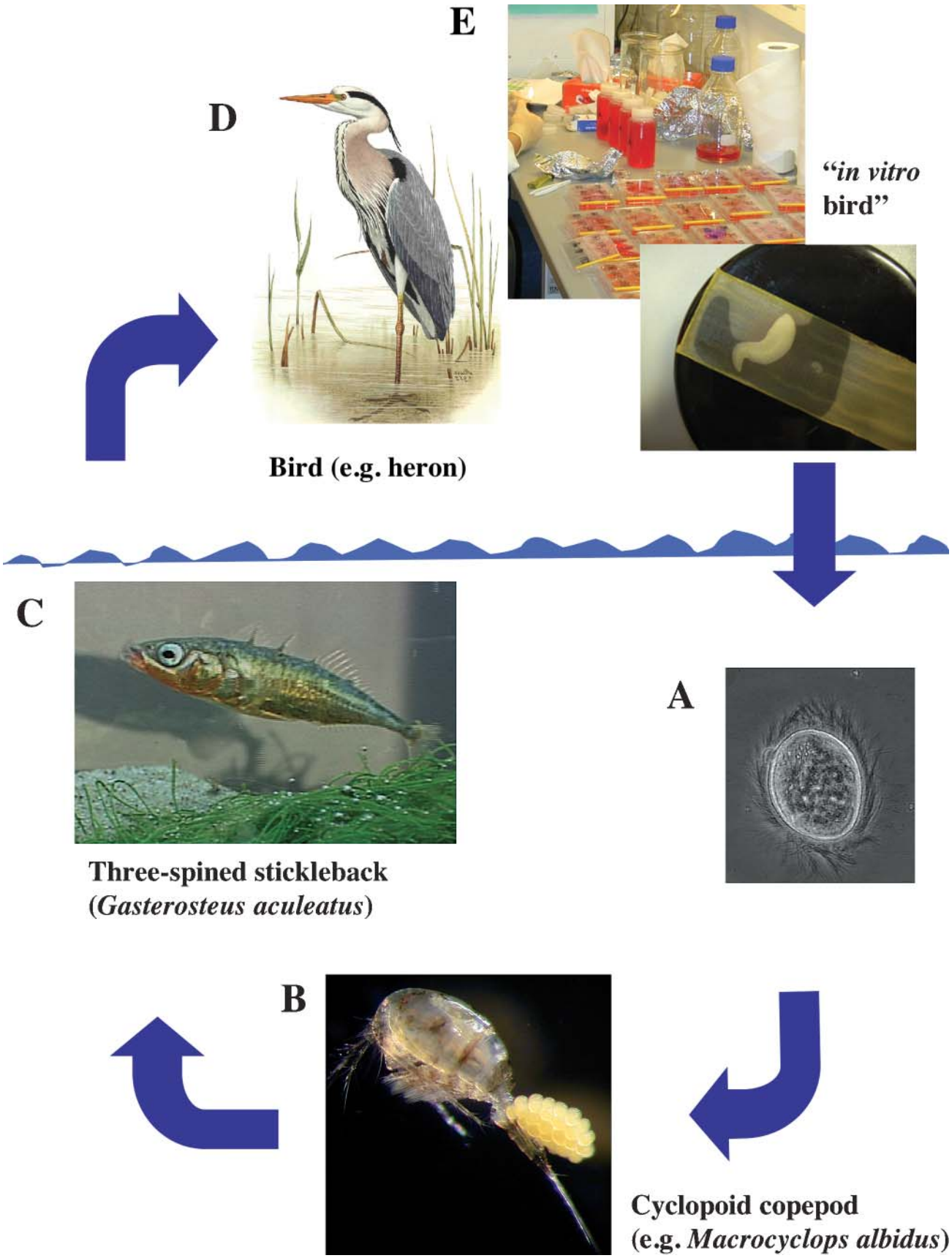
Setting the stage: a host-parasite model system

To test predictions about the evolutionary significance of sex in relation to host-parasite co-evolution, a model system which can be experimentally manipulated in the laboratory would be ideal. The pseudophyllidean tapeworm *Schistocephalus solidus* and its two intermediate hosts might be quite a perfect model (Fig. 1). The tapeworms and both intermediate hosts can be bred in the laboratory (Dubinina, 1957; Smyth, 1946; Wedekind, 1997). Several species of cyclopoid freshwater copepods serve as the first intermediate host, while the three-spined stickleback *(Gasterosteus aculeatus)* is the only suitable second intermediate host. Any species of fish-eating bird, e.g. heron, cormorant, or gull, can serve as the definitive host (Clarke, 1954). Tapeworms grow in the body cavity of the copepod (Fig. 2A–C) and stickleback intermediate hosts, while reproduction occurs in the bird gut. Tapeworm eggs are released into the water in the birds' faeces, and copepods prey upon the free-swimming larvae (coracidia). While both intermediate hosts suffer from an infection (Arme and Owen, 1967; Wedekind, 1997), birds are most likely not influenced strongly by the presence of these tapeworms in their gut. Therefore, to study host-parasite co-evolution in this system, the intermediate hosts are better suited than the definitive host (if there is no fitness reduction in the definitive host, the tapeworms are not supposed to induce any selection pressure on the host). For experimental set-ups, the bird host can be substituted by an *in vitro* breeding system (Fig. 1E; Smyth, 1946; Wedekind, 1997). The worms are kept in gauge nets in culture medium at 40 °C, a condition resembling the final hosts' gut. Eggs are collected and stored at 4 °C in the dark. For use, eggs are cultured at 18 °C for 3 weeks, and hatching is induced by exposure to light (Smyth, 1946; van der Veen and Kurtz, 2002; Wedekind, 1997). The *in vitro* system enables experimental manipulation and even observation of the worms while breeding (Lüscher and Wedekind, 2002).

