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HOST-SPECIFIC IN VITRO COLONISATION OF FISH EPITHELIA BY GYRODACTYLIDS

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Background. The monogeneans, *Gyrodactylus derjavini* Mikailov, 1975 and *G. salaris* Malmberg, 1957, are specific parasites of brown trout, *Salmo trutta* L., and Atlantic salmon, *S. salar* L., respectively. Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), is also susceptible to *G. derjavini* but, similarly like brown trout, mounts a host response against this parasite. Rainbow trout can also experience infections with *G. salaris* but experiences generally lower infection levels compared to Atlantic salmon. This in vitro work was conducted in order to elucidate if the parasites could find and select specific host scales without influence of live and mobile hosts.

Material and Methods. Using these two parasite species, a series of in vitro studies on host specificity were conducted. Scales with intact epithelia from rainbow trout, carp and salmon were cultivated and the two congeneric gyrodactylids (*Gyrodactylus derjavini* and *G. salaris*) were allowed to colonise the scales. Parasites attaching to the different scale types were monitored over 24 h.

Results. The gyrodactylids colonised fish scales to a lesser extent, in vitro, compared to experiments where live fish were exposed to parasites. However, the parasites attached to a higher extent to in vitro-cultured fish scales with epithelia from their specific hosts: *G. salaris* relocated more often to salmon scales compared to the scales from rainbow trout and common carp, *Cyprinus carpio* L. In contrast, *G. derjavini* colonised preferentially cultured scales from rainbow trout and, to a lesser degree, scales from salmon.

Conclusion. The work indicated that gyrodactylids colonising host epithelia are at least partly dependent on host movements but also have an ability to sense host-specific molecules due to their specific attachment to specific host scales.

Keywords: *Gyrodactylus derjavini*, *G. salaris*, host specificity, epithelium, in vitro, rainbow trout, carp, salmon, fish

INTRODUCTION

Host specificity of monogeneans is a well-described biological factor, which plays a major role in their ecology and population biology (Malmberg 1993, Whittington et al. 2000, Bakke et al. 2002, Buchmann and Lindenstrøm 2002). Two of the parasite-host systems exhibiting a pronounced predilection of specific monogeneans for certain hosts are the association between *G. derjavini* Mikailov, 1975 and the brown trout, *S. trutta* L., and the association between *G. salaris* Malmberg, 1957 and the Atlantic salmon, *S. salar* L. The rainbow trout, *O. mykiss* (Walbaum, 1792), was introduced into Northern Europe, which is an endemic area for *G. derjavini*, within the last 130 years. However, the latter host shows interactions with *G. derjavini* corresponding to the relations between brown trout and this gyrodactylid (Buchmann and Uldal 1997). Further, it was recently demonstrated experimentally that *G. derjavini* preferentially colonised rainbow trout to Atlantic salmon in a multiple-choice test and that *G. salaris* selected Atlantic salmon over rainbow trout when given the choice (Buchmann et al. 2004). These parasite-host associations are reflected under natural conditions. Thus, in endemic areas, brown trout are primarily infected with *G. derjavini* while salmon generally harbour *G. salaris* (cf. Mo 1991, 1993, 1997, Bakke et al. 2002). However, the basis for this specific host selection is not well elucidated. Chemical, mechanical, and behavioural mechanisms have been suggested to explain this host specificity (Whittington et al. 2000, Buchmann and Lindenstrøm 2002). Although chemical host substances are considered to offer a good explanation both among gyrodactylids (Buchmann 2001, Jørndrup and Buchmann 2005) and among other monogeneans (Yoshinaga et al. 2000, 2002, Hirazawa et al. 2003), it cannot be excluded that a range of abiotic and

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biotic factors (Kearn 1967) could also induce host colonisation. The present study was conducted on colonisation of *G. salaris* and *G. derjavini* of in vitro-cultured host epithelia in order to exclude other factors from the analysis.

