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Clonal structure of salmon parasite *Gyrodactylus salaris* on a coevolutionary gradient on Fennoscandian salmon (*Salmo salar*)

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The population structure of the Baltic salmon (*Salmo salar*) specific clade of *Gyrodactylus salaris* was studied using mitochondrial and nuclear DNA markers across a gradient of historical coadaptation. In the Onega and Ladoga lakes, the salmon was near to eliminating the parasite: just 5 of 548 inspected salmon juveniles carried a small number of parasites. In the northern Baltic Tornio River, *G. salaris* was observed as non-pathogenic in 23% of 765 fish. The population of naïve anadromous salmon in the Keret' River (White Sea) had almost perished after the parasite was imported from Lake Onega in 1992. The parasite clones defined by mtDNA were strongly spatially structured (F_{ST} = 0.548 in Keret'; F_{ST} = 0.484 in Tornio), suggesting competitive interactions via host defense. The prevalence and clonal structuring of *G. salaris* were concordant with the host resistance predicted from the suggested 132 000 years of common phylogeographic history in the Baltic refugia.

Introduction

Parasites and hosts experience an asymmetric tug-of-war evolution. The final outcome depends on the structure and demography of the species in question. The species pair Atlantic salmon (*Salmo salar*) and its specific monogenean ectoparasite *Gyrodactylus salaris* form an interesting evolutionary setting. Some extant salmon populations, on the Atlantic coast in Norway and in Russian Karelia on the White Sea, have been driven to the brink of extinction after introduc-

tions of the parasite, while the Baltic populations are tolerant and serve as permanent reservoirs of the parasite (Bakke *et al*. 2002). In tolerant salmon populations, the host–parasite communities have already reached an equilibrium, while in the susceptible populations the coevolution begins when they are first time inoculated with the parasite. The first steps of "coevolution" are expressed as an extremely high juvenile mortality of the host, to the extent of even threatening the survival of the parasite (Johnsen & Jensen 1991).

European salmon populations differ widely with respect to their resistance against or tolerance of *G. salaris* in laboratory experiments (reviewed in Bakke *et al*. 2002, 2004, 2007). Also, one North American stock (Bristol Cove) has been tested, but unfortunately, also the control fish died due to "social stress" during the experiment (Dalgaard *et al*. 2004). These differences have been explained by the differing periods that host and parasites have been in contact in different populations, however, until recently this hypothesis has been difficult to test.

Kuusela *et al*. (2007) provided evidence that the salmon-specific monophyletic mitochondrial clade of *G. salaris* (Meinilä *et al*. 2004) infecting Baltic salmon originated as a hybrid between two lineages of grayling parasites. A possible time for the hybridization event was during the Eemian interglacial, when the White Sea and Baltic Sea basins were briefly connected about 132 000 years ago (Funder *et al*. 2002). This connection was north of the present Lake Onega. Later, the area was covered by continental ice cap pushing aquatic fauna eastwards to the ice-dammed freshwater lakes (e.g., Kvasov 1979, Mangerud *et al*. 2001) which were large and permanent enough to maintain the refugial salmon populations from the brackish water pre-Eemian Baltic Sea. The palaeogeographic history of Baltic salmon populations has been elucidated recently. Instead of being of postglacial Atlantic origin, the Baltic salmon was shown to have been largely descendent from the freshwater refugia, most probably situated eastwards and/or southeast from the continental ice cap (Nilsson *et al*. 2001, Asplund *et al*. 2004, Tonteri *et al*. 2005).

When superimposed, the phylogeographic patterns of colonization of host and parasite allow the testing of the hypothesis that resistance differences are related to the time of coadaptation in the different populations. The easternmost freshwater salmon populations in Lake Onega and Lake Ladoga have the longest common history with the parasite. Lake Onega drains into the Baltic Basin, being the first lake to form after the recession of the ice some 11 000 years ago (Glückert 1995). Lake Ladoga was originally a gulf of the Baltic Ice lake, and was isolated from the southern Baltic (Gulf of Finland) by the end of the Ancylus lake phase, only about 8000 years ago (Saarnisto 1970, Björck 1995). The salmon stocks of the lakes Onega and Ladoga, and in the southern Baltic descend from the population which survived the glaciation in an eastern freshwater refugium (Koljonen *et al*. 1999, Nilsson *et al*. 2001, Asplund *et al*. 2004, Säisä *et al*. 2005, Tonteri *et al*. 2005, 2007).

The salmon populations in the northern part of the Baltic Sea are genetically different from those in the southern Baltic and large Russian lakes: they are also more variable (Nilsson *et al*. 2001, Asplund *et al*. 2004, Tonteri *et al*. 2005). According to Koljonen *et al*. (1999) and Säisä *et al*. (2005), the northern Baltic salmon populations have a clear Atlantic genetic component. The immigration and gene flow into the Baltic from the Atlantic salmon populations was not possible prior to the latest 10 000 years. It is to be expected that all the Baltic salmon populations were naturally challenged by *G. salaris* all this time.

