Molecular Ecology (2007) **16**, 5234–5245 doi: 10.1111/j.1365-294X.2007.03562.x

Hybrid origin of Baltic salmon-specific parasite *Gyrodactylus salaris*: a model for speciation by host switch for hemiclonal organisms

JUSSI KUUSELA,* MAREK S. ZIĘTARA†‡ and J. LUMME‡
*National Veterinary and Food Research Institute, Oulu Regional Unit, POB 517, FIN-90101 Oulu, Finland, †Gdańsk University Biological Station, Laboratory of Comparative Biochemistry, PL-80-680 Gdańsk-Sobieszewo, Poland, ‡Department of Biology, University of Oulu, POB 3000, FIN-90014 Oulu, Finland

Abstract

Host switching explains the high species number of ectoparasitic, viviparous, mainly parthenogenetic but potentially hermaphroditic flatworms of the genus *Gyrodactylus*. The starlike mitochondrial phylogeny of *Gyrodactylus salaris* suggested parallel divergence of several clades on grayling (also named as *Gyrodactylus thymalli*) and an embedded sister clade on Baltic salmon. The hypothesis that the parasite switched from grayling to salmon during the glacial diaspora was tested using a 493-bp nuclear DNA marker ADNAM1. The parasites on salmon in lakes Onega and Ladoga were heterozygous for divergent ADNAM1 alleles WS1 and BS1, found as nearly fixed in grayling parasites in the White Sea and Baltic Sea basins, respectively. In the Baltic salmon-specific mtDNA clade, the WS/BS heterozygosity was maintained in 23 out of the 24 local clones. The permanently heterozygous clade was endemic in the Baltic Sea basin, and it had accumulated variation in mtDNA (31 variable sites on 1600 bp) and in the alleles of the nuclear locus (two point mutations and three nucleotide conversions along 493 bp). Mendelian shuffling of the nuclear alleles between the local clones indicated rare sex within the clade, but the WS/BS heterozygosity was lost in only one salmon hatchery clone, which was heterozygous WS1/WS3. The Baltic salmon-specific *G. salaris* lineage was monophyletic, descending from a single historical hybridization and consequential host switch, frozen by permanent heterozygosity. A possible time for the hybridization of grayling parasite strains from the White Sea and Baltic Sea basins was during the Eemian interglacial 132 000 years BP. Strains having a separate divergent mtDNA observed on farmed rainbow trout, and on salmon in Russian lake Kuito were suggested to be clones derived from secondary and tertiary recombination events.

Keywords: balanced lethals, heterosis, homoploid hybrid speciation, host switch, hybridization, permanent heterozygosity

Received 27 May 2007; revision accepted 28 August 2007

Introduction

As a form of adaptive speciation, parasite evolution by host switch is an attractive option for explaining a fraction of species richness. Several theoretical analyses support the models of splitting one lineage into separate niches, even sympatrically among outcrossing species (Dieckmann *et al*. 2004). As an alternative, speciation by hybridization following polyploidization is well known in plants, while rare in animals. If the isolation is achieved by loss of sexuality, it is expected to lead to a dead end of evolution (Bell 1982; Kearney & Shine 2004). Homoploid hybrid speciation without chromosomal change is a less-known alternative (Coyne & Orr 2004; Seehausen 2004) which recently has been demonstrated in few occasions (Schwarz *et al*. 2005; Mavárez *et al*. 2006; Meyer *et al*. 2006).

The target organism of this study, the monogenean flatworm *Gyrodactylus salaris* Malmberg belongs to the *wageneri* species group, of the subgenus *Limnonephrotus*

Correspondence: Jaakko Lumme, Fax: +358 8553 1061; E-mail: jaakko.umme@oulu.fi

(family Gyrodactylidae, Malmberg, 1957). The species group has been characterized as an example of adaptive radiation, because of host switching among at least seven families of freshwater fish: Cyprinidae, Salmonidae, Percidae, Cottidae, Lucidae, Osmeridae, and Gasterosteidae. (Zięt̨ara & Lumme 2002, 2004). However, the parasites are strictly host specific in the ecological timescale. The phylogeny suggests that the host-switch events have been rare but definitive and irreversible. The specific case of *G. salaris*[1] presented in this study may suggest one explanation and mechanism for a sudden host switch, instant isolation and ultimately, speciation.

In Atlantic salmon (*Salmo salar*) populations along the Atlantic Ocean and the White Sea, *G. salaris* is the parasite causing gyrodactylosis, an industrially transmitted fish disease spread by commercial salmon aquaculture (OIE 2003). The parasite is native and the host co-adapted in the Baltic Basin salmon populations (e.g. Bakke *et al*. 2004). The pathogenic forms have been difficult to separate from similar-looking parasites on grayling (*Thymallus thymallus*), and even the internal transcribed spacer (ITS) sequencing left the question open (Cunningham 1997). The mtDNA improved the resolution (Meinilä *et al*. 2002, 2004), but the grayling and salmon parasites were still closely related, forming several sister clades in mitochondrial phylogenetic trees (Bakke *et al*. 2007; Hansen *et al*. 2007; Olstad *et al*. 2007).

On the basis of the mitochondrial phylogeny, it was hypothesized that the parasite lineage specific to Baltic salmon (and generally named as *G. salaris*) was monophyletic and probably switched to salmon from the plesiomorphic host grayling (Meinilä *et al*. 2004). Another divergent but monomorphic mtDNA lineage was found on rainbow trout (*Oncorhynchus mykiss*) farms (Hansen *et al*. 2003; Meinilä *et al*. 2004), on Arctic charr (*Salvelinus alpinus*) in Norway (Olstad *et al*. 2007; Robertsen *et al*. 2007), on salmon in Oslo Fjord (Hansen *et al*. 2003) and on salmon in Lake Kuito, Russian Karelia (Meinilä *et al*. 2004). In Göta River, Sweden, another mtDNA type has been reported in parasites on salmon (Hansen *et al*. 2003). These parasites have also been identified as *G. salaris*.

In this study, we tested the following hypothesis, based on the mitchondrial phylogenetic reconstruction and suggested by Meinilä *et al*. (2004): host switching from grayling to salmon occurred once, and was followed by instant sexual isolation. The model could also serve as an overt explanation of the high species number of *wageneri* group of *Gyrodactylus* (Zięt̨ara & Lumme 2002, 2004). Host switching of host-specific parasites without intermediate generalist phase is a logically difficult step, as are all models of sympatric speciation (Dieckmann *et al*. 2004). Yet, this is exactly what is seen in the sympatric host-specific strains of *Gyrodactylus* which still share (nearly) identical ITS of rDNA, and are impossible to separate by morphological inspection. They live on different hosts, and in well-resolved molecular phylogenies, all separate clades are strictly host specific, except the clade centred on rainbow trout farming (Hansen *et al*. 2007).

By utilizing a variable nuclear DNA marker, juxtaposed with the mitochondrial phylogenetic hypothesis of parasite strains on four host species: Atlantic and Baltic salmon (*S. salar*), grayling from the Baltic and White Sea basins (*T. thymallus*), and on farmed rainbow trout (*O. mykiss*), we wanted to elucidate the genetic relatedness of the mitochondrial sister lineages.

Materials and methods

Parasite sampling

White Sea basin grayling parasites were collected from the Oulanka River of the Lake Kovdozero system and in Penninki River, a tributary of the Pista River of the Kem' River system. From the Baltic basin, grayling parasites were from the Pyalma River, lake Onega, and from four widely spaced localities in the northern Baltic basin: Nagereatnu, Sweden, and Poroeno, Finland (tributaries of Tornionjoki), Niippa (Ounasjoki, tributary of Kemi River), and Soivio, Finland (tributary of the Iijoki River system). All these northern sites are located near the ancient shoreline of the Ancylus lake phase of the Baltic Sea and were probably colonized by grayling more than 9000 years ago (Meinilä *et al*. 2004). One sample on Baltic grayling was from a fish farm along river Radunia, tributary of Vistula in Poland. A map of sampling sites is presented in Fig. 1.

