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Biodiversity at the population genetic level: microsatellite DNA poplymorphism in the sea trout populations from southern Baltic

by

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ABSTRACT

An important component of biodiversity is a diversity between species and populations at the genetic level. Biodiversity conservation of species should include conservation of population genetic structures. In recent years, studies of microsatellite DNA polymorphism have become an exceptionally useful tool for identification and characterization of genetic diversity and differentiation within and among fish populations.

Brown trout (*Salmo trutta*) is an ecologically important and fishery (both sport and commercially) valuable species. Its migrating form: sea trout is dominant in the southern Baltic. In Poland, almost 90% of trouts are descendent from artificial enhancing of natural populations by stocking. Therefore, the genetic consequences of stocking on natural populations can be strong. Results of studies on genetic differentiation of the sea trout populations from six Polish rivers: Rega, Parsęta, Wieprza, Vistula, Słupia and Drwęca are presented in this paper. Five microsatellites *Ssa85*, *Ssa171*, *Ssa197*, *Str15*, *Str73* were studied. The presented results can be useful for future management of genetic resources of the sea trout in Poland.

INTRODUCTION

Most research related to problems of biodiversity protection deal with biodiversity at the ecological level. Changes in ecosystems and species composition as well as their geographic ranges in connection with environmental factors, including human activities have been studied extensively. Little attantion has been paid to a necessity of protection of genetic biodiversity. In other words, a necessity of protection and maintanance of genetic polymorphism and differenciation within and between populations. Genetic polymorphism of a species depends mainly on number and frequencies of alleles in its populations. Different alleles at the coding sequences in the genome can differ in their function in organisms, what is indicated by variation of biochemical properties of their products. Therefore, such genetic polymorphism is particularly important from the standpoint of adaptation capabilities and survival of a species. Destruction of the allelic polymorphism in populations as a result of human activities can cause lower potential for survival and even a total extinction of a species as a consequence. It is to remember, that the genetic polymorphism of a species in its adaptive component is a product of the evolutionary processes acting usually for long periods of time. Once it is reduced by human activities, its quick restitution is not possible.

It is important to develop proper techniques for identification of significant proportion of various alleles present in a species. Screening genetic polymorphism in populations through sequencing of large fragments of genome, theoretically proper, has got serious practical drawbacks: economical and practical. Most base pairs would not be changed in different

specimens. However, a quick identification of vast polymorphism located in selected regions of the genome is possible. Best example of such regions are VNTR (Variable Number of Tandem Repeat), including microsatellite DNA. Microsatellite loci may be involved in regulatory processes (Kashi and Soller, 1999).

Microsatellite polymorphism is a particularly useful tool in studies of sea fish species due to lower level of population structuring in comparison with fresh water fishes. Analysis of microsatellite alleles in fish populations can be used for studies and assesment of changes in populations resulted from management (Hansen et al. in press). Particularly interesting examples here can be exploited species as salmonids. Brown trout, and especially its migrating form sea trout (*Salmo trutta* m. *trutta*) populations in many countries in Europe have been intensly stocked.

In Poland catchments of sea trout exceed 300 tonns a year (Wenne et al., 2000). The intensive enhancment program has been developed in order to compensate for low natural reproduction. In recent years, 1.5 milion smolts and up to 5 milion alevins are released every year (Bartel, 1997). Changes in environmental conditions and especially dam contruction has limited natural reproduction of sea trout in Polish rivers down to 10% of the number 100 years ago. To decrease mixing of populations through the stocking program, to each river can be released only smolts originating from gametes collected from spawners caught in the same river. However, more than twenty years ago, some mixing between populations has occurred. Addtionally, in spite of strong homing some spawners strand and do not return to their natal rivers.

The main aim of this study is to compare populations of sea trout from six Polish rivers at the genetic level with the implications for the program of restitution of the Vistula river original population.