MATERIALS AND METHODS

Fish: Eggs of Atlantic salmon, *S. salar* (from the east Atlantic Scottish river Conon stock), and rainbow trout, *O. mykiss* (from the Danish trout farm Faarup mølle in Jutland), were hatched and reared at the salmon hatchery of Bornholm (Denmark). Following disinfection in iodophore (Actomar K30) the eggs were incubated in hatchery trays and hatched in municipal freshwater at pH 7–8 and at a temperature of 7°C. The fish were fed commercial pelleted fish feed (BioMar A/S, Denmark) at 10°C during the pre-experimental period. Common carp, *C. carpio* L., fry was obtained from a laboratory stock (Wageningen University, the Netherlands). All fish were transported to a pathogen-free university system (KVL, Frederiksberg, Denmark) and kept in recirculated water at least 2 months prior to experimentation.

Experimental temperature: All experiments were conducted at 12–13°C in a temperature-controlled room with constant artificial illumination.

Fish epithelia on scales for in vitro culture: Epithelium-covered scales were obtained from salmon (age 2+), rainbow trout (age 2+), and common carp (age 2+).

Parasites: A population of *G. salaris*, originating from the Norwegian river Laerdalselva, was imported to Denmark and kept on susceptible Scottish (Conon River) salmon, *S. salar* (age 1+), in recirculated water, in an isolated experimental fish tank unit, for two years before the experiment began. The *G. derjavini* population was collected on a Danish trout farm (Paelebro, Dambrug) and kept on rainbow trout *O. mykiss* (age 1+) in recirculated water for seven years before experimental start.

Isolation of parasites: Gyrodactylids were isolated by cutting heavily infected fins and placing these into tapwater filled Petri dishes. Parasites leaving the fins voluntarily, within 60 min, were then used for further experimentation without manipulating the worms with any tools.

In vitro cultivation of epithelial cells on scales: Scales from each species were removed with fine forceps from the dorsal region of the fish and rinsed in Hanks Balanced Salt Solution with a salinity of 0.9% (HBSS 14175-053, Invitrogen, UK) in Petri dishes (diameter 60 mm) for 15 min. Subsequently, the scales were transferred to tissue-culture medium L15 (Sigma L5520, USA) supplemented with antibiotics penicillin/streptomycin solution (penicillin 250 u · mL^{-1}, streptomycin 250 µg · mL^{-1}) (Sigma P0781, USA). This culture medium was diluted (1 : 8) using sterile water. After 24 h of pre-incubation, the scales were placed in groups in a Petri dish (60 mm diameter) with freshly diluted (1 : 8) L15 tissue-culture medium (Sigma L5520) and a normal atmosphere over the culture medium. The epithelia on scales were regularly checked (light microscope, magnification 40–100×) to ensure their integrity.

Parasite colonisation of epithelia in vitro

Experimental protocol: Two experiments were conducted under the dissection microscope (magnification 7–40×).

1) The first experiment was made with one type of scales (10 scales) in each Petri dish, either scales from Atlantic salmon (average scale size: 9.9 mm^2), rainbow trout (a-verage scale size: 7.4 mm^2), or carp (average scale size: 8.5 mm^2). Scales were placed in the periphery of the Petri dish. Following 24-h pre-incubation of scales, a number (9–15) of live specimens of *G. salaris* or *G. derjavini* was added to the centre (1 cm^2) of each Petri dish. The transfer of parasites from the isolation dish to the experimental dish was done by tilting the dish (resulting in the gyrodactylids concentrating in one pole of the dish) and by the use of a preparation needle. In order to decrease manipulation of the parasites they were then allowed to migrate from the isolation dish to the experimental dish voluntarily but they were guided by the preparation needle under the dissection microscope. Their numbers, however, were always watched to fit within the range of 9–15. Nine replicates were prepared for each scale type and each parasite species. The number of parasites attached to the scales was recorded at 1, 2, 3, 4, 5, 6, and 24 h post-exposure.