In this study, we utilized longer mtDNA segments than in earlier studies (Meinilä *et al*. 2004), in addition to the nuclear marker to re-analyse the salmon parasites from the Tornionjoki River (Baltic basin), lakes Onega and Ladoga (Baltic basin), and Keret' River (White Sea basin), as well as from several small rivers on the west coast of Sweden, where the salmon strains marginally belong to the Baltic population (Nilsson *et al*. 2001). One parasite sample is from the Vefsna River, representing the clone which caused the main Norwegian gyrodactylosis epidemic (Hansen *et al*. 2003). Only one parasite clone from a salmon hatchery was available (Iijoki, Raasakka, Finland). An interesting mtDNA sequence from a Macedonian farm strain of Ohrid trout (*Salmo letnica*) was included. Published results of *Gyrodactylus salaris* from Finnish rainbow trout farms, and from the derived non-anadromous salmon populations in Lake Kuito, Russian Karelia were also compared here (Zięt̨ara *et al*. 2006).

[1] The species name *Gyrodactylus thymalli* Žitňan (1960) is widely used for grayling-specific parasites, to emphasize the host specificity of different strains: the strains on grayling do not infect salmon, and vice versa. A comprehensive review was presented by Bakke *et al*. (2007).

Fig. 1 Map showing the sampling localities of salmon (pale dots) and grayling (dark dots) parasites. The Baltic Sea basin is marked with darker shading and a strong outline. The salmon parasites sampled from Vefsna (Norway) and Keret' (White Sea) were from infamous introduced infections, both originating from the Baltic area. The two separate localities Pikkuköngäs and Aventojoki are under the name Oulanka. In river Tornio, 12 rapids were sampled, while only four dots were drawn.

Molecular methods and data analysis

The analysis of mitochondrial DNA was conducted as earlier (Meinilä *et al*. 2002, 2004). The phylogenetic tree was based on an extended ~1600-bp fragment spanning from COI (1495 bp) over the $tRNA^{thr}$ to 16S rDNA (Ziętara *et al*. 2006; see Huyse *et al*. 2007 for the complete mitochondrial sequence of *G. salaris*). New primers FCox6 (5′-TTGGATCATAAGCGCATYGGTAT-3′) and 16S R (5′-CATTTAATCATGATGCAAAAGG-3′) were used to obtain the long sequence. The mitochondrial phylogenetic hypothesis displayed in Fig. 2 was constructed by MEGA 3.1 (Kumar *et al*. 2004), by utilizing Kimura's 2-parameter distance, and the neighbour-joining tree building method.

The anonymous nuclear marker ADNAM1 was sequenced according to Ziętara *et al*. (2006). The primers were InsF (5′-GATCTGCAATTCATCCTAAT-3′) and the reverse InsR (5′-TACAATTCGACCAAGGGTAG-3′) which amplify 493 bp (and 470 bp, as the short allele of the rainbow farm parasite) from an anonymous DNA locus. The alleles (haplotypes) of each phenotype (Figs 3 and 4) were resolved by utilizing a pair of selective primers, which amplify only the BS-class alleles (Ziętara *et al*. 2006), and by molecular cloning. These methods were needed to determine the *cis/trans*-relations of the variable nucleotides in heterozygotes. A minimum-spanning tree of the completely solved alleles was presented in Fig. 3.

The mitochondrial phenotypes, genotypes and haplotypes and the ADNAM1 alleles of each *G. salaris* clone studied in this paper are listed in Table 1, including the number of observed specimens and the GenBank Accession nos of ADNAM1 alleles and mtDNA sequences, as well as the ITS1–5.8S rDNA–ITS2 of the nuclear rDNA (shortly: ITS) sequences of most of the clones. A large number of the ITS sequences were new, produced to confirm the limited variation in this marker.

Results

Mitochondrial phylogeny of the salmon and grayling Gyrodactylus *parasites in the Baltic and White Sea basins*

The phylogenetic hypothesis of *Gyrodactylus* based on ~1600 bp of the maternally inherited mitochondrial COI gene sequences is presented in Fig. 2. The unrooted tree contains two grayling-specific clades described earlier from the Baltic and White Sea basin, and a new clade from Radunia, Pomerania, represented by two specimens. In addition to these clades, the recent study of Hansen *et al*. (2006) contained at least five separate sister clades parasitizing grayling in Norway, UK, Poland and Slovakia. Figure 2 contains only strains which were available for us, and in which the nuclear ADNAM1 marker and the long 1600 bp of mtDNA were analysed.

One mtDNA clade was found on grayling in two river systems in the White Sea basin, named as Oulanka and Penninki in the map in Fig. 1. The Baltic grayling-specific mitochondrial clade (97% support) was found in localities widely apart: in lake Onega (Pyalma), and in the northern part of the Baltic Sea Basin (Ancylus Lake level of Bothnian Bay: Nagereatnu, Poroeno, Niippa and Soivio in Fig. 1). The Polish parasite strain from Radunia was not a member of this mitochondrial clade, but it was related with the Vistula (Brda) sample of Hansen *et al*. (2007).

One of the main mitochondrial clades was specific for the Baltic salmon populations. As an introduced pathogen, this clade was also found in the White Sea (the Keret' River) and on the Atlantic coast of Norway (the Vefsna River). Considering all the evidence, the Baltic salmon parasite mtDNA clade with 99% bootstrap support was monophyletic. The most basal branch of the clade specific to salmon was found in Lake Onega (the Kumsha River), and the same mtDNA haplotype was also found (as an accidentally introduced and highly pathogenic) in the White Sea, the Keret' River.

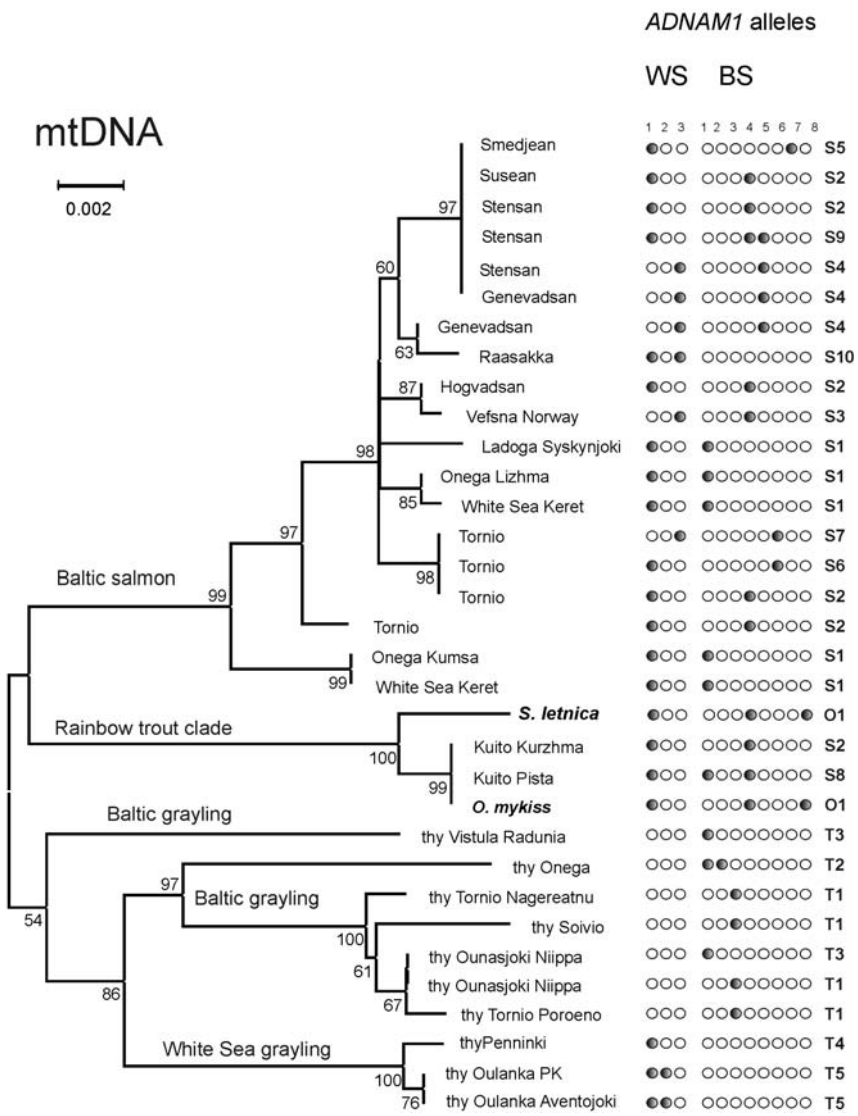

Fig. 2 Phylogenetic hypothesis of *Gyrodactylus salaris* based on 1600 bp of mitochondrial DNA. The separate clades on different host fishes or areas are marked. The tree is juxtaposed with the nuclear ADNAM1 genotype, expressed as dots in the right column. The ADNAM1 alleles are depicted in Fig. 3 and the genotypes in Fig. 4.