MATERIAL AND METHODS

Samples of sea trout spawners were collected from six Polish rivers (40 individuals for each river) in 1996. A sample from the Vistula River was collected in September, whereas samples from Pomeranian rivers (Słupia, Wieprza, Parsęta and Rega) and Drwęca were collected from October to December. Figure 1 shows location of these rivers on the Polish sea coast. Almost

all collection sites were situated a few km (2-38) up the river where fish were caught in traps. Only Vistula spawners were caught by commercial fishermen close to the river mouth. Trouts from the Parsęta River were cought by electrofishing at the location 40 km up the river mouth. All studied trouts belonged to year classes 2+ and 3+. Tail fin clippings were collected and preserved in 95% ethanol and kept at a 4°C until the DNA extraction was performed. The total genomic DNA was extracted using „Genomic DNA Prep Plus" kit (A & A Biotechnology, Gdynia, Poland). One tetranucleotide (*Ssa197*) and four dinucleotide (*Ssa171*, *Ssa85*, *Str15*, *Str73*) microsatellite loci were analyzed. The primer sequences were published by O' Reilly et al. (1996) and Estoup et al. (1993) (Gen Bank accession number U 43694, U 43693, U 43692, AB 001058, AB 001056 respectively). PCR annealing temperature, $MgCl_2$ and primer concentration are presented in Table 1. Concentration of Taq polymerase (0,4U/20µl Promega - Catalog# M 1861), nucleotides (0,2 mM) and termal profile for PCR (94°C 3 min., [94°C 30 sec, X°C* 30 sec, 72°C 30 sec] × 30, 94°C 30 sec, X°C 30 sec, 72°C 5 min.) were the same for all loci. PCR was followed by electrophoresis of products in 6% polyacrylamide with 3M urea denaturing gels (1,5µl PCR product/1,5µl formamide). Glass plate 40 cm × 33 cm with 0,33 mm thin was used. DNA fragments were visualized by silver staining. Silver staining conditions were as follows. Sticking gel was washed in 2 litter of solutions:

1. 10% acetic acid (15 min.)
2. Ultrapure water × 3 (5 min.)
3. Silver solution (30 min.) [3 g $AgNO_3$/ 2 l ; 3 ml formaldehyde]
4. Ultrapure water (30 sec)
5. Sodium carbonate anhydrous solution [15 g Na_2CO_3/2 l] (3-5 min.)

The microsatellites used in this study were recommeneded for population studies of sea trout within the EU „TROUTCONCERT" (FAIR CT 97-3882) project. The technique we used for microsatellite sudies was compared with a few other labs in Europe, in which various authomatic sequencers were used. The intercallibration was done within the „TROUTCONCERT" last year.

Arlequin program ver. 2.0 was used for statistical analises of the microsatellite data (Schneider et al. 2000).

* X°C - annealing temterature

RESULTS

Number of alleles was different in the five studied loci. 5 and 4 alleles were observed for loci *Ssa85* and *Str73*, and *Str15* respectively, 11 for *Ssa197* and 19 *Ssa171* 11 (Table 2). However differences in number of alleles for each locus between populations from particular rivers were moderate. Only for the *Ssa197* locus stronger variation was observed: 11 alleles in Vistula River, 10 in Drwęca, 9 in Rega and Parsęta, and 8 in Wieprza and Słupia. Two private alleles were observed. One was found at *Ssa171* (263 allele size) locus and second at *Str15* (231 allele size) locus in Rega population (Table 3). Population pairwise comparisons of F_{st} based on number of different composite alleles confirmed significance of differences only between Wieprza and Vistula rivers. Frequencies of alleles for 5 loci are presented in Table 3. Generally, differences in the frequencies of particular alleles among six rivers were not pronnounced, especially for *Ssa85*, *Str73* and *Str15*. Stronger differences occurred at *Ssa171* locus. The frequency of the 231 allele in the Vistula population was 0.1875, and 0.125 in Drwęca River population, whereas for other populations it was just a rare allele (Słupia and Rega) and was absent from the Wieprza and Parsęta populations. Population pairwise of comparisons of F_{st} based on conventional F-statistics from composite allele frequencies confirmed the statistically significant differences between Drwęca and the 3 other rivers: Wieprza, Słupia and Rega (Table 4). Out of 30 tests of Hardy-Weinberg equilibrium, 7 tests revealed significant deviations. For 4 cases it was a small deviation ($p < 0.05$) but in the 3 others significance was at the 0.01% (for 2 tests) and one at 0.001% level (Table 2). Almost all deviations from Hardy-Weinberg equilibrium (except for population from the Vistula River for *Str73* locus) showed a heterozygote deficiencies. Moreover, the 3 cases were concerned with the most variable microsatellite *Ssa171* for Słupia, Drwęca and Rega rivers. In pairwise population comparison of F_{st} based on frequecies of alleles significant differentiation between the Drwęca population and three Pomeranian populations from Rega, Wieprza and Słupia rivers.

