Gyrodactylus derjavini infections in four salmonids: comparative host susceptibility and site selection of parasites

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ABSTRACT: The susceptibility of 4 salmonids [rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, a Baltic (Iijoki) and an Atlantic (Conon) strain of *Salmo salar*] to infection with a Danish isolate of *Gyrodactylus derjavini* was tested in the laboratory (12 to 13°C). Initial parasite attachment numbers did not differ between species, but parasite populations increased significantly faster and to a higher level in rainbow trout compared to the other salmonids, with *S. trutta* exhibiting an intermediate position. The 2 strains of *S. salar* became only slightly infected during the following weeks despite a heavy infection pressure. The parasites' selection of microhabitat on the hosts also differed between species and changed over time on the same host species. The tail fin was the preferred site in all salmonids although the pectoral, pelvic and anal fins were important sites in the initial period of the 6 wk investigation. It is notable that the corneal surface, especially in the Baltic salmon, became increasingly more heavily infected in the later stages of the infection period. Explanations for the differing degrees of susceptibility are suggested and some of these were investigated. Lysozyme activity in the fin epithelia and the density of mucous cells in the fins from the hosts were compared. Marked lysozyme activity was detected in tail fins from the 4 salmonids, but the activity was not associated with host resistance to infection. In contrast, the density of superficial mucous cells, which showed regional variation on the tail fins, was negatively correlated with susceptibility to infection. Thus, fish with high density of epithelial mucous cells showed the lowest susceptibility to infection, and this finding is discussed in relation to host resistance.

KEY WORDS: Monogeneans · Gyrodactylids · Rainbow trout · Brown trout · Baltic salmon · Atlantic salmon · Susceptibility · Site selection

INTRODUCTION

The host specificity of monogeneans is a characteristic feature of this animal group clearly appreciated in the literature (Bychowski 1957, Kearn 1994). Even monogeneans able to infect a range of host species show a clear predilection for certain hosts (Jahn & Kuhn 1932, Nigrelli & Breder 1934). Some gyrodactylids exhibit an ability to infect a range of species in certain teleost families. *Gyrodactylus salaris* is able to colonize a number of salmonids with varying success (Bakke 1991, Bakke et al. 1992b). However, the host factors responsible for these differences are poorly elucidated. Both chemical communication elements

and host immune factors have been suggested to be of importance (Kearn 1976). Serum components were early demonstrated to affect survival of the ectoparasitic monogeneans *Benedenia* (*Epibdella*) *melleni* in vitro (Nigrelli & Breder 1934), and immunoglobulins, complement and lysozyme in serum were suggested by Vladimirov (1971) to affect survival of the gill parasite *Dactylogyrus* spp. in carp. Although a weak antibody reaction to monogenean antigens is found in fish (Buchmann 1993), other factors including cellular reactions should be considered. The mucous cell density in the tail fin of the rainbow trout has been demonstrated to decrease during the infection period with *G. colemanensis* and *G. salmonis* (Wells & Cone 1990), but whether this factor is related to host specificity is unknown. Differing emission of specific chem-

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ical stimuli extensively used in fish communication (Hara 1993) could be involved as well. The present paper describes the different degrees of susceptibility of 4 salmonids to infection with *G. derjavini* and the site specificity of the monogeneans, and contributes to the elucidation of the factors which are of importance to this differential susceptibility of salmonids to infections with gyrodactylids.

MATERIALS AND METHODS

Parasites. An isolated strain of *Gyrodactylus derjavini* was obtained from a Danish rainbow trout farm (Paelebro Dambrug) in the southeastern part of Jutland. Infected rainbow trout (total body length 10 to 15 cm) were transported to the laboratory in oxygenated water and kept as a laboratory host/parasite system in aerated 200 l aquaria at 12 to 13°C.