The second mtDNA clade which was not specific for grayling was monophyletic and it was also monotypic until this study. This mitochondrial lineage was found in several Finnish, Swedish and Danish rainbow trout farms and on the salmon population in Lake Kuito, Russian Karelia (the Pista and Kurzhma rivers; Ziętara *et al*. 2006). The same mtDNA was also found on salmon in the Oslo Fjord area of Norway (Hansen *et al*. 2003), and in Arctic charr (Robertsen *et al*. 2006; Olstad *et al*. 2007). All these strains had exactly identical ~800 bp CO1 sequence, suggesting a recent monoclonal origin. Interestingly, the parasite strain on Ohrid trout (*Salmo letnica*) was related but not identical with the widely distributed farm type known earlier (20 divergent nucleotides, 1.3% genetic distance).

It is to be emphasized that the basal nodes of the mitochondrial tree in Fig. 2 were not solved, and the positioning of the grayling parasites on the lower part and salmon parasites on the upper part is not to be understood as division to two monophyletic lineages, in spite of the 54% bootstrap support. In trees based on shorter mtDNA sequence but having many more lineages on grayling (Hansen *et al*. 2007), the intermingling of the sister clades among the hosts was clear (starlike phylogeny).

Phylogeny of the nuclear ADNAM1 marker alleles

The nuclear marker ADNAM1 was described earlier in rainbow trout farm-specific parasites (Ziętara *et al*. 2006). The marker was originally developed starting from the extra polymerase chain reaction (PCR) product of the degenerate primers BT1 and BT2R used by Collins *et al*. (2004) to amplify the β-tubulin gene. In addition to the target sequence, these primers produced a weak extra fragment of ~500 bp in rainbow trout farm *Gyrodactylus salaris*. When this fragment

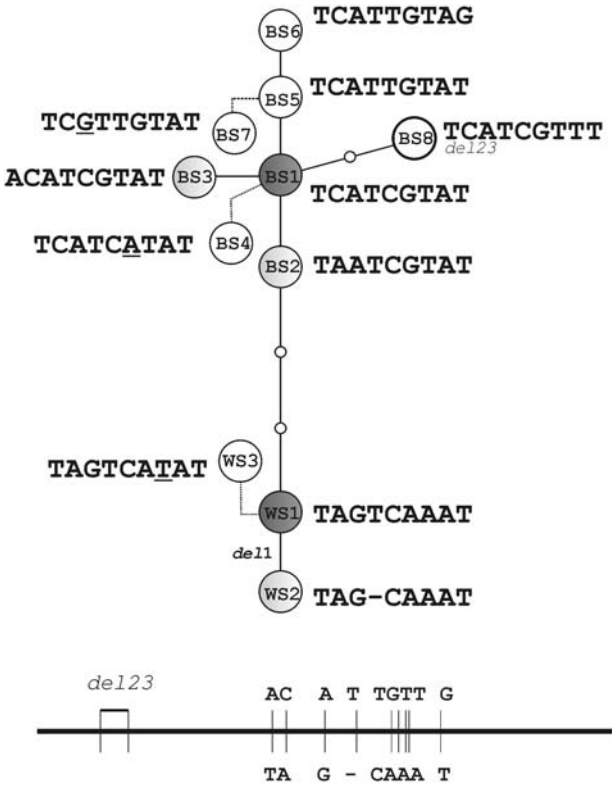

Fig. 3 The parsimonious network of the marker alleles (haplotypes) of the ADNAM1 locus found in *Gyrodactylus salaris*. The dark shadow indicates the two alleles found both on grayling and salmon, pale shadowing indicates those found only in grayling, and unshadowed alleles were only found in parasites on salmon. The short BS8 allele found only in parasites on rainbow trout and Ohrid trout is marked by bold outline. Small circles indicate intermediate haplotypes not observed. Dotted lines connect the haplotypes which were explained as nucleotide convertants (the converted nucleotide underlined in WS3, BS4 and BS7). The bottom panel depicts the relative position of the variable sites along the 493 bp of the ADNAM1 sequence.

was re-amplified and sequenced, it was observed that there was a heterozygous indel, destroying the sequence readings when direct sequencing was utilized from 5′ end. We developed specific primers (FBl, FBs) to solve this problem, sequencing the long and short fragments separately. The indel (later described as deletion) proved to be 23-bp long.

We sequenced the long fragment by specific internal primers, which amplified a fragment of 469 bp. The long fragment still proved to be heterozygous for several nucleotides, while the 'old' primers BT1 and BT2R of Collins *et al*. (2004) were selective, amplifying only one of the 'long' alleles. This allowed us to sequence the two long alleles separately, leading to the haplotype (allele) description WS1/BS4/BS8 (Genotype O1) presented for rainbow trout parasite in Figs 3 and 4.

The amplified DNA segment was nuclear, supposedly noncoding, but nonrepetitive DNA, with CG content as low as 33.9%, and showed no BLAST hits longer than ~15 bp. It was confirmed that the PCR product was not from fish tissue.

Subsequently, the marker was studied in Baltic salmon parasites, which were invariably observed to be heterozygous. And finally, the marker was also studied in parasites collected from grayling. The alleles predicted from heterozygous salmon parasites were found as homozygous, separately in the graylings in the White Sea and Baltic Sea basins.

From the heterozygous phenotypes (Fig. 4, Table 1) obtained by direct sequencing, the haplotypes (alleles) were solved by PCR utilizing selective primers, and by cloning as many molecules that the *cis/trans*-position of the variable nucleotides was solved. The alleles were found to be matching with the alleles observed in parasites on grayling. The designation of alleles (Fig. 3, Table 1) was based on all observed variable nucleotide sites, the long 23-bp deletion mentioned separately. For readability, the alleles were also named by short numbered symbols, BS and WS indicating the alleles grouped around the haplotypes found in the parasites on graylings in the Baltic Sea and White Sea basins, respectively (Fig. 4).

A minimum-spanning phylogeny of the ADNAM1 alleles (Fig. 3) was constructed without computer programs. The sequential nucleotide substitutions by mutation were unequivocally solved. Three inferred nucleotide reversions were marked by a crooked dotted line and by the underlined nucleotide in the respective haplotype in Fig. 3. Since the genotypes in salmon parasites were observed to be permanent heterozygotes, nucleotide conversion was a more parsimonious explanation for the reversions than back mutation. The genotypes of the observed parasite clones were depicted graphically in Fig. 4 utilizing the ADNAM1 haplotype tree where the alleles present were painted black.

The alleles found in separate parasite clones (Fig. 4) can all be derived from the two basal alleles, as two families centred around BS1 and WS1 haplotypes. The basal allele WS1 (TAGTCAAAT) was observed in the White Sea grayling parasites (Fig. 4), while BS1 (TCATCGTAT) was present in the grayling parasite found in the Pyalma River draining to Lake Onega, and as homozygous, in Radunia River, Poland, and Ounasjoki River, Finland.