Sexual reproduction and infection success of tapeworm parasites

S. solidus is a simultaneous hermaphrodite, i.e. each individual has male and female reproductive organs at the same time. The easiest way to produce offspring would therefore be to simply use self-sperm to fertilize the eggs (self-fertilization or 'selfing'). Yet, the worms seem not to like this option. In experimental pairs of worms, most prefer to have sex with their partner (cross-fertilization or 'outcrossing'; Lüscher and Milinski, 2003). They do so, in spite of a cost that arises from the conflict situation of the two worms, referred to as the 'hermaphrodite dilemma' (Leonard, 1990): both partners prefer

Fig. 1. Life cycle of the pseudophyllidean tapeworm *Schistocephalus solidus*. (**A**) Free-swimming larvae (coracidia) are ingested ► by a cyclopoid copepod (**B**), where they develop into procercoids. When copepods are eaten by three-spined sticklebacks (**C**), the tapeworm develops into a plerocercoid. Tapeworms produce eggs inside the gut of a fish-eating bird (**D**), which can be replaced in the laboratory by an *in vitro* cultivation system (**E**).


E
D
"in vitro bird"
Bird (e.g. heron)
C
A
Three-spined stickleback
(Gasterosteus aculeatus)
B
Cyclopoid copepod
(e.g. Macrocyclops albidus)

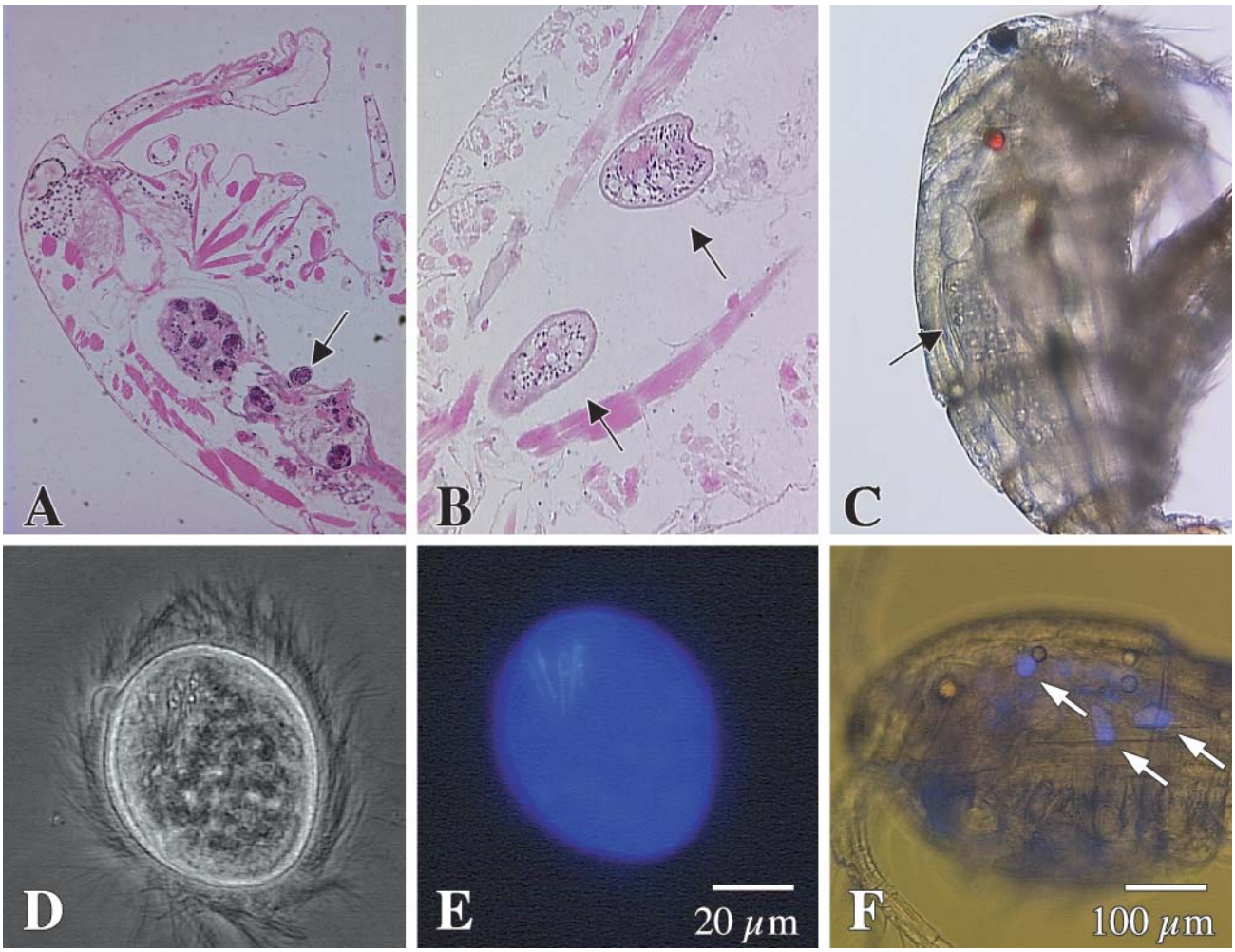


Fig. 2. Development of *S. solidus* tapeworms inside *Macrocyclops albidus* copepods. (**A**) Passage of tapeworms from the gut into the body cavity of copepods seems to be a decisive phase. (**B**) Once in the body cavity, the procercoids are rarely eliminated by the copepod defence system, and can reach a substantial size (**C**). To track individual tapeworms, coracidia (**D**) can be labelled with the blue fluorescent tracer dye CMAC (**E**). The label is retained during development into procercoids, which are thus visible inside the copepod (**F**) (E, F modified from Kurtz et al., 2002a).

to play the male role, i.e. they want to fertilize as many of the partner's eggs as possible, while saving their eggs for self-fertilization. This would maximize the number of offspring produced by such a 'selfish' worm. So, what keeps the worms from selfing, and what is the advantage of outcrossing, i.e. having sex with a partner? It should be noted that both modes of reproduction are sexual. However, during selfing recombination occurs only within the same individual. The consequences therefore resemble asexual reproduction.