Fish. Parasite-free salmonid fry (total body length 4 to 5 cm, 3 to 4 mo post-hatching) were obtained from different Danish hatcheries using recirculated well or tap water. Brown trout *Salmo trutta* fry were purchased from Haraldsted Production (Zealand); rainbow trout *Oncorhynchus mykiss* were delivered by Siglund Fish Production (Mön); Scottish Conon Atlantic salmon *Salmo salar* were obtained from Brusgaard Production (Jutland); and Finnish Iijoki Baltic salmon *Salmo salar* were delivered by Bornholms Salmon Hatchery (Nexö, Bornholm). All fish were acclimatized for more than a week in a thermostat-regulated cold room (12 to 13°C), as field data suggest this temperature to be suitable for *Gyrodactylus derjavini* population increase (Buchmann & Bresciani 1997). Fishes were kept in 200 l fish tanks until infection and were fed a restricted diet of commercial pelleted feed 3 times a week.

Water and fish tanks. For all purposes local tap water was used. This water has at delivery a pH of 7.43 to 7.46, a nitrate content of less than 10 mg l⁻¹, no nitrite and ammonia, and a total hardness of 24 degrees (Merck aquacant). During population studies fishes were held in continuously aerated glass aquaria (total volume 200 l) containing 100 l water. Aquarium water was recirculated by internal biofilters (Eheim) and checked regularly for pH and the presence of ammonia, nitrite and nitrate (Merck aquacant). Three-quarters of the water volume was exchanged twice a week.

Temperature. All experiments, including counting of parasites and infection procedures, were conducted in a thermostat-regulated cold room (12 to 13°C).

Light. Fish were kept under a natural light-dark cycle (14 to 16 h light and 8 to 10 h darkness).

Parasite counting. The number of *Gyrodactylus derjavini* were counted on anaesthetized (50 mg l⁻¹ MS 222) salmonids using a dissecting microscope with

subillumination (magnification 20× to 50×). During investigations fish were examined weekly over a 6 wk period.

Infection procedure. Infected rainbow trout (10 to 12 cm body length) with a known parasite burden were killed by a blow to the head whereafter scrapings of fins, body surfaces and the rest of the carcass were placed in a plastic aquarium (volume 10 l) containing 3 l water to secure that all the counted parasites were present and dispersed maximally. Such an aquarium containing a known number of monogeneans was used to infect 20 receptor fish. These were exposed to the parasites for 24 h. Thus, 2 small aquaria were used to infect 40 fish. After this period the fishes were examined for parasites and placed in 200 l aquaria with other fishes as described below (Table 1). Both rainbow trout and the salmonid to be tested were infected in this way for each experiment. Thus, 6 small aquaria were used to infect the rainbow trout, 2 small aquaria were used for the brown trout, 2 small aquaria were used for Scottish salmon and 2 small aquaria were used for the Baltic salmon infection procedure.

Monitoring of infection dynamics. After infection, the infected fishes were transferred to 200 l aquaria containing a total of 80 fish (40 rainbow trout as reference and additional 40 specimens of another salmonid to be tested; Table 1).

Site specificity. The numbers of parasites on different fins and body parts according to Fig. 1 were recorded on all fish immediately after infection (Week 1) and thereafter weekly for a total of 6 wk.

Lysozyme activity in fins. *Micrococcus lysodeikticus* agarose plates were prepared according to Lie et al. (1986). Tail fins from uninfected fish belonging to the same batches as were used for the infection experiments (10 rainbow trout as reference and 10 fins from another salmonid species) were placed on the agarose

Table 1. Experimental groups. Each aquarium (Aq.: 200 l) contained 100 l water and 80 fishes