The alleles WS1 and BS1 were the only ones shared by grayling and salmon parasites. The divergence between them was four nucleotides (two transitions, two transversions, 4/493 = 0.81%). In the ITS of rDNA, there was only one fixed nucleotide difference (0.08%) separating the White Sea grayling parasites from most other isolates reported (accession nos in Table 1). The alleles BS2, BS3, and WS2 were specific for grayling parasites and were never observed in salmon parasites, and the alleles WS3, and BS4, BS5, BS6, and BS7 were not seen in parasites on grayling.

Table 1 Localities, sea basins, host species and number of specimens (*N*) studied for clones of *Gyrodactylus salaris*. For each clone, the *ADNAM1* phenotype and the constituent haplotypes, the mitochondrial haplotypes, and ITS sequence accession numbers are given. Different *ADNAM1* phenotypes are separated by horizontal line. Host fish abbreviations *Ss* = *Salmo salar*, *Tt* = *Thymallus thymallus*, *Om* = *Oncorhynchus mykiss*, *Sl* = *Salmo letnica*. The countries are abbreviated as FI = Finland, SE = Sweden, NO = Norway, RU = Russia, PL = Poland, MA = Macedonia and the sea basins At = Atlantic, WS = White Sea, Ba = Baltic Sea, Kat = Kattegat (= Swedish west coast, which is marginally Baltic) and Ae = Aegean. Clones marked with asterisk were presented in Ziętara *et al*. (2006)

Locality, country	Sea basin	Host	N	*ADNAM1* phenotype	*ADNAM1* Genotype/alleles	Accession # *ADNAM1* alleles	mDNA haplotype	Accession # mtDNA	Accession # ITS1 5.8S ITS2
Tornio/Nagereatnu, SE	Ba	*Tt*	4	**T1**-ACATCGTAT	BS3-ACATCGTAT	DQ667944	ThyBa09	DQ180333	EF117867
Tornio/Poroeno, FI	Ba		1				ThyBa06	AF540903	EF117868
Iijoki/Soivio, FI	Ba		2				ThyBa08	AY472084	EF117869
Kemijoki, Niippa, FI	Ba		1				ThyBa11	EF612464	EF612463
Onega/Pyalma, RU	Ba	*Tt*	1	**T2**-TMATCGTAT	BS1-TCATCGTAT	DQ667945	ThyBa07	AF540901	EF117870
					BS2-TAATCGTAT	DQ667946			
Vistula/Radunia, PL	Ba	*Tt*	2	**T3**-TCATCGTAT	BS1-TCATCGTAT	EF495064	ThyBa10	EF495063	EF464678
Kemijoki, Niippa, FI	Ba		1				ThyBa11	EF612464	EF612463
Kuito/Penninki, FI	WS	*Tt*	2	**T4**-TAGTCAAAT	WS1-TAGTCAAAT	DQ667947	ThyWs05	AY472085	EF117871
Oulanka/Pikkuköngäs, FI	WS	*Tt*	6	**T5**-TAG(T/-)CAAAT	WS1-TAGTCAAAT	DQ667948	ThyWs03	AF540899	AF484544
Oulanka/Aventojoki, FI	WS		3		WS2-TAG-CAAAT	DQ667949	ThyWs03	DQ993195	EF117872
White Sea/Keret', RU	WS	*Ss*	20	**S1**-TMRTCRWAT	WS1-TAGTCAAAT	DQ468135	SalBa01	AF540891	EF117873
White Sea/Keret', RU	WS		15		BS1-TCATCGTAT	DQ468136	SalBa02	AF540892	EF117874
Onega/Lizhma, RU	Ba		2				SalBa03	AY840222	EF117875
Onega/Kumsha, RU	Ba		2				SalBa01	AY840223	EF117876
Ladoga, Syskynjoki, RU	Ba						SalBa11	EF117889	EF117887
Tornio, FI	Ba	*Ss*	46	**S2**-TMRTCAWAT	WS1-TAGTCAAAT	DQ468129	SalBa04	AF540905	EF117877
Tornio, FI	Ba		23		BS4-TCATCATAT	DQ468130	SalBa05	DQ468128	EF117878
Högvadsån, SE	Kat		3				SalBa10	DQ993194	EF117879
Suseån, SE	Kat		2				SalBa08	DQ993190	
Stensån, SE	Kat		5				SalBa08	DQ993191	
Kuito/Kurzhma, RU*	WS		6				RBT	DQ517533	EF117880
Vefsna, NO	At	*Ss*	6	**S3**-TMRTCATAT	WS3-TAGTCATAT	DQ667950	SalBa07	AF540906	EF117881
					BS4-TCATCATAT	DQ667951			
Genevadsån, SE	Kat	*Ss*	1	**S4**-TMRTYRTAT	WS3-TAGTCATAT	DQ667952	SalBa08	DQ993192	EF117882
Genevadsån, SE	Kat		1		BS5-TCATTGTAT	DQ667953	SalBa09	DQ993193	
Stensån, SE	Kat		2				SalBa08	DQ993191	
Smedjeån, SE	Kat	*Ss*	2	**S5**-TMGTYRWAT	WS1-TAGTCAAAT	DQ667954	SalBa08	AF540904	
					BS7-TCGTTGTAT	DQ667955			
Tornio, FI	Ba	*Ss*	20	**S6**-TMRTYRWAK	WS1-TAGTCAAAT	DQ468131	SalBa05	DQ468128	
					BS6-TCATTGTAG	DQ468132			
Tornio, FI	Ba	*Ss*	4	**S7**-TMRTYRTAK	WS3-TAGTCATAT	DQ468133	SalBa05	DQ468128	EF117883
					BS6-TCATTGTAG	DQ468134			
Kuito/Pista, RU*	WS	*Ss*	13	**S8**-TMRTCRWAT	WS1-TAGTCAAAT	DQ471291	RBT	DQ778628	EF117884
					BS1-TCATCGTAT	DQ471292			
					BS4-TCATCATAT	DQ471293			
Stensån, SE	Kat	*Ss*	9	**S9**-TMRTYRWAT	WS1-TAGTCAAAT	DQ667956	SalBa08	DQ993191	EF117885
					BS4-TCATCATAT	DQ667957			
					BS5-TCATTGTAT	DQ667958			
Raasakka hatchery, FI	Ba	*Ss*	13	**S10**-TAGTCAWAT	WS1-TAGTCAAAT	DQ667959	SalBa06	DQ993189	EF117886
					WS3-TAGTCATAT	DQ667960			
Many fish farms, FI*	Ba	*Om*	237	**O1**-TMRTCRWWT-del23	WS1-TAGTCAAAT	DQ436479	RBT	AF479750	AF328871
					BS4-TCATCATAT	DQ436478			
					BS8-TCATCGTTT	DQ436477			
Fish farm Vardar, MA	Ae	*Sl*	2	**O1**-TMRTCRWWT-del23	WS1-TAGTCAAAT	not submitted	specific	EF570120	EF570119
					BS4-TCATCATAT	separately			
					BS8-TCATCGTTT				