2) In the second experiment three epithelium-covered scales from each fish species (salmon, rainbow trout, carp) (total of nine scales) were placed in the same Petri dish. The scales were distinguished based on morphology. Nine to 15 specimens of *G. salaris* or *G. derjavini* were added to the dish and the numbers of attached parasites were recorded as in experiment 1.

Calculations and statistics: Within each of the different colonisation experiments the testing of parasite attachment the non-parametric Kruskal–Wallis test and Dunn's test were used to detect differences between experimental groups. The probability level was set at 5 percent in all tests.

RESULTS

G. salaris and *G. derjavini* colonised epithelium-covered scales with a maximum rate of relocation (from the Petri dish to fish scale) of 34% (minimum rate 1.4%). Some of the parasites died during incubation, whereas others released their embryos (Table 1). However, when attached *G. salaris* found scales from *S. salar* significantly more often ($P < 0.05$) than scales from rainbow trout and carp (Figs. 1, 2). After 1 h, more than 50% of all relocated *G. salaris* were found on salmon scales. This initial preference of *G. salaris* for *S. salar* increased in subsequent hours. Correspondingly, *G. derjavini* colonised scales from rainbow trout more often than salmon and carp scales ($P < 0.05$), both in single- and multiple-choice situations (Figs. 1, 2). Generally, it was observed that both *G. salaris* and *G. derjavini*, at least to some extent, would colonise scales from other hosts than their preferred ones (Tables 1, 2; Figs. 1, 2). The total colonisation rate did not increase when scales from the three different host species were exposed together. Thus maximum colonisation rates were between 16% and 18% in these cases (Table 3).

Table 1

Gyrodactylus salaris experiment with separate scales from salmon, rainbow trout, and carp; summary of 9 Petri dishes

		Hours post exposure						
		1	2	3	4	5	6	24
Separate salmon scales	No. of parasites used	114	110	106	111	101	92	72
	No. of parasites attached	18	24	27	29	26	31	17
	% attached	15.8	21.8	25.5	26.1	25.7	33.7	23.6
Separate rainbow trout scales	No. of parasites used	102	99	101	95	98	89	71
	No. of parasites attached	6	13	7	6	5	8	1
	% attached	5.9	13.1	6.9	6.3	5.1	9.0	1.4
Separate carp scales	No. of parasites used	109	107	101	96	97	98	60
	No. of parasites attached	8	6	6	6	9	13	2
	% attached	7.3	5.6	5.9	6.3	9.3	13.3	3.3

Table 2

Gyrodactylus derjavini experiment with separate scales from salmon, rainbow trout, and carp; summary of 10 Petri dishes for each species

		Hours post exposure						
		1	2	3	4	5	6	24
Separate salmon scales	No. of parasites used	94	89	70	56	55	51	38
	No. of parasites attached	4	12	8	6	6	7	5
	% attached	4.3	13.4	11.4	10.7	10.9	13.7	13.1
Separate rainbow trout scales	No. of parasites used	84	62	50	42	39	35	38
	No. of parasites attached	5	7	8	6	8	6	8
	% attached	5.9	11.3	16.0	14.3	20.5	17.1	21.1
Separate carp scales	No. of parasites used	99	69	59	53	51	43	47
	No. of parasites attached	3	5	8	5	1	3	3
	% attached	3.0	7.2	13.5	9.4	2.0	7.0	6.3

Table 3

Colonisation experiments with *G. salaris* or *G. derjavini* and using scales from three host species (salmon – 3 scales, rainbow trout – 3 scales, and carp – 3 scales) in each Petri dish; summary of 10 Petri dishes

		Hours post exposure						
		1	2	3	4	5	6	24
G. salaris on three types of scales	No. of parasites used	112	108	105	100	92	90	79
	No. of parasites attached	6	4	11	8	11	15	4
	% attached	5.4	3.7	10.4	8.0	12.0	16.6	5.0
G. derjavini on three types of scales	No. of parasites used	104	91	83	81	75	72	62
	No. of parasites attached	8	13	9	8	14	12	2
	% attached	7.6	14.2	10.8	9.9	18.6	16.6	3.2