Anadromous salmon populations found on the Atlantic coast of Norway have no genetic component from the freshwater glacial refugia, but rather descended from the continuum of sea-migrating populations along the French, Spanish and Portuguese coasts (Verspoor *et al*. 1999). In the White Sea, an origin from a northeastern refugia has been suggested, but also a mixture from the Atlantic populations is evident in the anadromous populations (Kazakov & Titov 1991, Asplund *et al*. 2004, Tonteri *et al*. 2005). The White Sea basin was free of salmon-specific *G. salaris* until 1992 (Kudersky *et al*. 2003). The high level of susceptibility of White Sea salmon to *G. salaris* was illustrated in the Keret' River where the parasite was introduced, probably in 1992, resulting in a 98% loss of juvenile produc-

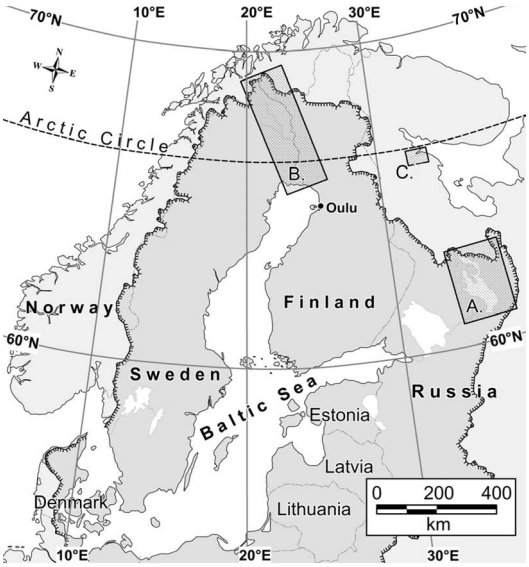

Fig. 1. Locations of the salmon sampling sites. The shading indicates the Baltic Sea basin and the squares delineate the areas presented in more detail in Figs. 2–4.

Fig. 2. Studied salmon spawning rivers of Lake Onega. The *G. salaris* parasite was found only in the Kumsa and Lizhma rivers. The hatchery suspected as the source of the Keret' infection is along the Shuya River.

tion (Kudersky *et al*. 2003). The case of Keret' parallels the Norwegian experience (Johnsen & Jensen 1991).

Thus, there is a palaeohistorical temporal gradient of co-occurrence of salmon and the salmon-specific strains of the *G. salaris* parasite: a maximum of 132 000 years in the refugial population colonizing Lake Onega and Lake Ladoga, perhaps only 10 000 years in the northern Baltic Sea, and no coadaptation at all in the anadromous populations on the White Sea. In this study, we analyze the population structure of the parasite *G. salaris* on the wild populations of the nominal host, *S. salar*, in these different, and predictably resistant versus susceptible populations.

Material and methods

Description of the salmon populations

Salmon populations from three different areas were studied (Fig. 1). The freshwater-migrating salmon populations in the lakes Onega and Ladoga, in Russian Karelia, were studied in six spawning rivers each during expeditions in July or August. The rivers are not very large, but they maintain fluctuating but healthy salmon stocks. Only one rapid was studied in each river.

Samples from lake Onega, clockwise from Petrozavodsk, were from the following rivers: Shuya 2004 (N_{fish} = 11), Lizhma 2001, 2002 and 2004 (N_{fish} = 98), Kumsa 2004 (N_{fish} = 16), Pyal'ma 2001 and 2004 (N_{fish} = 98), Tuba 2001 and 2004 (N_{fish} = 77) and Vama 2004 (N_{fish} = 16) (Fig. 2).

Samples from Ladoga were all collected in 2006, counterclockwise from Olonets: Vidlitsa (N_{fish} = 44), Tulema (N_{fish} = 66), Uuksunjoki (N_{fish} = 29), Syskynjoki (N_{fish} = 66), Hiitolanjoki (N_{fish} = 44), and Burnaya (Taipaleenjoki) (N_{fish} = 4). Because *G. salaris* was found only in one river (Syskynjoki), there is no map of Ladoga salmon rivers.

Samples from Tornionjoki and its tributaries Muonionjoki, Lätäseno and Könkämäeno (from here on collectively: Tornio; Fig. 3) were collected in 2000. Altogether, 765 fish were caught in 23 rapids along 468 km of the river (Anttila *et al*. 2008). The additional samples from 2006 were from four rapids (Table 1).