Based on a possible advantage of sexual reproduction in host-parasite co-evolution, we hypothesized that outcrossing *S. solidus* might have an advantage in the arms race with their hosts (Christen et al., 2002). The worms have relatively long generation times, normally a year, which is longer than the generation time of their copepod host, and comparable to the stickleback. When self-fertilizing the eggs, the young worms will be quite similar to their parents, and also among each other. Those copepod and stickleback hosts with the best immune defence against the parent worms will suffer less from tapeworm infection, and contribute more offspring to the next generation. Consequently, such host genotypes will have become more abundant the next year. Bad luck for offspring of worms that self-fertilized! Such offspring are expected to be similarly vulnerable to the host immune defence as their parents. In contrast, offspring from outcrossing worms will differ more strongly from their parents, and will be more diverse among each other. Most likely, therefore, some of them will have advantages in the interaction with their hosts.

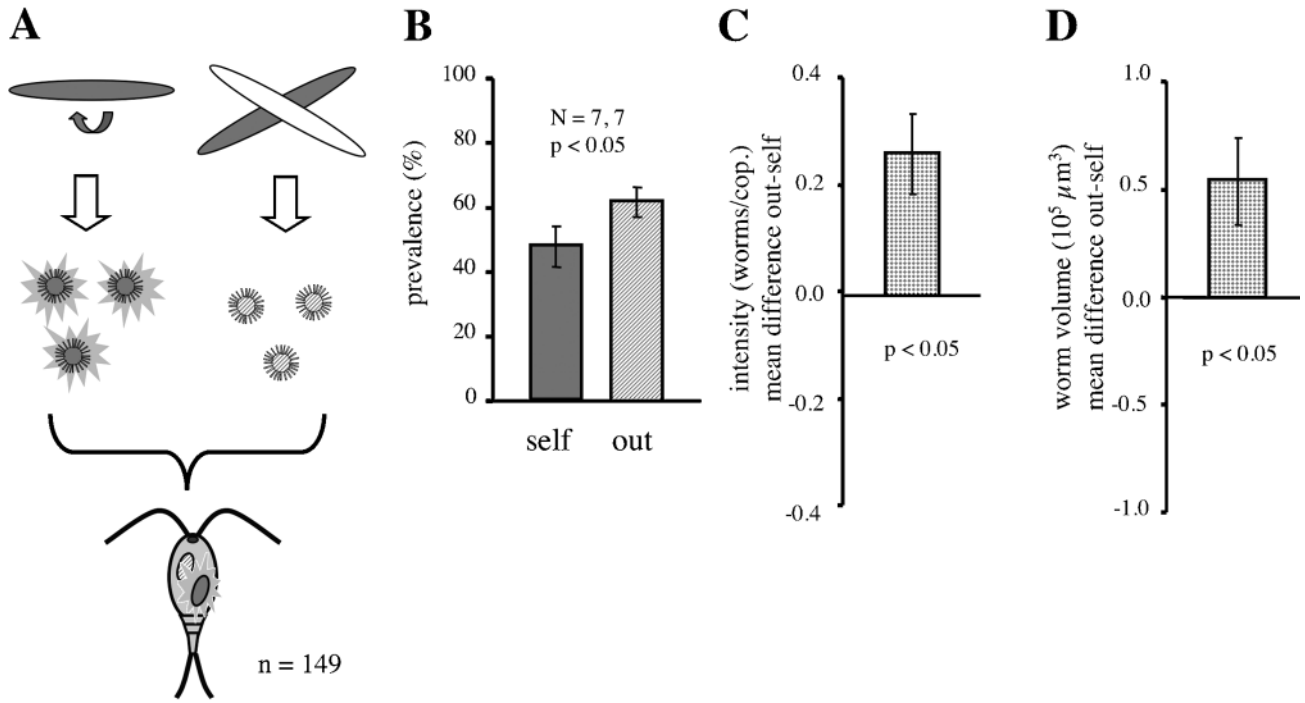


Fig. 3. Experimental analysis of outcrossing benefits for *S. solidus* tapeworms in their interaction with copepods. (**A**) Individual copepods were simultaneously exposed to tapeworm larvae produced by selfing and outcrossing parents, three of each type. For later discrimination, one type of larvae was fluorescently labelled. In half of the cases the selfed and in the other half the outcrossed larvae were labelled. (**B**) Six days post infection, more copepods were infected with outcrossed larvae. (**C**) Infected copepods harboured on average more outcrossed than selfed larvae. The bar shows the mean difference in intensity, i.e. the number of outcrossed worms minus the number of selfed worms inside each infected copepod. (**D**) Outcrossed larvae reached a larger size inside the copepods. The bar shows the mean difference in worm volume per copepod, i.e. the mean volume of outcrossed worms minus the volume of all selfed worms within each doubly infected copepod (data from Christen et al., 2002).

In a laboratory experiment we tested whether offspring of outcrossing *S. solidus* parents have a higher infection success and grow faster in their copepod intermediate host compared to offspring produced by self-fertilization (Fig. 3). For simplicity, offspring will further on be denoted as 'outcrossed' or 'selfed', respectively. Tapeworms were dissected from sticklebacks, caught from a large population of a lake connected to the Baltic Sea in Schleswig-Holstein, northern Germany. In order to breed tapeworms from selfing and outcrossing parents, the worms were either isolated for egg production, or paired immediately after collection from their stickleback hosts. The size of the worms was matched between the two worms of each pair, increasing their preference to mate with the partner (Lüscher and Milinski, 2003). In addition, sets of worm pairs and single worms were also matched with regard to the size of the single worm; that is, single worms matched both worms in a corresponding pair. This enabled pair-wise comparison, thereby controlling for possible size-related differences in infectivity. For infection, each individual copepod was exposed to both types of larvae, three selfed and three outcrossed, derived from the parasite sibships, which had been matched according to parent worm weight. This enabled direct competition between selfed and outcrossed parasites within the same host individual. To discriminate between them, selfed and outcrossed parasites had been labelled with a fluorescent tracer dye in varying combinations (see Fig. 3A; Kurtz et al., 2002a). Labelled coracidia maintain a stable fluorescent signal, which can be tracked into the procercoid stage in the copepod (Fig. 2D–F). The dye does not harm the tapeworms and the copepods, nor does it dissociate from the worm tissue, and is stable for up to two weeks if the experimental animals are kept in the dark.