DISCUSION

Drwęca is a tributary of the Vistula River. Sea trout population from the lover Vistula were separated from the upper Vistula populations and the Drwęca population by Włocławek dam. Migration of sea trout up the Vistula has been almost totally stopped by the dam. The lack of differences between the Vistula sea trout population and the Pomeranian population should be particularly stressed here. It confirms the presence of the population trout in the Vistula due to gene flow. Two seasons can explain lack of differentiation between Pomeranian populations and the Vistula population: stranding of Pomeranian trouts and stocking programe. Over tventy years ago smolts originated from the Pomeranian population of spawners were used for enhancement of the Vistula population (Bartel 1993). Additionally, present stocking does not exclude Pomeranian trouts from hatchery performed artificial reproduction.
The presented results of population analysis indicate that the original population from the Vistula River might be still represented as the isolated population in Drwęca River. Therefore, in the restitution program of the original Vistula population, gametes from the Drwęca population can be used. However, before any decisions are taken, studies of old archival scals performed by Prof. M. Łuczyński (Warmian-Mazurian University, Olsztyn) and colleagues should be completed. On the other hand, it can not be excluded that spawners entering the Vistula River in winter can in fact represent to higher degree the original population. Studies of temporal changes in genetic composition of Vistula River sea trout population are in progress.

Conclusions:

Microsatellite DNA can be used as a tool in studies related to improvement of population enhancement programes and therefore can be related to biodiversity protection of populations of sea trout under strong pressure from exploitation.

A recomendation from ICES is needed that studies of biodiversity of populations at the genetic level can be as useful for biodiversity protection as studies at ecosystem and species level.

REFERENCES

Bartel, R. 1993. Present situation of the Vistula sea trout. Arch. Ryb. Pol. 1:101-111

Bartel, R. 1997. Wymagania jakościowe w produkcji smoltów. XXII Krajowa Konferencja Hodowców Ryb Łososiowatych. Łeba, 1997: 39-52

Estoup, A., Presa, P., Krieg, F., Vaiman, D. and Guyomard, R. $(CT)_n$ and $(GT)_n$ microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity 71 1993: 488-496

Hansen, M. M., Ruzzante, E., Nielsen, E. E. and Mensberg, K-L. D. Brown trout (*Salmo trutta*) stocking impact assessment using microsatellite DNA markers. Ecological Applications (in press).

Kashi, Y., Soller, M. 1999. Functional roles of microsatellites and minisatellites. In: Microsatellites. Goldstein D. Band Schlöttwer C. (eds) Oxford University Press, Oxford, pp. 10-23

O' Reilly, P., Hamilton, L. C., McConnell, S. K. and Wright, J. M. 1996. Rapid detection of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences 53: 2292-2298.

Schneider, S., Roessil, D. and Excoffier, L. 2000. Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Wenne, R., Łuczyński, M. and Bartel, R. 2000. Ocena możliwości odróżnienia populacji troci wędrownej w Polsce metodami genetycznymi. Studia i Materiały MIR. Ser B (in press)

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Figure 1. Map of northern Poland with marked rivers which samples of sea trout were collected.