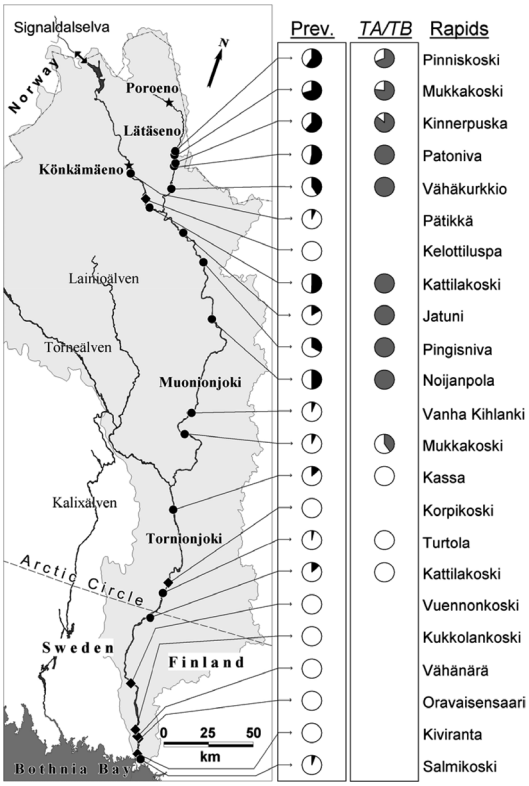

Fig. 3. The Tornio River and the inspected tributaries. The bifurcation between Könkämäeno and Signaldalselva at the border of Sweden, Finland and Norway is indicated by a small arrow. The distribution of *G. salaris* is illustrated in pie diagrams: the left column presents the prevalence of parasites on inspected salmon juveniles (the black sector denotes presence of the parasite), and the right column represents the proportion of the two mtDNA haplotypes *SalBa04* (TA, shaded) and *SalBa05* (TB, white). The two asterisks show the upstream localities where *G. salaris* has been collected on grayling: these mtDNA types were never observed on salmon.

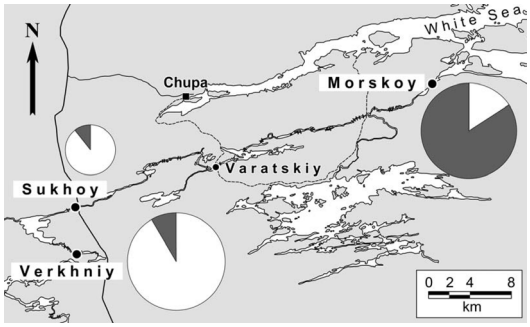

Fig. 4. The Keret' River system and the inspected rapids. The pie diagrams, drawn in scale with sample size, show the proportion of mitochondrial haplotypes *SalBa01* (shaded) and *SalBa02* (white sector).

The Keret' parasite samples were from three rapids in 2002: Morskoy, at the rivermouth (N_{fish} = 20); Sukhoy rapid, located 55 km from the sea (N_{fish} = 8); Verkhniy (Upper) rapid, 68 km from the sea (N_{fish} = 23). Two rapids were inspected in 2005: Morskoy (N_{fish} = 21) and Sukhoy (N_{fish} = 1). The location of the rapids is displayed in the map (Fig. 4).

Collection of *Gyrodactylus* parasites

After electrofishing, the salmon juveniles were killed, measured and stored for further examinations. For standardized parasite density estimates in the Keret' samples from 2002, and in all samples from Tornio, the dorsal fin and both pectoral fins were cut and transferred to the laboratory in 96% ethanol. The sensitivity of the method of examining only the three fins has been approximately 60% as compared with the examination of whole fish (Jensen & Johnsen 1992, Mo 1992). Thus, the Tornio data underestimate the prevalence.

At other sampling locations (Onega, Ladoga and Keret' 2006), whole fish were immediately preserved in the field in 96% ethanol. Every mm^2 of skin and fins was then inspected in the laboratory. Also, the ethanol in the vials or bottles was inspected, but *G. salaris* seldom drops from the skin due to ethanol shock. Parasites were counted under a preparation microscope and individual worms were collected for the DNA analysis.

During the study, accidental *Gyrodactylus* parasites, i.e., those not specific for salmon, were also identified, analyzed by molecular markers, and reported in another paper (Ziętara *et al.* 2008).

DNA extraction and sequencing

DNA was released from single specimens of *G. salaris* by digestion in a solution consisting of 0.45% Tween, 0.45% Igepal and 60 μg ml^{-1} proteinase K in 1 × concentrated PCR buffer. Digestion was carried out in a PCR machine

for 25 min at 65 °C, followed by inactivation of proteinase K for 10 min at 95 °C and cooling at 4 °C. Two μl of these digestions were used as templates for PCR amplification.

In the first phase of this study, a 588 bp fragment of mitochondrial CO1 sequence was amplified by primers *Keret'L1* 5´-GTTTT CGCTT CACCT GTCTGG and reverse *Keret'H1* 5´-TACAC CCACC ACACG ATTGG, based on published sequences (Meinilä *et al*. 2003). This fragment separates all major branches of *G. salaris*, specific on grayling, salmon and rainbow trout, as well as most of salmon specific haplotypes. The observations led to the hypothesis of clonal competition, and the resolution was improved by longer mitochondrial sequences, and also by utilizing the nuclear marker ADNAM1 (Ziętara *et al*. 2006, Kuusela *et al*. 2007).

The full length mitochondrial CO1 and its flanking tRNA segments were amplified with either *FCox6* (5-TTGGA TCATA AGCGC ATYGG TAT-3´) and *16SR* (5-CATTT AATCA TGATG CAAAA GG-3´) primers or *Trp1F* (5´-ATATA GACGA TTTGT TTTCA-3´) and *Thr1R* (5´-ACAGA TTACT TGGTA TTACA-3´) primers. *FCox6* primer is complementary to the 5´ end of COI, *16SR* primer to the 5´ end of 16S rDNA and the other two primers to transfer RNAs flanking the COI gene.