The results of this experiment were quite clear. Outcrossed tapeworms performed far better than selfed ones (Fig. 3). More copepods harboured outcrossed worms, which were also found in higher numbers in infected copepods. Furthermore, outcrossed worms also grew to a larger body size. Interestingly, such an advantage of outcrossing was not observed when copepods

were infected with either selfed or outcrossed tapeworms, instead of both types simultaneously (Christen et al., 2002). This indicates that competition for a common pool of resources within the same host is essential for an outcrossing advantage.
A similar experiment was recently performed with the second intermediate host, the stickleback. As in the copepod, an increased infection success of outcrossed tapeworms was observed in a competitive situation (Christen and Milinski, 2003).
These results show that sexual reproduction with a partner improves the vigour of the tapeworms in the interaction with their hosts. This could be due to the advantages in host-parasite co-evolution outlined above. On the other hand, self-fertilization also represents a high degree of inbreeding, and the observed effects might be a result of inbreeding depression. Inbreeding depression could reduce fitness, because after self-fertilization recessive deleterious mutations arrive at a homozygous state. However, a previous experiment in the same host-parasite system showed that heterogeneity among parasites enhances infection success in the copepod host (Wedekind and Rüetschi, 2000). All in all, it therefore seems likely that at least part of the positive effect of outcrossing in the context of host-parasite co-evolution is mediated by advantages resulting from offspring heterogeneity, not only inbreeding depression.
The idea of host-parasite co-evolution is based on the assumption that host immune defence is rather specific for different parasite genotypes. It is hard to imagine that evolutionary change of both parasite infectivity and host immune defence could be particularly rapid, when only non-specific defence mechanisms of hosts are involved. However, it is commonly assumed that invertebrate host defence is mediated by rather unspecific mechanisms. We therefore tried to find out how specific the defence of copepod hosts against tapeworm parasites might possibly be.

How specific is invertebrate immunity?

Inspired by our finding of considerable advantages resulting from outcrossing in *S. solidus* when infecting copepods, we challenged the paradigm that invertebrate host defence is purely unspecific. We tested the hypothesis that the defence system of copepods might enable more specific reactions against parasites than is currently assumed for invertebrates (Kurtz and Franz, 2003).
We performed a reinfection experiment, consecutively exposing copepod individuals to tapeworm larvae, with a time gap of three days between the exposures. To analyze the specificity of defence we made use of the fact that relatedness should determine antigenic similarity between individual parasites. The treatments consisted of either consecutive exposure to sibling parasites, or consecutive exposure to unrelated parasites (Fig. 4). To control for genetic differences in infectivity, two parasite sibships were always combined cross-wise, serving as the source of unrelated parasites for each other. If there is any kind of specific immune defence in the copepod hosts, we expect a reduced rate of reinfection for sibling parasites. As predicted, prior exposure to siblings reduced the secondary infection success compared to prior exposure to un-related parasites (Fig. 4).
The effect of a prior exposure to sibling parasites, i.e. reduction of reinfection, should increase with the antigenic similarity between the parasites. To vary the degree of similarity, we used tapeworm larvae derived from ‘selfing’ or ‘outcrossing’. Antigenic similarity should be higher after selfing. In contrast to the experiment described in the previous chapter aiming at advantages of outcrossing for the parasites, we were not interested in that aspect in the current experiment. Rather, we wanted to know whether the effect of the prior exposure to siblings would increase with the increased similarity when selfed worms were used. As predicted, such a trend was observed (Kurtz and Franz, 2003). This is relevant also with regard to our previous finding of outcrossing benefits for the parasite. Even when having to cope with an invertebrate immune system, outcrossing could be beneficial for the parasite, because it increases the chance to re-infect the host.
However, could such reduced reinfection also be caused by factors other than the host defence system? In particular, could the parasites, rather than the hosts, cause the reduced reinfection? This is unlikely because kin selection would favour cooperation between siblings, which would facilitate rather than reduce reinfection (Davies et al., 2002; Parker et al., 2003). If, on the other hand, within-host competition among siblings is particularly strong, only hosts which were previously infected could be affected (Sire et al., 1998; Wedekind and Rüetschi, 2000). However, excluding these hosts from our sample did not decrease the observed effect (Kurtz and Franz, 2003). Therefore, parasite-derived effects are unlikely to explain the reduced reinfection.
We concluded that the defence system of copepods is capable of reacting more efficiently when it had previously encountered antigenically similar parasites. This was the first example of such a high degree of specificity in the defence reaction of individual invertebrates.
Such a result was rather unexpected for an invertebrate host, while it would not be astonishing for a vertebrate. Acquired immunity of vertebrates is characterized by immunological memory and specificity (Janeway et al., 1999). The acquired immune system (also known as adaptive or specific immunity) is based on genetically