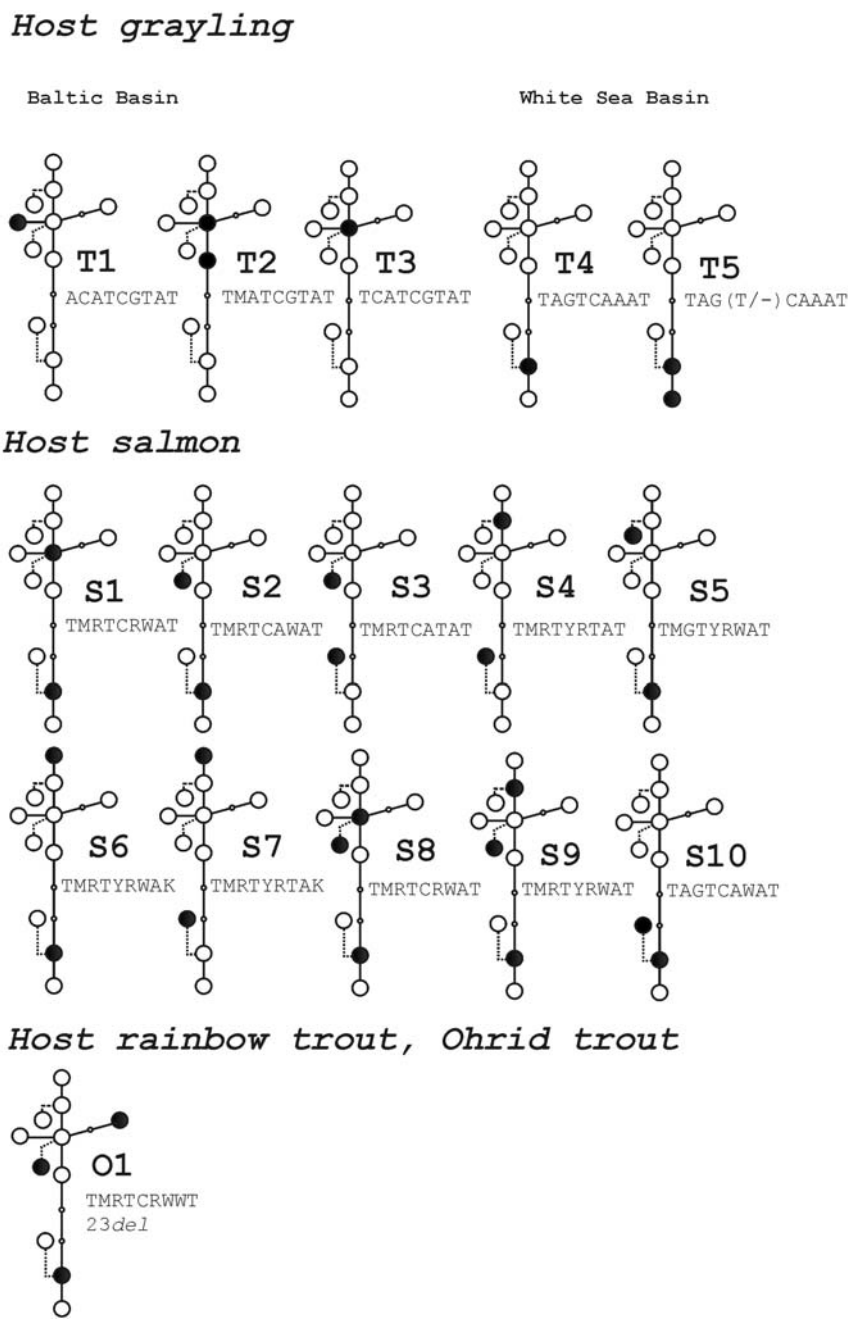

Fig. 4 ADNAM1 genotypes observed in parasites on different hosts. A black dot in the minimum-spanning tree figure (Fig. 3) indicates the observed allele. The names like TMRTCATAT (S3) are considered as phenotypes because they can be read by direct nucleotide sequencing.

ADNAM1 genotypes of the parasite clones

The ADNAM1 sequence 'phenotypes' in Fig. 4 and Table 1 were named following the International Union of Pure and Applied Chemistry (IUPAC) code (and by shorthand T1 via S1 to O1). Heterozygous nucleotides were indicated as follows: C/T = Y, A/G = R, A/T = W, G/T = K, A/C = M. These single nucleotide variants were readable from the direct sequencing. The genotype T5 was heterozygous for a single nucleotide deletion in the middle of the variable segment (T/–), causing a phase shift, and therefore cannot be expressed in a similar way. The 23-bp deletion of the short allele in genotype O1 was indicated as such, without listing the missing nucleotides. However, the phenotype O1 TMRTCRWWT could be obtained by direct sequencing from the 3' end of the marker.

In the course of this study, it came obvious that *Gyrodactylus* studied here is not a very sexual or panmictic species. The Mendelian combinations of the ADNAM1 alleles can be seen globally, while in each local population, only one or few genotypes were observed, and certainly not in Hardy–Weinberg equilibrium. This is a consequence of predominantly parthenogenetic propagation, which also can be concluded to be of a type maintaining heterozygosity.

However, the segregation patterns observed in Fig. 4 were fully compatible with the hypothesis that ADNAM1 is a single-copy nuclear DNA marker, and is inherited in Mendelian way in the sexual episodes.

In Fig. 4, the ADNAM1 genotypes of the parasites are displayed graphically, as ordered according to the hosts: grayling in the Baltic and White Sea basins, salmon, rainbow trout and Ohrid trout. Table 1 and the map in Fig. 1 help to localize each genotype geographically. The genotypes were also displayed as juxtaposed with the mitochondrial phylogenetic hypothesis in Fig. 2. The unidimensional presentation in Fig. 2, because of space limitation, is less readable than the radial phylogeny of ADNAM1 used in Fig. 4.

In all grayling samples from the Baltic Basin, Pyalma on Lake Onega, Radunia in Poland, and the northern rivers Nagereatnu, Poroeno, Ounasjoki and Soivio, the diverse mitochondrial lineages on grayling only contained three ADNAM1 genotypes, T1, T2 and T3. These genotypes comprised three alleles BS1, BS2 and BS3, differing by two steps of single nucleotide substitutions (Fig. 3). BS1 was the most widely distributed, BS2 was only found in Onega and BS3 was northern. Only T2 in Pyalma, Onega was heterozygous. The number of specimens studied is rather small, but the adequacy of sampling was ascertained by the wide geographical coverage: the extant specimens were connected by descent by thousands of individuals evidently carrying the same alleles since their last common ancestor.

In the White Sea basin, the grayling parasite samples were collected from the upper waters of the lakes Kovdozero (Oulanka) and Kuito (Penninki) watersheds (Fig. 1), from localities which were probably colonized during the highest freshwater phase of the White Sea Ice Lake, but have been well isolated several thousand years. Two genotypes (T4 and T5) combining two alleles (WS1 and WS2) were observed. The two alleles differed by a single nucleotide deletion, and all River Oulanka parasites from two tributaries were heterozygous for the deletion of WS2.

The data proved that all *G. salaris* parasites collected from the Baltic salmon populations were heterozygous with respect of ADNAM1 (Fig. 4).

The genotype S1 combining the alleles WS1 and BS1, which are central in the bipolar tree in Fig. 3, was found in the eastern salmon populations in the lakes Onega (Kumsha, Lizhma) and Ladoga (Syskynjoki), on three different mtDNA types (Fig. 2). The same nuclear genotype S1 was also found in the Keret' River on the two different mitochondrial types accidentally introduced from Lake Onega. The mitochondrial haplotypes in the two rivers of Onega differed from each other by 16 nucleotides along 1600 bp (Kimura's 2-parameter distance K2P = 1%).

The genotype S2, differing from S1 by one converted nucleotide in the allele BS4 was the most widely distributed among the Baltic salmon populations. It was recorded on two different mitochondrial haplotypes in the northern Tornio River and in several rivers in Swedish westcoast, also on two mtDNA types. The salmon parasite in the river Kurzhma, in Lake Kuito in Russian Karelia also had the genotype S2, while it was maternally derived from the rainbow trout-specific clone.

The two alleles of S2 (BS4 and WS1) were also present in all apparently triploid clones. The clone S8 was found in river Pista, Lake Kuito. This clone was the maternal half sister of S2 in river Kurzhma, but it had the BS1 allele, which was otherwise present only in Onega and Ladoga salmon parasites and on Baltic grayling (Ziętara *et al*. 2006). The clone S9 in Swedish river Stensån lived together with S2 and S4, all of them having identical mtDNA haplotype (Fig. 2). The possibility that S9 represented a mother-and-child union (S2 mother mated with S4) and not triploidy cannot be exluded in this specific case, because all *Gyrodactylus* species are viviparous. Nine specimens of S9 were observed.