The 20 μl PCR reaction consisted of 1 × PCR buffer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1 μM of

Table 1, Sampling data from the 23 rapids in the Tornionjoki and tributaries. The nuclear genotype was not determined for all specimens assigned to mtDNA types, and vice versa: *ADNAM1* genotype S2 was determined in an additional 21 specimens from Lätäseno (Mukkakoski and Kinnerpuska) without knowing the mtDNA type.

River	Rapid and year	Distance from sea (km)	N_{fish}	$N_{infected}$	Number of parasites						
					SalBa04			*SalBa05*			
					N	S2	S3	*N*	S2	S6	S7
Lätäseno	Pinniskoski 2000	468	15	9	11	3		7	7		
	Mukkakoski 2000	464	44	31	60	7		18	15		
	Kinnerpuska 2000	461	36	22	30	8		5	3		
	Patoniva 2000	459	15	8	14	2					
	Patoniva 2006		60	46	32	30	2	2	2		
	Vähäkurkkio 2000	438	32	13	11	2					
Könkämäeno	Pätikkä 2000	459	15	1							
	Kelottiluspa 2000	435	15	0							
	Kattilakoski 2000	431	55	28	11						
	Kattilakoski 2006		62	10	8	8					
Muonio	Jatuni 2000	397	18	3	8						
	Pingisniva 2000	364	43	14	16						
	Noijanpola 2000	330	60	30	31	10					
	Vanha Kihlanki 2000	255	33	2							
	Vanha Kihlanki 2006		60	3	1	1		1	1		
	Mukkakoski 2000	235	46	3	4	2		6		6	
Tornionjoki	Kassa 2000	170	52	7				11		8	
	Jarhoinen 2006	153	60	0							
	Korpikoski 2000	118	27	0							
	Turtola 2000	109	31	1				4			4
	Kattilakoski 2000	94	44	6				10		6	
	Vuennonkoski 2000	47	73	0							
	Kukkolankoski 2000	18	35	0							
	Vähänärä 2000	14	7	0							
	Oravaisensaari 2000	13	46	0							
	Kiviranta 2000	4	6	0							
	Salmikoski 2000	0	17	1							
Total			1007	238	237	73	2	64	28	20	4

each primer, 0.5 U of polymerase and 2 μl of the template prepared as described above. The PCR program was run as follows: 94 °C for 3 min, 38 cycles (94 °C for 30 s, 48 °C for 1 min and 72 °C for 1 min 50 sec), 72 °C for 7 min, and final cooling to 4 °C. Three additional internal primers were used to obtain the sequences: *RCox4* (5´-AGACA GGTGA AGCGA AAACA-3´), *LA* (5´-TAATC GGCGG GTTCG GTAA-3´) and *FCox3* (5´-GCCAA TAACC CAATC GTGTG-3´). The pair *FCox6*/*16SR* was used for 29 *G. salaris* specimens from Tornio, 2 specimens from Kumsa, 2 specimens from Lizhma, 2 specimens from Syskynjoki and 33 specimens from Keret'. *Trp1F*/*Thr1R* primers were used for 41 specimens from Tornio, 1 specimen from Lizhma, 1 specimen from Kumsa, 1 specimen from Syskynjoki and 2 specimens from Keret', for describing the haplotypes as completely as possible.

In this procedure, the sequencing errors were eliminated by consistently using direct sequencing, thus avoiding the PCR errors disturbing the cloning approach; and by repeating the sequencing several times from same specimens, using different primers. Final quality check was conducted during a most conservative comparison of aligned sequences: every variant has been observed in numerous independent specimens.

A nuclear single copy DNA marker (Anonymous DNA marker 1, *ADNAM1*) was developed and used here as described in Ziętara *et al*. (2006) and Kuusela *et al*. (2007). *ADNAM1* is a segment of obviously noncoding, but not repetitive DNA (CG content 33.9%, no BLAST hits longer than 15 bp), showing five identified alleles in the material of this study. *ADNAM1* was amplified with primers *InsF* (5´-GATCT GCAAT TCATC CTAAT-3´) and the reverse *InsR* (5´-TACAA TTCGA CCAAG GGTAG-3´). The 20 μl reactions consisted of 1 × PCR buffer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1 μM of each primer, 1 U of Taq polymerase and 2 μl of template prepared as above. The PCR program was run as follows: 94 °C for 3 min, 42 cycles (94 °C for 40 s, 50 °C for 30 s and 72 °C for 1 min), 72 °C for 7 min, and cooling to 4 °C. One additional *Ins1R* primer (5´-TTATT GACAT AGCAG CGTAT-3´) was used for sequencing. Altogether 155 specimens of *G. salaris* were sequenced from Tornio, 35 specimens from Keret', 2 specimens from Kumsa and 2 specimens from Lizhma. For ascertaining the haplotypes, a subsample from Keret' (one specimen, 6 colonies) and Tornio (4 specimens, 39 colonies) was cloned and sequenced.

Sequencing was based on Big Dye Terminator Cycle Sequencing kit protocol and the ABI 377 (PE Applied Biosystems) or ABI 3730 DNA Analyzer. (Applied Biosystems, Hitachi, Japan).