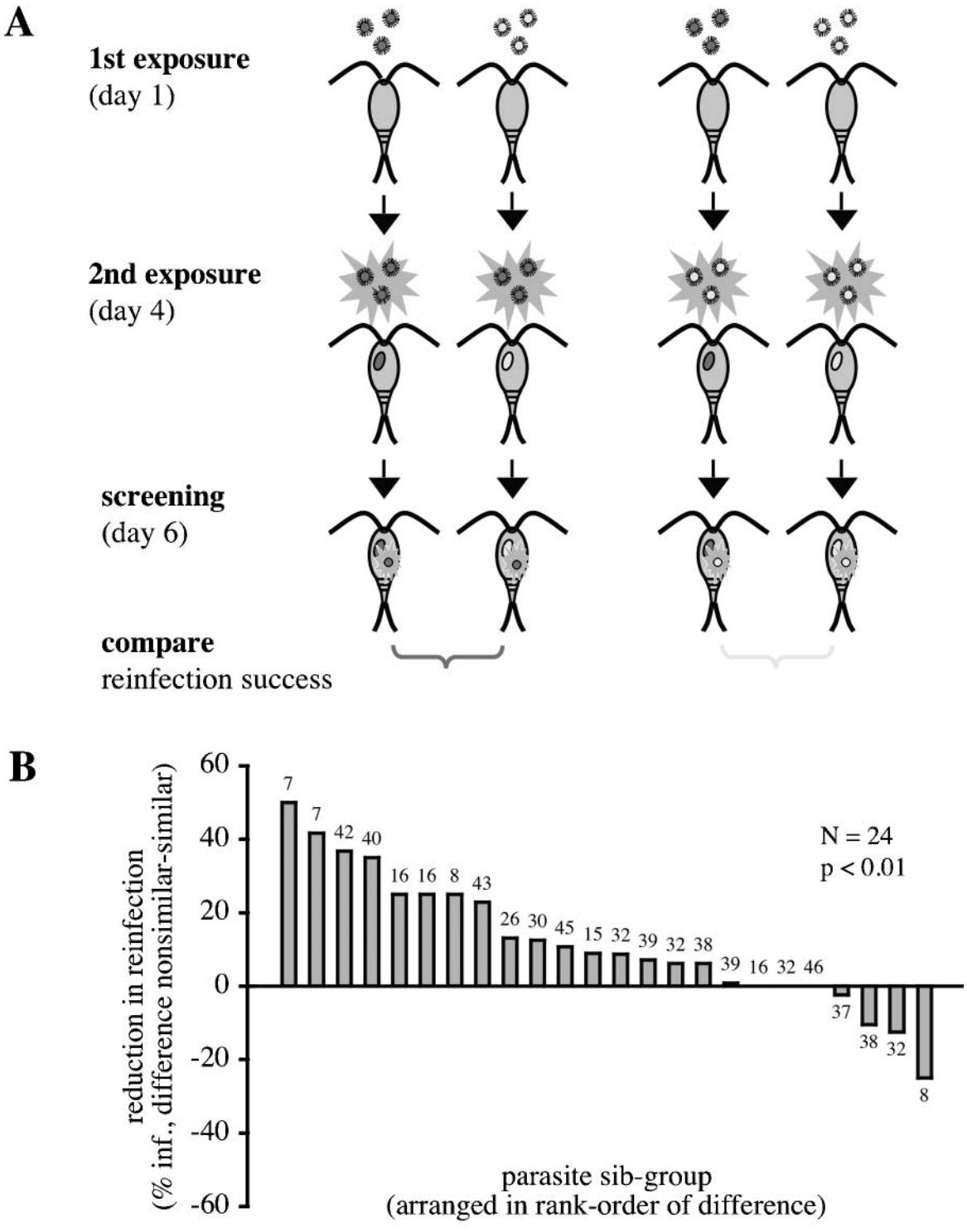


Fig. 4. Experimental demonstration of a high degree of specificity in the defence of copepods against tapeworms. (**A**) Copepods were exposed to tapeworms repeatedly. The treatments consisted of either consecutive exposure to sibling parasites, or consecutive exposure to unrelated parasites. (**B**) Each bar shows the difference in the infection success of a parasite sibship for copepods previously exposed to another or to the same sibship (numbers above bars indicate sample size, i.e. number of copepods exposed). Prior exposure to siblings reduced the secondary infection success compared to prior exposure to unrelated parasites in 17 of 24 tapeworm sibships. This indicates that the defence system of copepods was capable of reacting more efficiently when it had previously encountered antigenically similar parasites (modified from Kurtz and Franz, 2003).

rearranging genes, generating huge variability in T cell receptors and antibodies. This enables highly specific recognition of molecular details of antigens. In contrast, invertebrates rely solely on innate immunity. They are therefore believed to be devoid of any kind of highly specific recognition. However, there has been considerable debate about this issue, since invertebrates appear capable of induced defences against pathogens (Arala-Chaves and Sequeira, 2000; Cooper et al., 1992; Faulhaber and Karp, 1992; Klein, 1989; Kurtz et al., 2002b; Schmid-Hempel and Ebert, 2003). Yet these reactions are not very specific, resembling an up-regulation of quite unspecific immune components rather than acquired specific defences (Klein, 1989; Lemaitre et al., 1997). Indirect evidence for more specific interactions comes from populations of invertebrate hosts and their parasites, where genotype-specific interactions were recently described (Carius et al., 2001; Lively and Dybdahl, 2000; Schmid-Hempel and Ebert, 2003).

While there was so far no good direct evidence for specific recognition in the context of invertebrate host defence against parasites, invertebrates have been shown to be capable of highly specific recognition in another context: The rejection of foreign tissue transplants is based on highly specific recognition of molecular details, and has been demonstrated for invertebrates (Buss, 1982; Stoner and Weissman, 1996). However, in these cases, specificity is based on the recognition of particular compatibility factors (Scofield et al., 1982), and therefore not directly comparable with parasite defence, which faces a large and unpredictable range of molecular patterns. Inspired by the occurrence of such compatibility systems among invertebrates, it has been argued that the demand to preserve individuality, and to protect the germline against con-specific alien cells (Buss, 1982; Stoner and Weissman, 1996), could have been the original selection pressure leading to the evolution of highly specific recognition systems, while defence against parasites was acquired later (De Boer, 1995; Rinkevich, 1999). Our study, demonstrating a high degree of specificity in invertebrates' defence against pathogens, rather than tissue compatibility, might somewhat weaken this interesting idea.