Aq.	Fishes in 200 l aquaria	Infection method (24 h exposure) with small (10 l) aquaria each containing 20 fish
1	40 rainbow trout and 40 brown trout	4 aquaria each with 150–200 parasites (2 for rainbow trout and 2 for brown trout)
2	40 rainbow trout and 40 Atlantic salmon (Scottish, Conon)	4 aquaria each with 80–100 parasites (2 for rainbow trout and 2 for Atlantic salmon)
3	40 rainbow trout and 40 Baltic salmon (Finnish, Iijoki)	4 aquaria each with 80–100 parasites (2 for rainbow trout and 2 for Baltic salmon)

and incubated for 17 h in humid chambers at room temperature, after which plates were pressed, dried, stained with methyl violet and lugol and destained in ethanol. The maximum width of the lysed zone perpendicular to the middle of the tail fin (fork) was then measured (0.1 mm accuracy). The lysed zone was expressed as percent of the lysed zone of reference rainbow trout fins on the same agarose plate (see Table 2).

Mucous cell count. In order to elucidate species differences in mucous cell density (independently of infection) and importance for the initial susceptibility to infection these cells were enumerated as follows. Tail fins from 10 uninfected fishes from the same batches as were used for the infection experiments were fixed in 4% phosphate-buffered formaldehyde for 7 d, washed in distilled water, stained with Alcian Blue (1%) in 3% acetic acid, rinsed in distilled water and finally mounted in Aquamount. The number of superficial mucous cells per area unit in the epithelium was then determined using a compound microscope (magnification 200 $\times$). In order to locate regional differences in cell density 5 adjacent zones (1 to 5) located from the axial fin edge (fork) to the tail peduncle, each covering 0.61 mm², were counted in each fish.

Data processing and statistical procedures. The infection level was expressed as prevalence (percentage of fish infected), abundance (number of parasites per fish infected and uninfected) and intensity (number of parasites per infected fish) according to Margolis et al. (1982). Variance to mean ratio was calculated. The site selection of monogeneans was expressed as percentage of the parasite population registered in a particular site. Differences between means of samples were tested using a Student's *t*-test (level of significance was 0.05).

RESULTS

Population dynamics of *Gyrodactylus derjavini* on various salmonid hosts

The infection procedure was reproducible as judged from the uniform rainbow trout acceptance of parasites following the six 24 h exposures in small aquaria of this host. *Gyrodactylus derjavini* populations on rainbow trout increased in all three 200 l aquaria to high levels within 3 to 4 wk post-infection followed by a slight population decline. Brown trout experienced a marked increase of the parasite burden, which, however, peaked at a significantly lower level. Only a slight parasite population increase on both Baltic and Atlantic salmon was recorded (Fig. 2). It is noteworthy that almost all fishes initially became infected during the

Fig. 1. Body parts on which the number of monogeneans were registered. 1: caudal fin, 2: adipose fin, 3: dorsal fin, 4: pectoral fin, 5: pelvic fin, 6: anal fin, 7: anterior dorsal body, 8: anterior ventral body, 9: posterior dorsal body, 10: posterior ventral body, 11: head, 12: cornea

infection procedure (no significant difference in abundance and prevalence in Week 1) but only the rainbow trout sustained a 100% infection in the following weeks. The variance to mean ratio was highest in the rainbow trout groups in all experiments. However, no easily interpretable variation of this parameter was deduced in any group, although the elevated variance to mean ratio in the last weeks of the period indicates that the fish groups contained fish with very different abilities to combat the infection. Some mortality was recorded in all groups, especially 3 wk after the initial infection (Fig. 2). In all these comparisons rainbow trout and the other salmonid group to be tested were kept together in the same aquarium providing a comparable infection pressure.

Site specificity

The initial infection of the salmonids was predominantly located on pectoral, tail, pelvic and anal fins. However, during the population increase the tail fin in rainbow trout, brown trout and Atlantic salmon became increasingly more heavily infected and harboured the larger proportion of the parasites. Further, in Baltic salmon the tail fin was the most frequently selected site during the first 3 wk but in the last weeks of infection the corneal surface was the primary choice of the gyrodactylids. The cornea was also an important microhabitat in the other salmonids for the monogeneans in the later weeks. In addition, the hind part of the body proper and the head in all salmonids tested became increasingly more heavily infected in that period (Fig. 3).