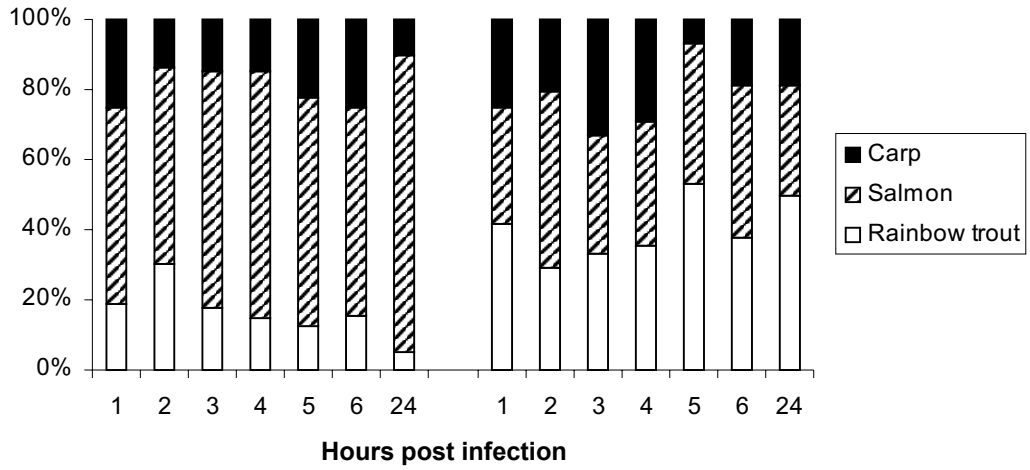

Fig. 1. In vitro colonisation by *Gyrodactylus salaris* (left) or *Gyrodactylus derjavini* (right) of fish scales (salmon, rainbow trout, carp); one scale type per exposure; within 24 h; scale-attached parasites only; relative percentage of parasites on a specific scale type in relation to all attached parasites is shown; each column represents a summary of 9 Petri dishes

DISCUSSION

The results of the present in vitro work, compared to previous in vivo experiments (Buchmann et al. 2004) indicate, that both *G. derjavini* and *G. salaris* are less efficient in their colonisation of host material (scales) in vitro. A possible explanation could be a decrease of host substances released from the scales due to the preincubation period of 24 h. Further, the artificial environment including tissue culture medium and antibiotics could also be suggested to influence the host findings. Finally, the worms could be partly dependent on host movements to obtain a higher host colonisation rate. However, despite this low colonisation rate, it was in fact noted that the parasites are able to discern in vitro to some extent between epithelium-covered scales from different fish species. Thus, *G. derjavini* preferentially colonised epithelia from rainbow trout and *G. salaris* mainly relocated to scales from salmon. Scales from an atypical host (carp) became colonised at a significantly lower rate. This corresponds to the observations, conducted in vivo by Buchmann et al. (2004), where *G. salaris* and *G. derjavini* showed predilection for live representatives of Atlantic salmon and rainbow trout, respectively. Brown trout, *S. trutta*, is the natural host for *G. derjavini* while rainbow trout, *O. mykiss*, is a host once introduced to Europe, where *G. derjavini* is endemic. However, it has been shown that rainbow trout