It is not clear which defence mechanism might cause specific recognition in invertebrate defence against parasites. In the defence of copepods against tapeworms, passage of worms from the gut into the body cavity (Fig. 2A) seems to be a crucial phase (van der Veen, 2003; van der Veen and Kurtz, 2002). It is possible that the differential up-regulation of fairly specific immune components such as lectins might account for the observed specificity at this stage (Marques and Barracco, 2000). Known receptors of the innate immune system do not appear to have the capacity for highly specific recognition (Hoffmann and Reichhart, 2002; Janeway and Medzhitov, 2002; Salzet, 2001). It is unlikely that a system homologous to vertebrate adaptive immunity, based on an immense diversity in T cell receptors and antibodies, is involved (Klein, 1989). However, this does not exclude the possibility that a specific innate defence system comparable to the one observed here might also exist in vertebrates, in addition to the acquired immune system.

Recognition of specific pathogens will be adaptive in the evolutionary sense, because it enables hosts to track a world of constantly changing pathogens. A more specific defence might reduce the costs associated with mounting an immune reaction (Lochmiller and Deerenberg, 2000; Moret and Schmid-Hempel, 2000; Råberg et al., 2002; von Schantz et al., 1999).

An optimal immune response might involve a quick, but unspecific first reaction, followed by a more specific, fine-tuned response later during the course of an infection. The vertebrate immune system makes use of such a strategy with its innate and adaptive arms of defence (Janeway et al., 1999). To analyze the optimality of immune responses in more detail, I will now concentrate on the second intermediate host of *S. solidus*, the three-spined stickleback *G. aculeatus*. In particular, I will focus on the question whether female choice of mates might optimize the immune system of the offspring.

Mate choice for optimal immunity in sticklebacks

The previous experiments demonstrate the benefits of sexual reproduction for a parasite in the evolutionary arms race with its host's defence system. Advantages can arise from the random recombination of parental traits during sexual reproduction, leading to new combinations of traits in more heterogeneous offspring. However, on top of random combination, advantages of sexual reproduction could be even more pronounced when characters are combined which make up a particularly good mix.

In many species, mating is not random (Andersson, 1994; Darwin, 1871). Especially females seem to be very choosy when picking a mate. It has been suggested that mate choice leads to offspring with an especially good mix of genes. Because of the evolutionary arms race with parasites, immune genes are likely targets of such mating decisions (Ebert and Hamilton, 1996; Hamilton et al., 1990).

Previous studies have shown that stickleback females optimize diversity in a particular set of immune genes in their offspring when choosing their mates (Aeschlimann et al., 2003; Reusch et al., 2001). These genes of the major histocompatibility complex (MHC) play a central role in the presentation of antigens to the adaptive immune system (Janeway et al., 1999).

MHC proteins bind foreign peptides (e.g. pathogen-derived antigens) and subsequently interact with a particular cell type of the adaptive immune system, T lymphocytes. These T cells activate B-lymphocytes to produce antibodies, which are specific for the antigen. The MHC is the most polymorphic gene cluster in the human genome (Janeway et al., 1999). MHC polymorphisms have also been demonstrated in several other vertebrate species, including fish (Apanius et al., 1997; Bernatchez and Landry, 2003; Edwards and Hedrick, 1998). Often, the number of different MHC alleles present in a population is extremely high (Apanius et al., 1997; Edwards and Hedrick, 1998; Klein, 1986). It could be expected that a high number of MHC molecules in an individual would enable recognition of a large spectrum of different pathogen antigens. However, the number of different MHC molecules expressed in an individual seems to be comparatively small. Humans, for example, normally express six different MHC class I and six different MHC class II alleles (Janeway et al., 1999). In sticklebacks, single-strand conformation polymorphism (SSCP) of the peptide-binding region reveals that individuals typically express about six different alleles, at an estimated number of six MHC class II loci (Binz et al., 2001), but the number of alleles detected in field-caught sticklebacks can be as low as two or as high as eight (Reusch et al., 2001).

What is the reason for the relatively low number of MHC molecules per individual? MHC molecules do not only present pathogen-derived peptides, but also self-peptides. Increasing the number of MHC molecules would increase the amount of possible combinations of self-peptides with MHC proteins. To avoid auto-reactivity of the immune system, T cell clones reacting with these combinations have to be eliminated by a process of negative selection (Janeway et al., 1999; Sebzda et al., 1999). Consequently, to prevent excessive elimination of auto-reactive T cell clones, while still enabling the recognition of a sufficiently diverse set of parasite antigens, there might be an optimal number of MHC molecules in an individual (De Boer and Perelson, 1993; Nowak et al., 1992). However, this theoretical expectation is still controversial (Borghans et al., 2003), and has not yet been proven experimentally.

MHC genes influence odour preferences for certain con-specifics in humans and in several animal taxa (Jacob et al., 2002; Penn and Potts, 1999; Potts et al., 1991; Wedekind et al., 1995; Yamazaki et al., 1976). In most studies, MHC-dissimilar mating partners were preferred over similar ones. The reason for such a preference might not be the optimization of the immune system of offspring. Instead, MHC might be used as an indicator of relatedness, and MHC-dissimilar mating might aim at avoiding to breed with close kin.