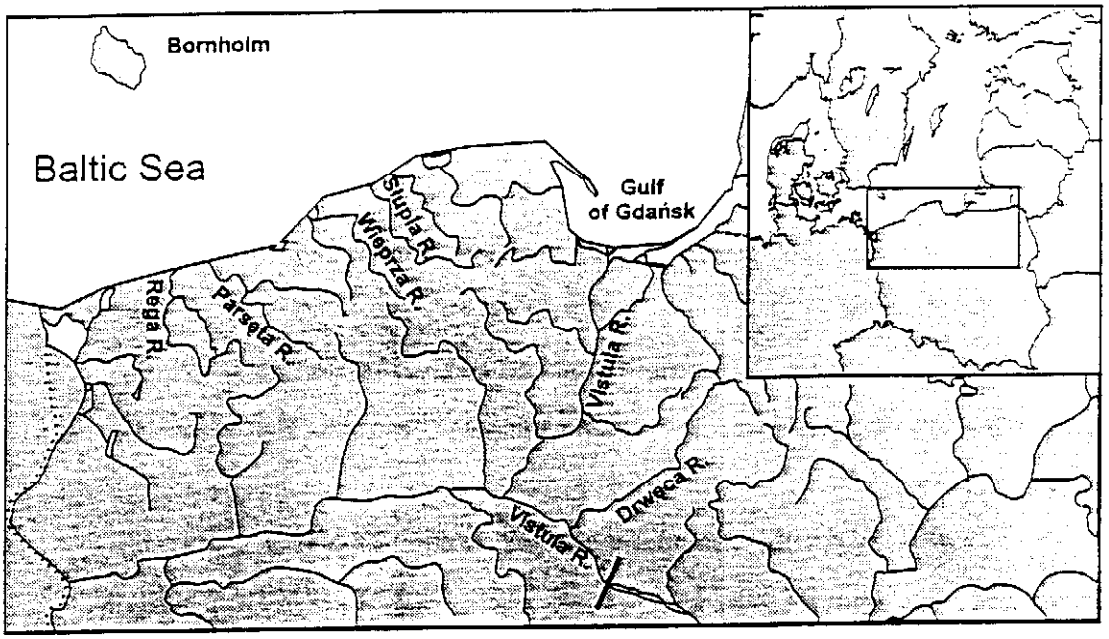

Table 1. Conditions of PCR amplification for five studied microsatellites. Annealing temparatures, $MgCl_2$ and primer concentrations.

	Ssa197	Ssa171	Str15	Str73	Ssa85
annealing temp.	60°C	54°C	58°C	58°C	62°C
$MgCl_2$ conc. (mM)	1,5	1,5	1,5	1,5	1,5
primer conc. (μM)	0,2	0,3	0,2	0,2	0,2

Table 2. Number of allels per locus (N), results of tests for deviations from expected Hardy-Weinberg poportions, expected (H_e) and (H_o) bserved heterozygosity, p value (p) and sample size (n) of the studied populations. Significant deviations from Hardy-Weinberg equilibrium marked •.

microsattelite	Vistula-96	Wieprza-96	Slupia-96	Drwęca-96	Rega-96	Parsęta-96
Ssa171 Total no. allels 19						
N	14	14	14	14	16	16
H_o	0,800	0,850	0,775	0,850	0,850	0,925
H_e	0,899	0,903	0,893	0,925	0,928	0,920
p	0,064	0,359	0,005•	0,0000•	0,017•	0,468
n	40	40	40	40	40	40
Ssa197 Total no. allels 11						
N	11	8	8	10	9	9
H_o	0,775	0,975	0,825	0,850	0,750	0,875
H_e	0,821	0,793	0,818	0,768	0,823	0,830
p	0,049•	0,082	0,761	0,093	0,826	0,981
n	40	40	40	40	40	40
Str15 Total no. allels 5						
N	4	4	4	4	5	4
H_o	0,775	0,575	0,800	0,675	0,650	0,725
H_e	0,748	0,747	0,751	0,720	0,734	0,736
p	0,403	0,071	0,904	0,0003•	0,505	0,469
n	40	40	40	40	40	40
Str73 Total no. allels 4						
N	4	4	4	4	4	4
H_o	0,675	0,650	0,600	0,550	0,400	0,650
H_e	0,573	0,596	0,591	0,588	0,562	0,573
p	0,698	0,048•	0,134	0,194	0,144	0,617
n	40	40	40	40	40	40
Ssa85 Total no. allels 5						
N	5	4	5	4	5	4
H_o	0,564	0,550	0,675	0,459	0,675	0,675
H_e	0,699	0,668	0,717	0,638	0,698	0,720
p	0,070	0,076	0,045•	0,053	0,860	0,761
n	39	40	40	37	40	40

Table 3. Frequences of alleles at five microsatellite loci for six studied populations of sea trout. Sizes of alleles and inferred number of repeats are presented.