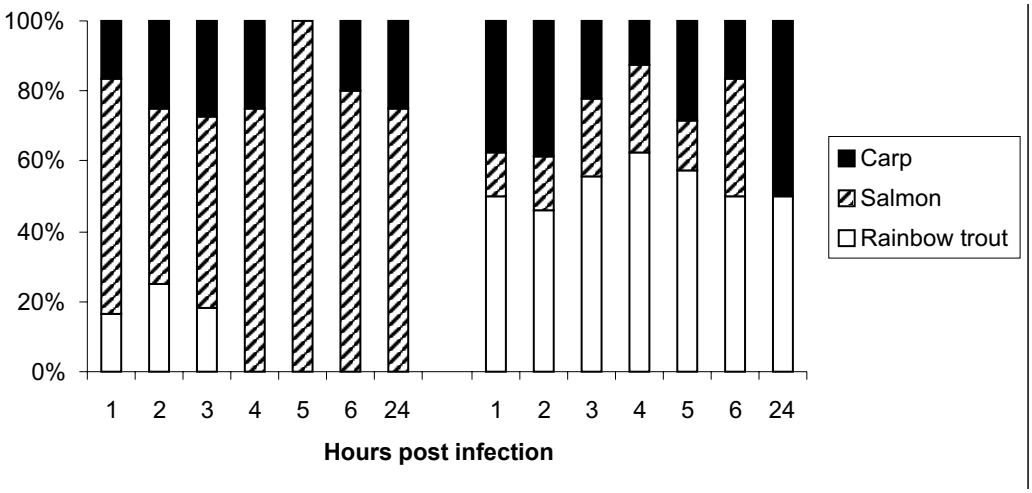

Fig. 2. In vitro preference by *Gyrodactylus salaris* (left) or *Gyrodactylus derjavini* (right) of fish scales when simultaneously exposed to three scale types (salmon, rainbow trout, carp), in one Petri dish, within 24 h; scale-attached parasites only; relative percentage of parasites on a specific scale type in relation to all attached parasites is shown; each column represents a summary of 10 Petri dishes

exhibits comparable interactions with this parasite (Buchmann and Uldal 1997) and rainbow trout/*G. derjavini* system is a suitable laboratory model for a susceptible but eventually responding host. Due to lack of totally pathogen-free specimens of *S. trutta* we decided to use rainbow trout material in the present investigation. During the colonisation experiments it was noted in several cases that the total number of parasites in the Petri dishes decreased due to some parasite mortality. However, some of the live parasites were bearing developed embryos in their uteri, which subsequently were born in vitro. Therefore, the total number fluctuated, increasing at five occasions.

Studies on monogenean host selection in vitro were previously conducted by Kearn (1967), who showed that oncomiracidia of *Entobdella soleae* preferred scales of its natural host common sole, *Solea solea* (L.), over those of many other species. This will add to the notion that the host specificity of monogeneans is, to some part, associated with a recognition system between the host and the parasite.

It is likely that host behaviour (movements) influenced the attachment of parasites in the in vivo study described by Buchmann et al. (2004), by increasing the chances of direct contact between host and parasite. In the present in vitro work, however, only isolated cell-covered scales were used. This indicates that the stimuli, initiating the observed host-specific colonisation, are connected with factors present in the scales. Although, it cannot be excluded that gyrodactylids recognise the specific host cell micro-architecture (Buchmann and Bresciani 1998), a likely explanation is that the scales covered with epidermal tissue are producing substances, which can be detected by the host-specific parasite. Monogeneans, in general, are known to possess sensory organs (Lyons 1969, Watson and Rohde 1994) and have been suggested to involve lectin–carbohydrate recognition (Buchmann 2001, Yoshinaga et al. 2000, 2002, Jørndrup and Buchmann 2005), pH-related interactions (Hirazawa et al. 2003) and other compounds (Whittington et al. 2000) in their communication with their microhabitat in the fish. Host movements are probably necessary to secure parasite–host contacts whereby these short-range chemo-attractants can play a role. In fact, the total colonisation rate experienced in this in vitro system (<34%) was much lower compared to in vivo colonisation rates (>80%) experienced by Buchmann et al. (2004). This will stress that chemoattraction is not the only important factor when gyrodactylids are colonising their hosts. In order to elucidate to what extent host movements are responsible for the additional host colonisation a series of studies should be conducted with live but immobilised host.

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