Data analyses

The sequences were aligned and inspected using MEGA3.1 (Kumar *et al*. 2004). In the data analysis, haplotype (clonal) diversity was calculated as $H = (n/(n-1)) \times (1 - \sum p^2)$. Conventional $F_{ST} = (H_T - H_S)/H_T$ statistics based on haplotype frequencies were used to estimate the degree of population differentiation. The software Arlequin 2.000 was used for calculations (Schneider *et al*. 2000). For demonstrative purposes, the F_{ST} was transformed to the number of (female) migrants per generation (Hudson *et al*. 1992) $Nm_F = 0.5(1/F_{ST} - 1)$.

For testing the apparent negative interference (competition) between the parasite (mitochondrial) clones, a permutation test program was written to estimate the expected number of fish with mixed infection and the probability of randomly obtaining the observed number, or less. The calculation was based on repeated random sampling from a virtual population having the observed haplotype frequencies, using the same distribution of sample size per fish as in the actual data.

Results

Long mtDNA sequences, including the complete 1503 bp of cytochrome oxidase subunit 1 and the transfer RNAs for tryptophan and threonine, of all haplotypes observed in this study are deposited in GenBank (AF540891 and AF540892 Keret'; AY840222 Onega/Lizhma; AY840223 Onega/Kumsa; EF117889 Ladoga/Syskynjoki; AF540905, DQ468128 and EU304825 Tornio). The alleles (haplotypes) of *ADNAM1* recorded in this study are deposited in GenBank with acces-

sion numbers DQ468129-DQ468136 (Kuusela *et al*. 2007).

After 130 000 years of coevolution: Lake Onega and Lake Ladoga

Among the fish sampled from six Lake Onega spawning rivers (Fig. 2) during three separate visits (2001, 2002, 2004), the *G. salaris* infection was very rare. The total number of the inspected salmon parr was 293, of which three fish were infected (prevalence 1.0%). Also, the intensity (number of parasites per fish) was very low. In the Lizhma River, a single *Gyrodactylus* specimen was detected in the year 2002 (N_{fish} = 15); in the year 2004, twelve parasites were found on a single infected fish (N_{fish} = 24). In the Kumsa River, ten parasites were found on one fish in 2004 (N_{fish} = 50). All the parasites were observed on second-year juveniles (1+), and the first-year fish were clean.

In the Lizhma River, the mtDNA haplotype *SalBa03* (AY840222) was almost identical to the *SalBa02* found in Keret' (AF540892), differing from it by two T/C transitions along 1606 bp. The haplotype in the Kumsa River *SalBa01* (AF840223) was identical to the second haplotype from Keret' (AF540891).

Both widely divergent mitochondrial clones in Lake Onega had the heterozygous nuclear *ADNAM1* phenotype S1, TMRTCRWAT, consisting of alleles TCATCGTAT (DQ468136) and TAGTCAAAT (DQ468135). This nuclear genotype was also identical in all parasites in the Keret' River and in the two in Lake Ladoga basin.

The mitochondrially dimorphic population of *G. salaris* in Lake Onega rivers was subdivided into the extreme: two clones existed but were completely isolated, and four possible spawning rivers (Fig. 2) were free of parasites during the sampling: Shuya (N_{fish} = 11), Pyal'ma (N_{fish} = 98), Tuba (N_{fish} = 77) and Vama (N_{fish} = 16).

Six spawning rivers (N_{fish} = 255) were inspected along Lake Ladoga in July 2006. Two specimens of *G. salaris* were observed on 2 of 68 fish from the Syskynjoki population. The two parasites in Syskynjoki carried the nuclear *ADNAM1* genotype S1, identical to the parasites in Lake Onega. The mitochondrial haplotype *SalBa11* was unique, but belonged to the clade specific to the Baltic salmon (EF117889). Other samples were completely free of parasites: Vidlitsa (N_{fish} = 44), Tulema (N_{fish} = 66), Uuksunjoki (N_{fish} = 29), Hiitolanjoki (N_{fish} = 44), and Burnaya (Taipaleenjoki) (N_{fish} = 4).

Postglacial coevolution: the Tornio River system

The 765 fish caught in 2000 in the Tornio River and its tributaries carried a total of 5089 *Gyrodactylus* worms, counted on the pectoral and dorsal fins only. The parasite was observed in 16 of 23 rapids. The prevalence (proportion of infected fish) was 23.4%, and the incidence (mean number of parasites) on the three fins of infected fish was 28.4 (median = 8, range = 1–643). The prevalence (and intensity) of the infection was clearly higher in upstream rapids (Fig. 4). A detailed description of the epidemiology and analysis of the environmental factors influencing *G. salaris* in the river was given by Anttila *et al*. (2008).