In the northern Baltic Tornio River, all *G. salaris* individuals were also heterozygous, showing three different genotypes: S6, S7, or S2, on two mitochondrial types which differed by 10 nucleotides along 1600 bp. Together, these two markers defined four clones of parasites. (In the map in Fig. 1, there are only few dots along the Tornio River, for clarity, but *G. salaris* samples were taken from 12 rapids, along 470 km of the river). The clones SalBa04-S2 ($N = 46$), and SalBa05-S2 ($N = 28$) existed together in the same three neighbouring upstream rapids. The clones SalBa05-S6 ($N = 20$) and SalBa05-S7 ($N = 4$) were separated, the latter one found in only one weakly infected rapid.

In the Norwegian Vefsna River, the phenotype of *G. salaris* was a unique S3. As identified by the mitochondrial sequence, this sample represented the main Norwegian epidemic of *G. salaris* (Hansen *et al*. 2003). The exact provenance of this clone was not yet found in the Baltic Basin.

The last addition to the phenotype/genotype collection of parasites on Baltic salmon was a clone found in a salmon hatchery (Raasakka, the river Iijoki, Finland), which was an unusual combination of two alleles from the WS group, S10. It is to be noted that the WS3 allele was a gene conversion product, reverted in a heterozygous WS/BS salmon parasite. Actually, WS1/WS3 was an expected segregation product from the cross between genotypes S6 and S7, which were present in clones found geographically closest to the Raasakka hatchery in the Tornionjoki River. However, the hatchery clone had its own, but salmon parasite-specific type of mtDNA (Fig. 2)

O1 was the only genotype found in a large sample of Finnish rainbow trout farm parasites which also have a specific mitochondrial haplotype (RBT clone). O1 carried a strange short allele BS8 of the Baltic family and a divergent mitohondrial type. As a surprise, the short BS8 allele on O1 phenotype was also observed in a parasite from Ohrid

trout (*S. letnica*) from Macedonia. Even more surprising was that this clone had a 1.3% divergent mtDNA haplotype belonging to the same clade than the RBT clone, which earlier had been monotypic (Fig. 2). It also had four heterozygous nucleotides in the ITS, which will be further analysed in another study. The fact that the alleles WS1 and salmon specific BS4 are found in these aberrant clones and in the progeny of the RBT clone in Lake Kuito confirmed that O1 genotypes are secondary hybrids, derived paternally from Baltic salmon parasites.

Discussion

Monoclonal hybrid origin of Baltic salmon-specific Gyrodactylus salaris

The phylogenetic and phylogeographical analysis of *Gyrodactylus salaris* by mitochondrial sequencing (Meinilä *et al*. 2002, 2004; Hansen *et al*. 2003) distinguished six sister clades in the *G. salaris* cluster. Four of the mitochondrial clades were specific to grayling. Recently, Hansen *et al*. (2007) increased the number of grayling specific mtDNA clades to eight, by including new samples from England, Poland and Slovakia. In the mitochondrial phylogeny based on a ~800-bp sequence, both salmon-infecting clades were still separate sister groups among the grayling-specific clades. A very basal haplotype was observed on salmon in Göta River, Sweden, not belonging to any other groups. Here, with a longer sequence of 1600 bp, the clades found on salmon are still two sister lineages separated from the three lineages on grayling, showing that they have a different matrilinear descent.

On the basis of the phylogeography of the different clades and strains in the previously glaciated areas of Northern Europe, Meinilä *et al*. (2004) suggested that the *G. salaris* cluster was undergoing the initial steps of a speciation-like evolution, simultaneously by two means. First, the five grayling clades were differentiating during and after an apparent glacial diaspora on the plesiomorphic (phylogenetically ancestral) host, and second, the Baltic salmon-specific clade lived in sympatry with the Baltic clade on grayling, as a consequence of the hypothetical *irreversible* host switch from grayling to salmon. The switch was suggested to have occurred when the predecessors of Baltic salmon populations were isolated in an eastern freshwater glacial refugium, situated at the edge of the continental ice cap somewhere in present Russia (Phylogeography of Salmon, Nilsson *et al*. 2001; Asplund *et al*. 2004; Säisä *et al*. 2005). This suggested scheme was still lacking the explanation for the host switch: the hybrid origin of the salmon-specific clade which was demonstrated here.

By using the nuclear DNA marker sequence ADNAM1, we here confirmed that the Baltic salmon-specific *G. salaris* is indeed monophyletic. As a summary of the results, most of the Baltic salmon parasite clones, as well as the harmful secondary introductions to the White Sea (Keret') and Atlantic Norway (Vefsna) showed two alleles originating from the White Sea and Baltic Basin graylings. The apparent triploids were also WS/BS heterozygotes. Together with the monophyletic mtDNA clade, this demonstrated that the Baltic salmon-specific lineage originated as monoclonal, from a single hybridization event of two grayling-specific strains. The alleles which were permanently heterozygous in the salmon parasites were found in the extant descendants of the tentative parental grayling parasites, which at present live geographically isolated on opposite sides of the Baltic and White Sea divide.

The two divergent ADNAM1 alleles combined in this single hybridization event were still maintained as heterozygous in all but one of the extant Baltic salmon-specific parasite clones. The suggested original alleles BS1 and WS1 have accumulated minor modifications by mutation: two single nucleotide substitutions per 493 bp in the BS group (Fig. 3) and at least three events of single nucleotide gene conversions. The number of nuclear changes is much less than the observed 31 variable sites in the 1600 bp in the mitochondria of the clade. The amount of accumulated nuclear and mitochondrial variation is more than could be expected if the hybridization was postglacial.

On the basis of palynological analysis of sediments and comparative studies on molluscan and crustacean fossils, Funder *et al*. (2002) suggested that the White Sea and the Baltic Sea were briefly (< 3000 years) connected during the early Eemian interglacial period approximately 132–130 000 years ago. Two narrow straits crossed the present divide north of Lake Onega, evident as brackish water fauna introduced into the Baltic basin in the northern parts of the Lake Onega. This event probably is the most reasonable calibration point for the origin of the salmon-specific lineage of *G. salaris,* and it allows estimation of the substitution rate of different DNA sequences. The maximum genetic divergence (Kimura's 2-parameter distance) of the mtDNA of salmon-specific parasites reported in GenBank was estimated to be 1.7%, between haplotypes from Swedish Göta and Ätran rivers). The Göta sequence was very basal, but was not included in the tree in Fig. 2, because the ADNAM1 genotype was not available, and therefore, it was not known, whether it belongs to the same hybrid clade. If it does, this divergence value leads to an upper limit estimate of divergence rate of mtDNA 13.1% per million years. The genetic distance of the two 1600-bp long haplotypes found in Lake Onega was 1.0%; if their divergence started just after the fusion of parents 132 000 years ago, the estimated rate is 7.6% per million years. This is certainly the lower boundary of the divergence rate. By using these substitution rates, the maximum divergence 3.1% between the main grayling and salmon-specific clades transforms to 400 000–240 000 years.

The parasite lineage found on rainbow trout in farms is a secondary hybrid

The other mtDNA clade found in parasites commonly named as *G. salaris* has been observed in parasite strains in geographically distant salmon populations in Oslo Fjord, Norway (Hansen *et al*. 2003), and Lake Kuito, Russian Karelia (Meinilä *et al*. 2004). This mtDNA was also found on Arctic charr (*Salvelinus alpinus*) in lake Pålsbufjorden, Norway (Robertsen *et al*. 2006; Olstad *et al*. 2007). Most commonly this mtDNA clade was observed in Finnish rainbow trout farms, where it was present always as ADNAM1 genotype O1, which was an apparently triploid, nonsegregating clone (Ziętara *et al*. 2006).