Lysozyme activity in fins

A marked lysozyme activity in the fish fins appearing as clear zones surrounding the fins in the *Micrococcus*

Fig. 2. Course of infection level (prevalence, abundance, variance to mean ratio) and cumulative mortality in the 4 salmonids during 6 wk: (a) rainbow trout/brown trout, (b) rainbow trout/Baltic salmon (Finnish, Iijoki), (c) rainbow trout/Atlantic salmon (Scottish, Conon). The abundance differed significantly between rainbow trout and the tested group of salmonids in all weeks except for Week 1

Table 2. Lysozyme activity in tail fins from 4 salmonids

Fish	No. of fins	Lysozyme activity (lysed zone): percent of rainbow trout activity
Rainbow trout	30	100
Brown trout	10	55.0
Baltic salmon (Finnish, Iijoki)	10	70.0
Atlantic salmon (Scottish, Conon)	10	57.1

lysodeicticus agarose gels was demonstrated. However, the activity was not correlated to the parasite susceptibility in rainbow trout and the other salmonids. Thus, rainbow trout fins exhibited in all cases a much stronger lysozyme activity (Table 2).

Mucous cells in tail fins

The number of superficial mucous cells per 0.61 mm² varied significantly in the different fin zones of a single fish species. Thus, the edge of the fins (zone 1) exhibited in all cases a lower number of cells. Among host species the rainbow trout tail fins had a lower cell density than the brown trout tail fins, which in turn scored lower than the 2 salmon strains (Table 3).

DISCUSSION

The decisive factors of the host specificity found in most monogenean-fish host systems are still insufficiently elucidated. However, the present study may provide some clues for further efforts to explain this fundamental aspect of monogenean biology. Basically, this work has clearly documented that various salmonid hosts exhibit highly differing degrees of suscep-

tibility to the Danish strain of *Gyrodactylus derjavini* tested. Rainbow trout was clearly the most susceptible species and brown trout a more resistant species. Baltic and Atlantic salmon both showed low susceptibility to the parasite although both strains were able to support a minor population. This gyrodactylid has previously been reported from rainbow trout (Buchmann et al. 1995, Shinn et al. 1995), from brown trout (Ergens 1983, Malmberg & Malmberg 1993, Shinn et al. 1995) and from Atlantic salmon (Malmberg & Malmberg 1993, Mo 1993, Shinn et al. 1995). Under natural conditions all these species and strains are able to sustain a population of this parasite although at different levels. It has previously been indicated that a genetic factor is involved in the differences of host susceptibility to infection with a *Gyrodactylus* species (Madhavi & Anderson 1985, Bakke et al. 1990, Bakke & MacKenzie 1993) and it is evident that both the genes of the parasite (virulence) and the genes of the host (resistance factors) are important. In support of this, the susceptibility of salmonids to infection with a Norwegian strain of *G. salaris* was previously found to differ significantly: A Norwegian and a Baltic stock of the Atlantic salmon exhibited totally different susceptibilities (Bakke et al. 1990); the Scottish Conon stock showed low resistance (Bakke & MacKenzie 1993); the brown trout showed a poor ability to sustain a *G. salaris* population (Jansen & Bakke 1995); and the rainbow trout individuals varied considerably in susceptibility to infection but sustained as a population significant parasite numbers (Bakke et al. 1991).