Only two *G. salaris* mitochondrial haplotypes, *SalBa04* (76.9%) and *SalBa05* (23.1%), were detected in the Tornio River system (N = 255; Fig. 3). *SalBa04* and *SalBa05* differed by ten nucleotides along 1606 bp (accession numbers AF540905 and DQ468128, respectively). In only four of 13 rapids examined by sequencing the mtDNA of parasites, both mitochondrial clones were present simultaneously. The distribution of mitochondrial haplotypes is illustrated in Fig. 3, and the nuclear data (*ADNAM1* genotypes) are presented in Table 1. In the three uppermost rapids in Lätäseno (468–461 km), *SalBa04* (N = 101) and *SalBa05* (N = 28) haplotypes were observed together. In these rapids, both mtDNA haplotypes had the same nuclear genotype S2 TMRTCAWAT (N = 69, alleles TCATCATAT/TAGTCAAAT, accession numbers of the alleles DQ468129 and DQ468130). In the lower part of the river, in Mukkakoski (235 km), where only three fish out of 46 carried parasites, mitochondrial types *SalBa04* and *SalBa05* were also observed together, but carried different *ADNAM1* genotypes: *SalBa4* + S2

TMRTCAWAT (in one fish, $N = 2$) and *SalBa05* + S6 TMRTYRWAK (in two fish, $N = 6$). Both combinations were found in other rapids as well, up and downstream from Mukkakoski, respectively (Table 1).

The mitochondrial haplotype *SalBa05* carried altogether three different *ADNAM1* genotypes, in different rapids: in Lätäseno S2 TMRTCAWAT ($N = 25$, *see* above), in Mukkakoski (235 km), Kassa (170 km) and Kattilakoski (94 km) S6 TMRTYRWAK ($N = 20$, TAGTCAAAT/TCATTGTAG, DQ468131/DQ468132). A unique genotype S7 TMRTYRTAK ($N = 4$, TAGTCATAT/TCATTGTAG, DQ468133/DQ468134) was observed on the single infected fish from the Turtola rapid (109 km). This genotype differed from S6 TMRTYRWAK by one converted nucleotide.

Overall, the F_{ST} value calculated over the mtDNA haplotype frequencies in different rapids was 0.484 ($H_T = 0.357$, $N = 255$, $P = 0$). This was transformed to the number of female migrants per generation $Nm_F = 0.533$. The corresponding estimate comparing the river segments above and below the Tornio–Muonio River confluence $Nm_F = 0.132$ is even smaller.

A simultaneous infection by *SalBa04* and *SalBa05* was observed on only four fish in the year 2000 sample. This occurred in Mukkakoski and Kinnerpuska, where the nuclear *ADNAM1* genotype in both mitochondrial haplotypes was S2 TMRTCAWAT. In a permutation test, simulated random resampling using the actual sample sizes per fish, four or fewer mixed infections were never observed in a million repeats. Considering only the four rapids where both mitochondrial haplotypes were observed, 18.1 double infections were expected, and four or fewer occurred in 2.4% of the random trials.

The rapids Patoniva (Lätäseno), Kattilakoski (Könkämäeno), Vanha Kihlanki (Muonionjoki) and Jarhoinen (Tornionjoki) were re-inspected in 2006. One new mtDNA haplotype, *SalBa12* (EU304825), was found that differed from the previously determined *SalBa04* by one nucleotide outside of the commonly sequenced 800 bp. Also, a new combination of nuclear alleles was found: S3 TMRTCATAT ($N = 2$, TCATCATAT/TAGTCATAT). This combination can be explained as a recombinant between S2 and S7, and it was identical to the nuclear genotype recorded from the Vefsna River, Norway. The S3 clone in Patoniva differs in mtDNA from Vefsna (Kuusela *et al*. 2007).

The Keret' River: an introduced parasite on a susceptible host

In the Keret' River, every fish caught in the year 2002 in the Morskoy ($N_{fish} = 20$), Sukhoy ($N_{fish} = 8$) and Verkhniy rapids ($N_{fish} = 23$) was infected. Only three fish were caught from the Varatskiy rapid, and they were parasite free. In other rapids, each inspected fish was severely infected, with the number of parasites on fins varying from about 100 to several hundred.

In the Keret' River, the two mitochondrial haplotypes *SalBa01* (43%) and *SalBa02* (57%) were almost equally common, but unevenly distributed (Fig. 4). *SalBa1* and *SalBa02* sequences differed by 16 nucleotides on 1606 bp, and they were unique among the sequences of *Gyrodactylus salaris* observed in *Salmo salar* (Meinilä *et al*. 2004). Haplotype *SalBa02* dominated the lower Morskoy rapid (84%, $N_{parasites} = 68$, $N_{fish} = 9$). Haplotype *SalBa01* replaced it in the upper section, in the Sukhoy rapid, located 55 km from the sea (89%, $N_{parasites} = 19$, $N_{fish} = 3$), and in the Verkhniy rapid, 68 km from the sea (90%, $N_{parasites} = 63$, $N_{fish} = 9$). The fixation index F_{ST} calculated over haplotype frequencies was 0.562 (unweighted) or 0.548 (mean H_S weighted by sample sizes, $H_T = 0.494$, $N_{parasites} = 150$). The transformation to the number of migrants resulted in the value $Nm_F = 0.412$. The similarity of haplotype frequencies in Verkhniy and Sukhoy deflates the F_{ST}, which between Verkhniy and Morskoy was $F_{ST} = 0.703$, transforming to $Nm_F = 0.211$. Thus, the parasite clones were not randomly distributed in the Keret' River, rather they were in strong disequilibrium with respect of drift and migration.