The relations of rainbow trout parasites (RBT clone) and the parasites on Kuito salmon were explored by Ziętara *et al*. (2006), who suggested that the RBT clone had been sexual as a female, when meeting another parasite clone in the Lake Kuito (or in some intermediary fish farm), and had mothered the two different clones found in separate spawning rivers in Kuito (ADNAM1 genotypes S2 and S8). We want to point that the recombination again coincided with the host switch, this time back to salmon. In the river Tornio and elsewhere in the Baltic basin, the RBT clone was never observed on salmon, in spite of recurrent stocking and escaping of rainbow trout in this area.

In previous reports, the mitochondrial DNA found in RBT clone was monotypic in all other hosts and localities as well, suggesting recent monoclonal origin. The Baltic salmon-specific strain was suggested to be the paternal parent, and it was hypothetized that the maternal parent was also supplying the hybrid with the short ADNAM1 allele BS8, which was not found in any other parasite strains (Ziętara *et al*. 2006). In the present study, the same ADNAM1 short allele BS8 was found also in parasites on Ohrid trout (*Salmo letnica*), but as a surprise, on a slightly different mtDNA. Using the above calibration, the mtDNA of parasites on *S. letnica* have diverged from the RBT clone some 170 000 years ago. The evolutionary history of this strange combination will be explored elsewhere, together with a description of some other Polish and Macedonian *G. salaris* strains from fish farms (Rokicka *et al*. 2007).

Homoploid hybrid speciation: a convenient alternative for hemiclonal organism

The regular presence of penis and sperm in *Gyrodactylus* specimens has prevented considering the species as largely asexual (Bakke *et al*. 2007). This study is the first to use a variable nuclear gene to show how limited their sexuality is, a fact already suggested by Harris (1998) when studying a related *Gyrodactylus gasterostei*. The life cycle of *Gyrodactylus* is best described as hemiclonal, and the sexuality seems not to be regular or cyclic (Bell 1982; D'Souza *et al*. 2003). This hypothesis was consistent with the observations in this study.

Homoploid hybrid speciation without chromosomal change is not a common or well-known alternative explaining adaptive radiation, and there are a handful of animal examples, however, on normally bisexual organisms (Coyne & Orr 2004; Seehausen 2004; Schwarz *et al*. 2005; Mavarez *et al*. 2006; Meyer *et al*. 2006). Essential in this hypothetical scheme is an empty, productive or marginal niche, which will be utilized by a hybrid between existing species or lineages, which as adapted specialists stay in their own niches (Buerkle *et al*. 2000). For herbivores, invasive plants may represent the ecological opportunities for switching (Schwarz *et al*. 2005). Stocked or farmed alien fish certainly may appear as a resource for a parasite. Modern human-aided trafficking of plants, animals and people causes numerous invasions and colonizations both for hosts and parasites (Taraschewski 2006). The episodes of faunal displacement during geological cycles may have created many opportunities by shuffling consumers and resources. In the present hypothesis, the salmon of the Baltic Sea was the underexploited resource, utilized by a genetically novel parasite created by recombination of genomes of two grayling specific strains of *G. salaris*.

Interestingly, the Baltic salmon superclone of *G. salaris* described here is the only *Gyrodactylus* parasite specific for Baltic salmon. Either, the niche really was empty, or the new superior parasite outcompeted other *Gyrodactylus* species capable of infecting salmon. Many other fish species carry several *Gyrodactylus* species, even in the same sea basin, or sympatrically (Ziętara & Lumme 2004).

Balanced lethal system, strong heterosis, or epistatic/ pleiotropic host recognition?

All Baltic salmon parasites observed were heterozygous; most of the clones carrying WS/BS alleles, and only one clone (S10) had two WS alleles. Also, the polymorphic clones (where detection of heterozygosity was possible) found in grayling (T2 and T5) contained only heterozygous individuals. This shows that the reproductive system, automictic parthenogenesis (Cable & Harris 2002) must be of a type maintaining heterozygosity. The cytology has not yet been studied, but there are some alternatives for such a system, for example, the centric fusion of the products of first meiotic division, without recombination by crossing over (Suomalainen *et al*. 1987).

Shuffling of the nuclear alleles among the mitochondrially differentiated parasite clones was evident (Fig. 2). In fact, in the total material, almost all Mendelian combinations of the nuclear alleles were seen, while not in locally segregating Mendelian proportions. Therefore, it was obvious that the parasite strains express sexuality every now and

then. However, the only deviation from the obligatory WS/BS heterozygosity in the Baltic salmon-specific clade was the unusual genotypic combination S10 of two alleles WS1/WS3 in a clone found in a salmon hatchery in Raasakka. If the clones have ordinary sex at all, then also combinations carrying only WS or BS alleles are necessarily produced. Because they are rarely observed, we conclude that the homozygotes are not fit enough to survive in the wild. The infection by the S10 clone in the Raasakka salmon hatchery was the first case observed in farms in many years, while all salmon hatcheries in northern Finland have been monitored regularly since 1984 (Rintamäki-Kinnunen & Valtonen 1996).

The observed obligatory heterozygosity may be maintained if both marker alleles are linked with lethal genes. Without crossing over, the marker and the lethal factor only need to be on the same chromosome.

Very strong overdominance or epistatic interactions could have similar consequences and could be modelled as a balanced lethal system as well. If a heterozygous gene combination is definitive for the correct host recognition, then segregation is a risk. Every attempt to recombine, by selfing (geitonogamy, sex within a clone, Reusch 2001), or by outcrossing between the clones, leads to a segregation load of one-half, because half of the progeny are expected to be homozygous for lethals, or not able to find the correct host.

A *Gyrodactylus* female may give birth to only three to four first degree progeny during her lifetime because of the high cost of hyperviviparity: she carries the daughters until they are full grown and already pregnant with the next embryo (Cable & Harris 2002; Bakke *et al*. 2007). At least the first two or three daughters are supposedly produced asexually or parthenogenetically. Risking the homozygosity of the last of three or four embryos by sexual intercourse means the loss of lifetime fitness by a value of 0.167–0.125, which in the long run is enough to select against sexual affairs, at least among clones recognized as 'self'.

Thus, the balanced-lethal hypothesis may well explain the obvious reluctance of the clones to participate in sex. In an influential paper titled *What's Wrong with a Little Sex*, Peck & Waxman (2000) demonstrated that asexual clonal growth can lead to large ecological advantage (in the case of *G. salaris*, to the utilization of a new, naive host). Maximum utilization of overdominance or unique epistatic interactions between alleles is only possible when recombination is suppressed. If the frequency of sexuality is pressed below a certain threshold, it eventually becomes disadvantageous. In the case of *G. salaris*, heterosis may be expressed as a specific adaptation or just as orientation to the salmon host, and thus the successful hybrid clone can no longer segregate anymore without being lost (Archetti 2004).

Acknowledgements

Dr Päivi Rintamäki found the unusual *Gyrodactylus salaris* sample from the salmon hatchery, and Dr Göran Malmberg transmitted the parasites from the Swedish west coast. Magdalena Rokicka collected the Radunia parasites, and together with Dr S. Stojanovski, the Ohrid trout parasites. The key parasites from Lake Onega and Lake Ladoga were collected by authors together with Alexei Veselov. Pasi Anttila submitted the two worms from Ounasjoki, Niippa. Professor Outi Savolainen suggested important clarifications to the text. The work has been supported by the Finnish Academy and the Ministry of Forestry and Agriculture, as well as the Oulanka Biological Station of Oulu University.

References

Archetti M (2004) Recombination and loss of complementation: a more than twofold cost for parthenogenesis. *Journal of Evolutionary Biology*, **17**, 1084–1097.

Asplund T, Veselov A, Primmer C *et al*. (2004) Geographical structure and postglacial history of mtDNA haplotype variation in Atlantic salmon (*Salmo salar* L.) among rivers of the White and Barents Sea basins. *Annales Zoologici Fennici*, **44**, 465–475.