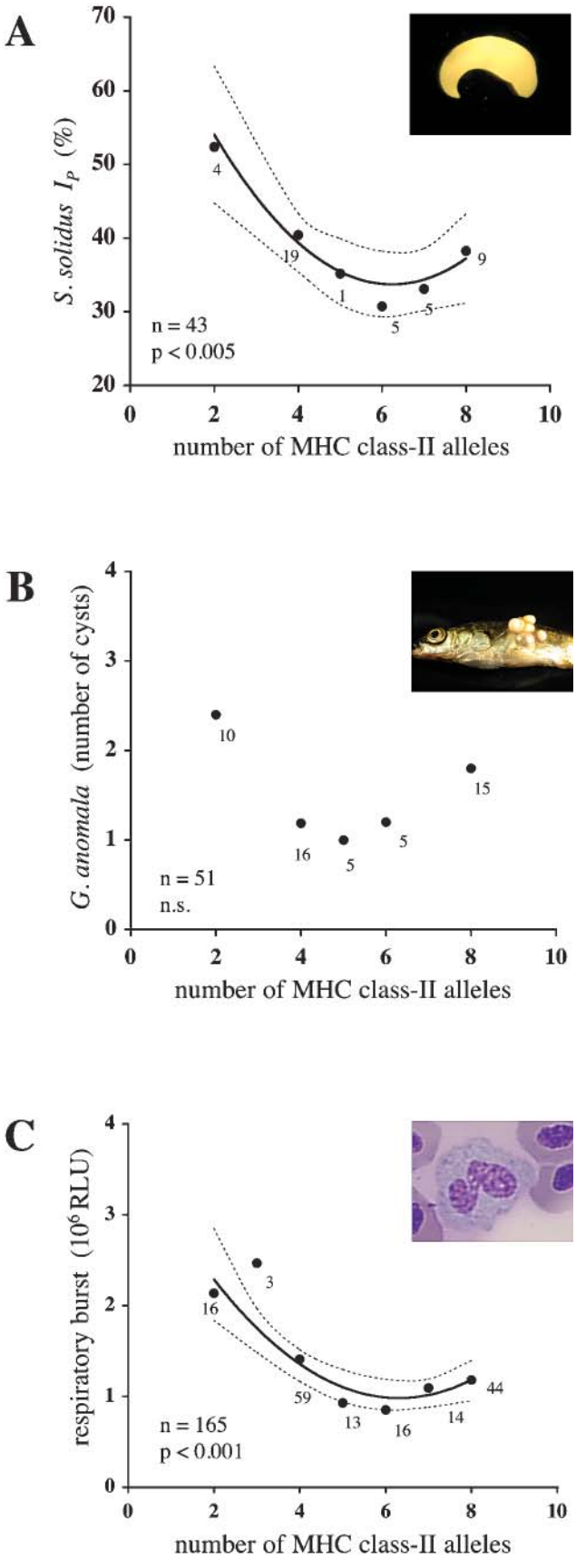
In natural populations of sticklebacks, the risk to breed with kin is very low (Reusch et al., 2001). When choosing a mate, sticklebacks seem to behave exactly as predicted by immunological theory. In flow-channel experiments, stickleback females with a low number of MHC alleles prefer the odour of males with a high number of different MHC alleles, while those females which already have many alleles go for males with low diversity. In essence, stickleback females seem to optimize the combined MHC diversity, aiming at offspring with optimally six different MHC alleles (Aeschlimann et al., 2003).

Do females indeed benefit from such a choice strategy? We predicted that the immune system should work optimally with a moderately high individual MHC diversity. This should lead to an optimal adaptive immune response and therefore a lower parasite burden. In line with this prediction, sticklebacks with an average allelic diversity of 5.2 different MHC alleles harboured the lowest parasite species diversity in natural populations (Wegner et al., 2003a). However, this field data is correlative, and factors other than MHC might influence parasite burden. We therefore tested experimentally whether there is an optimal individual MHC diversity with regard to parasite resistance and immune defence (Kurtz et al., in press).

Sticklebacks which had been bred in the laboratory were exposed twice (separated by two weeks) to two species of parasites, the tapeworm *S. solidus* and the microsporidian *Glugea anomala*. Nine weeks after the second exposure, we determined the degree of parasite infection, parameters of immune defence and the number of MHC alleles.

The probability of tapeworm infection was not significantly influenced by MHC diversity. However, those sticklebacks that were infected with tapeworms suffered less from the infection when they had a moderately high MHC diversity (Kurtz et al., in press). Tapeworm mass (in relation to host mass, i.e., the parasite index, I_P) was lowest in sticklebacks with 6.2 different MHC alleles (Fig. 5A). Similarly, but not significantly so, sticklebacks with an intermediate number of MHC alleles developed the lowest number of *Glugea* cysts (Fig. 5B). Recent experimental evidence with three other parasites, *Diplostomum spathacaeum*, *Camallanus lacustris* and *Anguillicola crassus*, supports our hypothesis that optimal rather than maximal MHC diversity confers the highest level of parasite resistance (Wegner et al. 2003b).

Why should sticklebacks with optimal MHC diversity be unable to prevent a tapeworm infection, while they can limit the extent of an infection? Different immune mechanisms could be involved in preventing an infection and in limiting the degree of the infection. In particular, innate immune functions might be most important to destroy parasites in an early stage of infestation.



Later, antibodies might be directed against the parasites and limit their growth.

Can we find differences in the immune system relating to MHC diversity? We expected that sticklebacks with optimal MHC diversity have a superior adaptive immune response. To evaluate the immunological status of individual sticklebacks, we isolated leukocytes from the head kidney, the major hemopoietic and lymphoid organ of fish (Iwama and Nakanishi, 1996). As a measure of the activation status of the innate relative to the adaptive immune system, we microscopically determined the ratio of granulocytes to lymphocytes. Granulocytes are involved in innate immunity while lymphocytes are the most important cells of the adaptive immune system. As expected, sticklebacks with optimal MHC diversity had a lower amount of granulocytes (Kurtz et al., in press). This indicates a relatively lower activation status of the innate immune system in MHC-optimal fish. As a functional measure of innate immune activity, we quantified the 'respiratory burst' reaction. During the respiratory burst, reactive oxygen intermediates are generated to kill pathogens (Janeway et al., 1999). We analyzed the respiratory burst in head kidney cell cultures, associated with phagocytosis of zymosan particles *in vitro*. Sticklebacks with optimal MHC diversity showed a weaker respiratory burst (Fig. 5C). Respiratory burst increased with the proportion of granulocytes and with an infection. However, these effects alone were not sufficient to explain the effect of MHC diversity on the respiratory burst (Kurtz et al., in press). These results suggest that individuals with a sub-optimal MHC diversity might be unable to turn an initial innate immune response into an adaptive response (Janeway et al., 1999; Luster, 2002). Are there any consequences of such a sustained activation of the innate immune system? Immune reactions themselves have