Ssa171

repeat number	allel size	Wisła	Wieprza	Słupia	Drwęca	Rega	Parsęta
55	225	0	0,0250	0	0	0,0125	0,0375
56	227	0	0	0,0125	0	0	0,0125
57	229	0,0125	0,0750	0	0,0250	0,0375	0,0125
58	231	0,1875	0	0,0625	0,1250	0,0625	0
59	233	0,0500	0,0125	0,0250	0,0875	0,0375	0,0125
60	235	0	0	0,0375	0,0250	0,0500	0,0250
61	237	0,1500	0,1000	0,0625	0,1250	0,0750	0,1375
62	239	0,1500	0,1000	0,2375	0,1125	0,1250	0,1000
63	241	0,1125	0,1750	0,1625	0,1000	0,0750	0,1000
64	243	0,0625	0,0500	0,0625	0,0625	0,0625	0,0875
65	245	0,0875	0,0250	0,0375	0,0625	0,1250	0,0875
66	247	0,0125	0	0,0625	0	0	0,0250
67	249	0,0750	0,1250	0,0500	0,0875	0,1375	0,1250
68	251	0,0375	0,1625	0,0875	0,0375	0,0875	0,1125
69	253	0,0125	0,0250	0,0750	0,0750	0	0,0500
70	255	0,0125	0,0375	0	0,0125	0,0500	0,0250
71	257	0,0375	0,0750	0,0250	0,0625	0,0375	0,0500
72	259	0	0,0125	0	0	0,0125	0
74	263	0	0	0	0	0,0125	0
	allel number	S=80	S=80	S=80	S=80	S=80	S=80

Ssa197

repeat number	allel size	Wisła	Wieprza	Słupia	Drwęca	Rega	Parsęta
14	128	0,0250	0	0	0,0250	0	0
15	132	0,1500	0,1625	0,1000	0,2750	0,1875	0,1250
16	136	0,3125	0,2625	0,3500	0,3750	0,3500	0,3250
17	140	0,0875	0,3250	0,1875	0,1625	0,1375	0,2000
18	144	0,2500	0,0625	0,1375	0,0750	0,0625	0,1000
19	148	0,0500	0,0625	0,0375	0,0125	0,0625	0,0500
20	152	0,0250	0,0125	0,0750	0,0125	0,0500	0,0875
21	156	0,0250	0,0875	0,0750	0,0125	0,0625	0,0750
22	160	0,0375	0	0,0375	0,0375	0,0250	0,0125
23	164	0,0125	0,0250	0	0,0125	0,0625	0,0250
24	168	0,0250	0	0	0	0	0
	allel number	S=80	S=80	S=80	S=80	S=80	S=80

Str15

repeat number	allel size	Wisła	Wieprza	Słupia	Drwęca	Rega	Parsęta
15	221	0,2625	0,2625	0,2375	0,3000	0,2250	0,3125
16	223	0,2750	0,2125	0,2375	0,2625	0,1500	0,1875
17	225	0,1625	0,1750	0,2000	0,0875	0,1875	0,1625
18	227	0,3000	0,3500	0,3250	0,3500	0,4250	0,3375
19	231	0	0	0	0	0,0125	0
	allel number	S=80	S=80	S=80	S=80	S=80	S=80

Str 73

repeat number	allel size	Wisła	Wieprza	Słupia	Drwęca	Rega	Parsęta
12	142	0,0250	0,0250	0,0125	0,0125	0,0375	0,0625
13	144	0,0500	0,0750	0,0750	0,0750	0,0125	0,0250
14	146	0,5000	0,4500	0,4000	0,3875	0,4250	0,3750
15	148	0,4250	0,4500	0,5125	0,5250	0,5250	0,5375
	allel number	S=80	S=80	S=80	S=80	S=80	S=80

Ssa85

repeat number	allel size	Wisła	Wieprza	Słupia	Drwęca	Rega	Parsęta
13	108	0,0641	0,0250	0,0750	0,0135	0,0375	0,1000
17	116	0,2948	0,2750	0,3625	0,3648	0,2875	0,3125
18	118	0,4487	0,4625	0,3375	0,4594	0,4250	0,4000
19	120	0,1538	0,2375	0,2125	0,1621	0,2375	0,1875
20	124	0,0384	0	0,0125	0	0,0125	0
	allel number	S=78	S=80	S=80	S=74	S=80	S=80

Table 4. Population parwise Fst based on computing conventional F-Statistics from composite allele frequencies for five microsatellites. Significant difference marked •.

	Wisła	Wieprza	Słupia	Drwęca	Rega	Parsęta
Wisła	0,00000					
Wieprza	0,00049	0,00000				
Słupia	0,00080	0,00063	0,00000			
Drwęca	0,00114	0,00175•	0,00206•	0,00000		
Rega	0,00096	0,00002	0,00017	0,00176•	0,00000	
Parsęta	0,00002	0,00017	0,00016	0,00113	-0,00093	0,00000