Bakke TA, Harris PD, Hansen H, Cable J, Hansen LP (2004) Susceptibility of Baltic and East Atlantic salmon *Salmo salar* stocks to *Gyrodactylus salaris* (Monogenea). *Diseases of Aquatic Organisms*, **58**, 171–177.

Bakke TA, Cable J, Harris PD (2007) The biology of gyrodactylid monogeneans: The 'Russian-Doll killers'. *Advances in Parasitology*, **64**, 161–377.

Bell G (1982) *The Masterpiece of Nature*. *The Evolution and Genetics of Sexuality*. University of California Press, Berkeley, California.

Buerkle CA, Morris RJ, Asmussen MA, Rieseberg LH (2000) The likelihood of homoploid hybrid speciation. *Heredity*, **84**, 441–451.

Cable J, Harris PD (2002) Gyrodactylid developmental biology, historical review, current status and future trends. *International Journal for Parasitology*, **32**, 255–280.

Collins C, Miller KA, Cunningham CO (2004) Characterization of a beta-tubulin gene from the monogenean parasite, *Gyrodactylus Salaris* Malmberg, 1957. *Parasitology Research*, **92**, 390–399.

Coyne JA, Orr HA (2004) *Speciation*. Sinauer & Associates, Sunderland, Massachusetts.

Cunningham CO (1997) Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. *Journal of Parasitology*, **83**, 215–219.

D'Souza TG, Storhas M, Schulenburg H, Beukeboom LW, Michiels NK (2003) Occasional sex in an 'asexual' polyploid hermaphrodite. *Proceedings of the Royal Society B: Biological Sciences*, **271**, 1001–1007.

Dieckmann U, Doebeli M, Metz JAJ, Tautz D, eds (2004) *Adaptive Speciation*. Cambridge University Press, Cambridge, UK.

Funder S, Demidov I, Yelovicheva Y (2002) Hydrography and mollusc faunas of the Baltic and the White Sea-North Sea seaway in the Eemian. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **184**, 275–304.

Hansen H, Bachmann L, Bakke TA (2003) Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling and rainbow trout in Norway and Sweden. *International Journal for Parasitology*, **33**, 1471–1478.

Hansen H, Martinsen L, Bakke TA, Bachman L (2006) The incongruence of nuclear and mitochondrial DNA variation supports conspecificity of the monogenean parasites *Gyrodactylus salaris* and *G. thymalli*. *Parasitology*, **133**, 639–650.

Hansen H, Bakke TA, Bachmann L (2007) Mitochondrial haplotype diversity of *Gyrodactylus thymalli* (Paltyhelminthes; Monogenea): extended geographic sampling in United Kingdom, Poland and Norway reveals further lineages. *Parasitology Research*, **100**, 1389–1394.

Harris PD (1998) Ecological and genetic evidence for clonal reproduction in *Gyrodactylus gasterostei* Gläser, 1974. *International Journal for Parasitology*, **28**, 1595–1607.

Huyse T, Plaisance L, Webster BL *et al*. (2007) The mitochondrial genome of *Gyrodactylus salaris* (Platyhelminthes: Monogenea), a pathogen of Atlantic salmon (*Salmo salar*). *Parasitology*, **134**, 736–747.

Kearney M, Shine R (2004) Morphological and physiological correlates of hybrid parthenogenesis. *American Naturalist*, **164**, 803–813.

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

Malmberg G (1956) Om förekomsten av *Gyrodactylus* på svenska fiskar. *Skrifter utgivna av Södra Sveriges Fiskeriförening, Årskrift*, **1956**, 19–76.

Mavárez J, Salazar CA, Bermingham E, Salcedo C, Jiggins CD, Linares M (2006) Speciation by hybridization in *Heliconius* butterflies. *Nature*, **441**, 868–871.

Meinilä M, Kuusela J, Ziętara MS, Lumme J (2002) Primers for amplifying ~820 bp of highly polymorphic mitochondrial COI gene of *Gyrodactylus salaris*. *Hereditas*, **137**, 72–74.

Meinilä M, Kuusela J, Ziętara MS, Lumme J (2004) Initial steps of speciation by geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea: Gyrodactylidae). *International Journal for Parasitology*, **34**, 515–526.

Meyer A, Salzburger W, Schartl M (2006) Sexual selection driven hybrid origin of a new species of swordtail (Teleostei: *Xiphophorus*). *Molecular Ecology*, **15**, 721–730.

Nilsson J, Gross R, Asplund T *et al*. (2001) Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonization of the Baltic Sea area. *Molecular Ecology*, **10**, 89–102.

OIE (2003) *Manual of Diagnostic Tests for Aquatic Animals*, 4th edn. Published by The Office International des Epizooties, Paris.

Olstad K, Robertsen G, Bachmann L, Bakke TA (2007) Variation in host preference within *Gyrodactylus salaris* (Monogenea): and experimental approach. *Parasitology*, **134**, 589–597.

Peck JR, Waxman D (2000) What's wrong with a little sex? *Journal of Evolutionary Biology*, **13**, 63–69.

Reusch TBH (2001) Fitness-consequences of geitonogamous selfing in a clonal marine angiosperm (*Zostera marina*). *Journal of Evolutionary Biology*, **14**, 129–138.

Rintamäki-Kinnunen P, Valtonen ET (1996) Finnish salmon resistant to *Gyrodactylus salaris*: a long-term study at fish farms. *International Journal for Parasitology*, **26**, 723–732.

Robertsen G, Hansen H, Bachman L, Bakke TA (2007) Arctic charr (*Salvelinus alpinus*) is a suitable host for *Gyrodactylus salaris* (Monogenea, Gyrodactylidae) in Norway. *Parasitology*, **134**, 257–267.

Rokicka M, Lumme J, Ziętara MS (2007) Identification of *Gyrodactylus* ectoparasites in Polish salmonid farms by PCR-RFLP of the nuclear ITS segment of ribosomal DNA (Monogenea: Gyrodactylidae). *Acta Parasitologica*, **52**, 185–195.

Säisä M, Koljonen M-L, Gross R *et al*. (2005) Population genetic structure and postglacial colonization of Atlantic salmon (*Salmo salar*) in the Baltic Sea area based on microsatellite DNA variation. *Canadian Journal for Fisheries and Aquatic Sciences*, **62**, 1887–1904.

Schwarz D, Matta BM, Shakir-Botteri NL, McPheron BA (2005) Host shift to an invasive plant triggers rapid animal hybrid speciation. *Nature*, **436**, 546–549.

Seehausen O (2004) Hybridization and adaptive radiation. *Trends in Ecology & Evolution*, **19**, 198–207.

Suomalainen E, Saura A, Lokki. J (1987) *Cytology and Evolution in Parthenogenesis*. CRC Press, Boca Raton, Florida.

Taraschewski H (2006) Hosts and parasites as aliens. *Journal of Helminthology*, **80**, 99–128.

Ziętara MS, Lumme J (2002) Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea: Gyrodactylidae). *Evolution*, **56**, 2445–2458.

Ziętara MS, Lumme J (2004) Comparison of molecular phylogeny and morphological systematics in fish parasite genus *Gyrodactylus* Nordmann, 1832 (Monogenea, Gyrodactylidae). *Zoologica Poloniae*, **49**, 5–28.

Ziętara MS, Kuusela J, Lumme J (2006) Escape from an evolutionary dead-end: a triploid clone of *Gyrodactylus salaris* is able to revert to sex and switch host (Platyhelminthes, Monogenea, Gyrodactylidae). *Hereditas*, **143**, 86–92.

Žitňan R (1960) *Gyrodactylus thymalli* sp. nov. aus den Flossen der Äsche (*Thymallus thymallus*). *Helminthologia*, **2**, 266–269.

Jussi Kuusela is interested in fish and fishing in general, and he completed this work as a part of his PhD thesis in University of Oulu. Marek Ziętara is a biochemist by training, and worked as a post doc researcher first in Leuven, Belgium, and during this project in Oulu, but is back in Gdańsk now. Jaakko Lumme took part in this great parasitological adventure from a background of *Drosophila* research.