For first time in this paper, similar mtDNA haplotypes are also reported from Lake Onega. As mentioned above, all parasites in the Keret' River had an identical heterozygous nuclear *ADNAM1* genotype S1 TMRTCRWAT, the same as in the lakes Onega and Ladoga. This prevents observing possible sexual exchange between the clones, which was evident in Tornio. However, no homozygotes were observed.

In the Keret' River, the two mitochondrial clones existed at all sampling sites, though in different proportions. Based on the observed haplotype frequencies in the three rapids, the expected number of double infections was 12.1 on 20 fish where two or more parasites were sequenced. Eight double infections were detected. The probability of observing eight or fewer double infections in a random sample was $P_{(rnd \leq obs)} = 0.050$ (one million permutations).

In 2005, the Keret' River was visited once again. In the Morskoy rapid, 21 salmon parr were inspected, and three of them were infected, each by the mtDNA clone *SalBa01,* which was present as a minority clone in that rapid in 2002. The Sukhoy rapid had almost no salmon juveniles, but the single 18 cm long precocious male observed was infected by parasites belonging to the *SalBa02* clone. *ADNAM1* genotypes remained unchanged.

Discussion

This study is the first to examine *G. salaris* genetic population structuring across a gradient host/parasite coadaptation timescale. We inspected a large number of salmon juveniles, in ecologically different circumstances, on resistant and susceptible hosts, utilizing molecular markers to reveal the population structure. To obtain a snapshot of the dynamics of the native and introduced parasite populations, it was important to screen the populations with a "frustratingly" low parasite density millimeter by millimeter. Only the relatively highly infected localities were studied by the "standard method" of three fins.

We used molecular identification of the parasite clones, allowing us to correctly place them into the phylogeographic framework of *G. salaris,* and to eliminate the possibility of misidentification. Actually, the salmon juveniles caught during the Lake Ladoga expedition in 2005 carried more non-specific *Gyrodactylus* parasites than *G. salaris*: a few specimens of *G. aphyae* and *G. papernai* (parasites of Eurasian minnow *Phoxinus phoxinus* and loach *Noemacheilus barbatulus*, respectively) were observed, most probably as accidental visitors (Ziętara *et al.* 2008). In the Tornio River, a specimen of *G. lotae* (parasite of burbot, *Lota lota*) was similarly observed in year 2006 on a single salmon parr (EU523133).

Grayling parasites, separable by mtDNA and ADNAM1, which have been traditionally named as *G. thymalli* (Žitňan 1960), were not observed on salmon in this study, while the two hosts live sympatrically in all localities studied. The parasite lineages, while historically related and sharing the same gene pool, are strictly host specific and reluctantly sexual (Kuusela *et al.* 2007, Anttila *et al.* 2008).

For checking the Hardy-Weinberg equilibrium (HWE) and linkage equilibrium in the *G. salaris* populations, we utilized here an anonymous nuclear DNA marker (Ziętara *et al.* 2006). The *a priori* expectation was that the parthenogenetic clonal episodes of reproduction may lead to deviations from HWE, especially in strongly fragmented host populations. The first, qualitative conclusion was that the nuclear gene marker of salmon-specific strains of *G. salaris* was diploid, variable, and permanently heterozygous in all individuals. It was not segregating in a Mendelian manner and thus, not in HW equilibrium (Kuusela *et al.* 2007).

Gyrodactylus salaris was rare in the historically coadapted host population

In Lake Onega, *G. salaris* was assumed to be native. The White Sea and Lake Onega (representing now the Baltic Sea basin) were connected during the Eemian interglacial 132 000 years ago, and this was suggested to be the earliest possible time for the White Sea and Baltic Sea grayling parasites to hybridize (Kuusela *et al.* 2007). The genetic divergence between the two mtDNA haplotypes observed on Lake Onega salmon was large (1%), suggesting that the parasite population in this lake is old, surely predating the earliest postglacial recolonization of the recovering lake twelve thousand years ago (Nilsson *et al.* 2001, Asplund *et al.* 2004). Still, the nuclear genotype of all parasites was uniform in Onega, Keret' and Ladoga. The existence of three distant mtDNA haplotypes and no intermediate forms indicates pruning of clones (= selection), by some kind of direct or indirect competition.

The fact that the two mtDNA clones have coexisted for so long may be considered as a consequence of the fugitive strategy. When a spawning river is occupied by a parasite, there is no chance for the other clones to invade. On the other hand, the fugitive strategy leads to the reduction of clones and to low clonal diversity in open populations.

Kudersky *et al*. (2003) reported a much higher prevalence of *G. salaris* in the rivers of Lake Onega. In Lizhma and Kumsha, infection was detected in 25% of the fish. In the Pyal'ma River, the prevalence was once observed to be 73%, while we have sampled the Pyal'ma River twice (4 August 2001, $N = 48$; 6 July 2004, $N = 50$) without seeing a single parasite on salmon (but a single one was collected on grayling, Kuusela *et al*. 2007). This wide fluctuation shows the high-amplitude seasonal demographic cycles of the parasite, and supports the metapopulation model of a fugitive parasite. On the other hand, in Lake Ladoga there is only one earlier report of *G. salaris* on salmon (Ergens 1983), but the observation was later corrected as it was found to originate from a fish farm, not from the wild (Kudersky *et al*. 2003).