In our study an isolated strain of *Gyrodactylus derjavini* from a single trout farm in Denmark was multiplied in the laboratory and used as the infection source in standardized procedures for infection of the various salmonids. Therefore, different strains of parasites are unlikely to explain the recorded differences. Macroenvironmental factors may influence the performance of gyrodactylids (Malmberg 1993). However, in the present study these parameters were kept constant, and in all fish tanks rainbow trout were kept as

Table 3. Mucous cell density (no. of cells per 0.61 mm²) (mean and SD) in tail fins from 4 salmonids. Tail fins were divided into zones 1 to 5 from edge to tail peduncle. A total of 10 fins were counted in each group. *Significantly higher than rainbow trout; **significantly higher than brown trout

Salmonid	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
Rainbow trout	23.9 (10.5)	70.1 (14.9)	89.0 (15.9)	101.6 (18.6)	115.7 (29.6)
Brown trout	70.5 (28.3)*	124.3 (41.1)*	128.1 (33.8)*	140.4 (36.9)*	144.0 (45.6)*
Baltic salmon (Finnish, Iijoki)	102.6 (34.6)**	209.9 (44.4)**	241.1 (88.9)**	276.7 (116.9)**	283.0 (137.6)**
Atlantic salmon (Scottish, Conon)	152.5 (32.4)**	227.0 (31.3)**	220.0 (35.8)**	272.8 (65.5)**	276.6 (60.1)**

Fig. 3. Site selection of *Gyrodactylus derjavini* on the 4 salmonids during 6 wk post-infection. Three groups of rainbow trout were registered and did not differ. Therefore only one [the group kept with the Baltic (Iijoki) salmon] is presented here

reference and received equal infection pressure throughout the experiments. Therefore, the experimental results should be explained by differences in the hosts susceptibilities, which probably result from a large variety of factors. One possibility is that different hosts differ with respect to the chemical composition of the superficial epidermis and mucus (Harris et al. 1973) or chemical communication substances (Hara 1993) and that monogeneans with a well-developed sensory and nerve system (Lyons 1969, Reuter 1987) are attracted by certain favourite hosts. Indeed, specific blood antigens in fish mucus could be part of this process (O'Rourke 1961).

We found that the initial attachment of parasites (Week 1) did not differ significantly between rainbow trout, brown trout and the tested strains of salmon. The different degrees of susceptibility became evident only in the following weeks. This suggests that these different salmonid hosts emit a gyrodactylid attractant to the same extent and that additional factors in the host epidermis are responsible for the varying degrees of resistance. A number of factors can be suggested which may account for this. The anatomy of the host epidermis could differ among salmonid species and provide microhabitats better suited for certain parasites than for others. The well-documented non-specific defence mechanisms (Fletcher 1982) and specific immune response of the fish host (St. Louis-Cormier et al. 1984) could also influence resistance to monogenean invasion. Thus, a number of active non-specific substances such as proteases (Hjelmeland et al. 1983), trypsin (Braun et al. 1990), lysozyme (Lie et al. 1989) and specific antibodies (Fletcher & Grant 1969, Bradshaw et al. 1971, Lobb & Clem 1981, St. Louis-Cormier et al. 1984) have been detected in fish mucus. As both the resistance of brown trout to ectoparasite invasion and the epidermis change seasonally (Pickering 1977, Pickering & Christie 1980) and the composition of fish mucus varies during a *Gyrodactylus* sp. infection (Moore et al. 1994), the epidermal surface should be a relevant subject for study in this context. Actually, the present study demonstrated that salmonids with high mucous cell density on the fins showed the highest resistance to infection with *G. derjavini*, suggesting that the mucous cells affect this parameter. The Scottish salmon tested in Norway did not show the same degree of susceptibility to *G. salaris* infection (Bakke et al. 1992b, Bakke & MacKenzie 1993) as we found to *G. derjavini*, which suggests that a more complex explanation is needed. However, these authors did not record the density of mucous cells in their experimental hosts. When rainbow trout were infected with *G. colemanensis*, a decrease in the mucous cell density throughout the infection period was seen (Wells & Cone 1990), suggesting an exhaustion of the epidermis

during infection. That lysozyme production of fishes plays a role in resisting monogenean invasion has been suggested by Vladimirov (1971). However, the lysozyme activity of fins from salmonids in this experiment was not positively correlated with resistance to *G. derjavini* infection, as we found the most susceptible species (rainbow trout) to possess the strongest lysozyme activity in the fins.