◄ **Fig. 5.** Influence of MHC diversity on parasite resistance and innate immunity in the three-spined stickleback (*G. aculeatus*). Sticklebacks differing in the number of MHC class II alleles had repeatedly been exposed to both *S. solidus* tapeworms and *G. anomala* microsporidians. (**A**) Sticklebacks with an intermediate number of MHC alleles developed relatively smaller tapeworms (the figure shows the weight of the worms in % of fish weight for tapeworm-infected fish). (**B**) Sticklebacks with intermediately high MHC diversity had, in tendency, a lower number of *G. anomala* cysts (the figure shows the mean number of cysts per stickleback infected with microsporidians). (**C**) The expression of an innate immune trait, the respiratory burst reaction upon phagocytosis, was lowest in fish with intermediate MHC diversity, indicating that an optimal adaptive immune system enables lower expression of unspecific, innate immunity. Regression lines with their 95% confidence limits are shown, numbers next to data points indicate the sample sizes (modified from Kurtz et al., in press).

been shown to be (energetically) costly (Lochmiller and Deerenberg, 2000; Moret and Schmid-Hempel, 2000). This seems to be especially true for innate immunity, as mutant mice that have to rely solely on innate immunity have an increased metabolic rate (Råberg et al., 2002). Secondly, and probably even more important, immune defence can have harmful side-effects (von Schantz et al., 1999). Such collateral damage might be reduced with a highly specific immune reaction. Reactive oxygen intermediates that are produced during the respiratory burst can contribute to ageing, cancer and immune or brain disorders (Ames et al., 1993). Hosts make use of radical scavengers such as carotenoids to protect their tissues from these harmful side-effects.
Interestingly, carotenoids are also used in male breeding signals, which play an important role in female choice in sticklebacks and in many other animal taxa (Lozano, 1994; Milinski and Bakker, 1990; Olson and Owens, 1998). Female sticklebacks prefer males with bright red throats rich in carotenoids (Milinski and Bakker, 1990). These carry fewer parasites after experimental infection with *Ichthyophthirius multifiliis* (Milinski and Bakker, 1991), and have offspring which are more resistant against *S. solidus* (Barber et al., 2001). Our results give a possible explanation for these findings. Males with optimal immunogenetics might decrease respiratory burst, thereby reducing the demands for oxygen scavengers such as carotenoids to buffer oxidative stress. Surplus carotenoids could then be allocated to breeding ornamentation. This scenario proposes a mate choice mechanism that uses both visual and olfactory cues to optimize the immune system of offspring.

Conclusions

The co-evolution of hosts with their parasites seems to be one reason for the predominance of sexual reproduction throughout the animal kingdom. In our experimental model system of a tapeworm parasite and its copepod and stickleback intermediate hosts, benefits of sexual reproduction were found for both parasites and hosts. For the hermaphroditic tapeworm, sexual reproduction with a partner (outcrossing) increased the infection success in its copepod host. Such benefits are mainly expected when the interface between parasites and hosts involves specific interactions. Parasite defence of the copepods was found to be indeed highly specific, which was rather unexpected for an invertebrate host, and casts some doubt on the currently held view that invertebrate immune systems are unspecific. In addition to random recombination of traits, sexual reproduction can lead to further benefits when mates are chosen with regard to particular combinations of genes in the offspring. Stickleback females choose mates in such a way that an optimal combination of immune genes in the offspring is achieved. Such an optimal diversity of the MHC leads to a reduced impact of the tapeworm parasite, and a lower activation status of the innate immune system, possibly reducing the costs associated with unspecific immune defence. All in all, benefits of sexual reproduction seem to be relevant for both hosts and parasites in their co-evolutionary arms race. This does, however, not imply that other possible benefits of sexual reproduction, such as the elimination of deleterious mutations are of minor importance for the maintenance of sex. The relevance of the various possible benefits of sex might vary between systems, and it is as yet far from clear which factors are most important.

Acknowledgements

I would like to thank the Deutsche Zoologische Gesellschaft and the Deutsche Gesellschaft für Parasitologie for the opportunity to present this paper at the 96th Annual Meeting in Berlin. I am particularly grateful to R. Lucius for the invitation and to R. A. Steinbrecht for his editorial work on this special issue. I would like to thank all those involved in discussing, planning and carrying out the various experiments described in this review. M. Milinski inspired, directed and enabled all experiments. The experiment on benefits of outcrossing in tapeworms was done together with M. Christen, specificity in copepod defence was studied together with K. Franz, and the stickleback experiment was done in collaboration with M. Kalbe, P. B. Aeschlimann, M. A. Häberli, K. M. Wegner, T. B. H. Reusch and M. Milinski. M. Kalbe helped with catching, rearing and dissecting sticklebacks, determining parasites, and in many other ways. M. Christen and I. T. van der Veen were generous with advice and help with the copepod cultures. S. Schjørring gave eggs from lab-bred tapeworms to be used for copepod infection. A. Lüscher, S. Schjørring, I. T. van der Veen and others helped breeding the tapeworms. I would like to thank G. Augustin, R. Leipnitz, L. Jahnke, W.-R. Wulff, H. Luttmann, S. Liedtke, C. Schmuck, I. Schultz, D. Lemcke and others for technical assistance. I am grateful to C. Wedekind for introducing to us the techniques to breed tapeworms, J. Scharsack, V. Stefanski, H. Engler and L. Dawils for initial help with developing immune assays for sticklebacks, A. Hämmerli for statistical advice, and T. Boehm, D. Hasselquist, J. Rolffs, M. T. Siva-Jothy, M. Michaud and many others for discussion. M. Christen, P. B. Aeschlimann and M. A. Häberli were supported by the Swiss National Fund.

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