It is to be pointed out that *G. salaris* has no specific aestivating or hibernating phases in the life cycle. The extremely low population densities in summer have to be understood to indicate a low effective population size, genetic bottlenecks and high risk of local extinction.

The difference of the lake salmon populations in comparison to the Tornio is that the spawning rivers are not connected by regular seasonal migrations like the rapids in a river. This may amplify extinction–recolonization cycles. The adult fish may meet during the feeding migration. The structured pattern observed in the Tornio River basin was amplified to the extreme in Lake Onega. Two parasite clones were detected, but they were in different rivers, and four of the six rivers were clean, supposedly not recolonized by the parasites after local extinction events. In Lake Ladoga, the two parasites found among 255 fish certainly were on the verge of extinction, but the unique mtDNA suggested that the clone was endemic.

Moderately coadapted host–parasite community in the northern Baltic basin

The epidemiological and ecological analysis of the Tornio *Gyrodactylus* infection was published by Anttila *et al*. (2008). The infection was stable in the river system over the time period of 2000–2004, and the sample from 2006 confirmed this stability. The most important fact in constructing the demographic model is to know that the down-migrating smolts were the most heavily infected cohort: 82.5% were infected, and some of them with a high intensity. Up to 566 parasites were counted on the three standard fins of an individual fish (Anttila *et al*. 2008).

In the Tornio drainage there were only two mitochondrial haplotypes and four clones detected by using nuclear markers. These numbers were raised by one mtDNA haplotype and one clone in 2006. Yet the population is open for parasite immigration from the whole area of the Baltic Sea, when the feeding migrations of salmon extend to the southern part of the sea. The extent of the genetic variation in *G. salaris* in the Baltic Sea basin has not been throroughly explored, but the two closely related mitochondrial haplotypes certainly are a small fraction of the available variation (Hansen *et al*. 2003, Kuusela *et al*. 2007). Another important observation was that rainbow trout-specific clones were not observed on salmon in the Tornio system. Similarly, the mitochondrial haplotypes found on upstream grayling populations were never observed on salmon.

The field observations are in accordance with the laboratory susceptibility tests made by Bakke *et al*. (2004) with Baltic salmon from Indalsälven. Somewhat surprisingly, the salmon stock of Indalsälven was only moderately resistant against *G. salaris*, at least in comparison to the "standard Baltic" stock from Neva, which is from an extinct population maintained in hatcheries in Lake Ladoga and in Finland (Bakke *et al*. 2002, 2004). Rintamäki-Kinnunen and Valtonen (1996) interpreted northern Baltic salmon in hatcheries as resistant, because *G. salaris* infection appeared and disappeared, usually without any pathological symptoms. Only once has a disease outbreak been reported (Rintamäki 1989).

Population structure of the introduced parasite on a susceptible host

The well-known example of parasite introduction is the Norwegian epidemic of *G. salaris*, which started in the Lakselva River and spread throughout central Norway, demonstrating the enormous dispersal capacity of the parasite when unconsciously aided by man (Johnsen & Jensen 1991). The Norwegian west-coast epidemic was obviously caused by one clone, as suggested by mtDNA (Hansen *et al*. 2003) and also by nuclear IGS (Intergenic spacer of ribosomal DNA: Cunningham *et al*. 2003, Hansen *et al*. 2006). The rapid expansion of *G. salaris* in Norway is a reference to the high dispersal capacity of the parasite, but in our native populations, dispersal capacity looks rather weak.

The Keret' River was our example of a susceptible host and an introduced parasite. The origin of infection was clearly from Lake Onega. The two mitochondrial haplotypes were almost identical to those observed in Lizhma and Kumsa, and not found elsewhere. The parasites most probably were brought together in the Shuya hatchery, the assumed intermediate source of the Keret' infection (Kudersky *et al*. 2003). In spite of the short time between the introduction and sampling, the two clones had reached an almost vicariant structure and clearly divided the river into temporary territories. The process can be described as a random drift, but considering the migrations of the host, the drift has to be amplified by either direct or secondary clonal repulsion.

The resampling in the Keret' River in July 2005 presented a complementary picture. The salmon parr density was very low. Only four of 22 fish were infected, each of them mildly, and the dominant mitochondrial haplotype *SalBa02* in Morskoy had changed to *SalBa01*. *SalBa02* was observed on the single precocious male caught in the Sukhoy rapid, where the other haplotype dominated in 2002. The change is not statistically significant, because only four fish were infected, but it further emphasizes the stochasticity of the demography of *G. salaris*, especially on a low host density.

The competition between clones most probably is mediated by host defense reactions (Bakke *et al*. 2002). The resistance of the host indicates the intrinsic ability of fish to develop a growth-restricting defense reaction against the parasite population (Buchmann & Lindenstrøm 2002). Between the rivers Tornio and Keret', there was a significant difference in the frequency of individual fish infected simultaneously by two clones (Tornio: 4 observed, 18 expected, Keret': 8 observed, 12 expected, $G^2 = 5.219$, $P < 0.05$). The sign shown by the difference is correct. This suggests that the resistance vs. susceptibility difference of the coadapted and naïve hosts contributed to the structuring of the parasite clones.

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