The *Gyrodactylus derjavini* population variation expressed in both rainbow trout and brown trout experimental groups followed the typical pattern previously seen in other fish host/*Gyrodactylus* spp. systems. The initial multiplication of the ectoparasites to a peak followed by a more or less strong decline is known from *Gyrodactylus alexanderi* on *Gasterosteus aculeatus* (Lester & Adams 1974), from *G. bullatarudis* on *Poecilia reticulata* (Scott 1985), *G. colemanensis* and *G. salmonis* on *Oncorhynchus mykiss* (Cusack 1986, Cone & Cusack 1988), and *G. salaris* on *Salmo salar* (Bakke et al. 1990, Bakke & MacKenzie 1993) and on *Salvelinus fontinalis* (Bakke et al. 1992a). A clear-cut explanation for this variation has not yet been provided but may include a range of immunological processes and parameters. Likewise, the characteristic microhabitat selection of *G. derjavini* on the 4 salmonids tested is not readily explained. The initial invasion of pectoral fins, pelvic fins and anal fins could be explained by the infection method, as these parts of the fish make first contact with the parasites on the aquarium bottom. The subsequent transfer of *G. derjavini* to the tail fin involves an active movement of parasites and corresponds to the behaviour of *G. colemanensis* on rainbow trout (Cone & Cusack 1989). It differs markedly from the site selection of *G. salaris* on *Salmo salar*, where the dorsal fin and pectoral fins are preferred (Jensen & Johnsen 1992). Normally *G. salaris* is not recovered from the gills, but recently this organ was also shown under some circumstances to be vulnerable to infection with *G. salaris* (Appleby & Mo 1997). *G. turnbulli* on *P. reticulata* also showed a dynamic migration posteriorly after a random initial infection (Harris 1988). Improved feeding and dispersal capacity result from this behaviour, which during evolution could have been encoded into the parasite genome. However, in other non-gyrodactylid monogeneans the specialized microhabitat selection can be a strategy for optimization of intraspecific contact-facilitating mating (Rohde 1977, Buchmann 1989). The change of parasite distribution on the host during the phase of population increase could be explained by a crowding effect triggering the migration of parasites to new foraging sites. Alternatively, the recorded change of habitat during the population decrease could also be explained by the parasites' escape from localized epithelial immune reactions or pathologically changed

structures, a mechanism which also was indicated by Paperna et al. (1984) in the *Benedenia monticelli/Liza* spp. association. Thus, following a heavy infection with *G. derjavini* on the tail fin of rainbow trout, the total parasite population on the host declines concomitant with an increase of the subpopulation on the corneal surface on the fish. Likewise, Baltic salmon in particular experienced a marked colonization of parasites on the cornea in the final period. Although the cornea normally is an unsuitable and exposed microhabitat for the parasite, this simple anatomical structure without mucous cells, lymphocytes and macrophages (Hibiya 1982) may serve as a refugium with a reduced immune reaction towards the ectoparasites during this phase.

The present findings about host susceptibility may have some practical application, as restocking of Danish waters with *Salmo trutta* and *Salmo salar* (both the Iijoki and the Conon stock) is in progress and raises questions about parasite contamination from rainbow trout farms. Thus, it appears that, where *Gyrodactylus derjavini* is concerned, the salmon restocking programme is not threatened and the brown trout seems able to cope with infections with this organism.

Gyrodactylus derjavini is probably an endemic species in native brown trout in Denmark, which could explain the relative resistance of this host to that particular organism. Rainbow trout, in contrast, were introduced in the late 1880s and did not develop an innate resistance to infection with this gyrodactylid during evolution. The decrease in parasite counts in the later stages of infection in rainbow trout and the migration of parasites to the cornea suggest, nonetheless, that this host is capable of mounting an immune response during infection.

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