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Rafal Bernaś, Anita Poćwierz-Kotus, Piotr Dębowski, Roman Wenne. The genetic relationship between extirpated and contemporary Atlantic salmon *Salmo salar* L. lines from the southern Baltic Sea. *Genetics Selection Evolution*, 2016, 48 (1), pp.29. 10.1186/s12711-016-0208-y . hal-01341363

HAL Id: hal-01341363

<https://hal.science/hal-01341363>

Submitted on 4 Jul 2016

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SHORT COMMUNICATION

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The genetic relationship between extirpated and contemporary Atlantic salmon *Salmo salar* L. lines from the southern Baltic Sea

Rafał Bernaś^{1*}, Anita Poćwierz-Kotus², Piotr Dębowski¹ and Roman Wenne²

Abstract

Background: The genetic relationship between original Atlantic salmon populations that are now extinct in the southern Baltic Sea and the present-day populations has long been controversial. To investigate and clarify this issue, we successfully genotyped individuals of the historical populations from the Oder and Vistula Rivers using DNA extracted from dried scales with the Atlantic salmon single nucleotide polymorphism array.

Results: Our results showed a global F_{ST} of 0.2515 for all pairs of loci, which indicates a high level of genetic differentiation among the groups analyzed in this study. Pairwise F_{ST} values were significant for all comparisons and the highest values were found between present-day reintroduced Slupia River salmon and extinct Vistula River Atlantic salmon. Bayesian analysis of genetic structure revealed the existence of substructures in the extirpated Polish populations and three main clades among studied stocks.

Conclusions: The historical salmon population from the Oder River was genetically closer to present-day salmon from the Neman River than to the historical salmon from the Vistula River. Vistula salmon clearly separated from all other analyzed salmon stocks. It is likely that the origins of the Atlantic salmon population from the Morrum River and the Polish historical native populations are different.

Background

Currently, the European distribution of the Atlantic salmon *Salmo salar* L. ranges from northern Portugal to the Pechora River in northwest Russia and encompasses Iceland, the British Isles and the Baltic Sea [1]. Baltic salmon populations reproduce in about 45 river systems of which at least 29 hold native salmon populations or partly mixed following stocking practices [2]. The native Atlantic salmon in Poland became extinct in the middle of the twentieth century. Salmon disappeared from the upper Vistula River in the 1950s, from its lower course after the Włocławek Dam was opened in the 1960s and from most Pomeranian rivers during the same period.

Salmon also disappeared from the Drawa River (Oder basin) at the end of the 1980s.

Historically, the largest population of salmon ascended the Vistula River and migrated to the tributaries of the upper Vistula River [3]. In the Oder River, salmon migrated upstream to the mountain tributaries, but due to dam construction on these rivers during the second half of the twentieth century, salmon spawning grounds were observed mainly in the Drawa River [3]. The present Polish wild salmon population in the Slupia River originated from the Latvian Daugava River, from which individuals were collected for a recovery program that was launched in the 1990s [3]. To date, the genetic make-up of the Polish salmon breeding population is still very similar to that of the Daugava stock [4].

Ongoing discussions about the post-glacial colonization routes that were followed by the Baltic salmon

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(*Salmo salar*) have, to date, resulted in three hypotheses. The first one argues that Baltic salmon may have derived from a refugial population that survived in eastern preglacial lakes and colonized the Baltic basin before a marine strait connected the Baltic Sea to the North Sea [5, 6]. The second hypothesis suggests that Baltic salmon have a western Atlantic origin [7], whereas the third one proposes that colonization spread from both east and west areas [8]. Knowledge on how the southern Baltic Sea was colonized by Atlantic salmon populations is still scarce and the only published results concern populations from the southern Swedish rivers, Morrum and Eman [9] and the southeast Neman River [10]. Until now, extinction of Polish populations has prevented analysis of these lines. However, preserved salmon scales and tissue fragments that were collected before 1970 can be used to extract DNA samples.

Using the Atlantic salmon Illumina 7K single nucleotide polymorphism (SNP) array as in [11], our aim was to investigate the genetic relationships between Southern Baltic salmon stocks (Oder and Vistula river basins) using preserved archival material and current salmon

populations in this region. All methods and sampling are described in detail in Additional file 1 and on Fig. 1.

Results

Analysis of genotyping data showed that the number of polymorphic SNPs for each population ranged from 874 (Vistula) to 1608 (Morrum) and that the mean number of alleles ranged from 1.571 (Neman) to 1.786 (Morrum). Observed heterozygosity per locus/population ranged from 0.235 (Vistula) to 0.339 (Neman) and expected heterozygosity from 0.241 (Vistula) to 0.32 (Slupia and Neman) (Table 1). Allelic richness A_R and private allelic richness P_{AR} ranged from 1.116 to 1.230 and 0.010 to 0.050, respectively. We detected 17 SNPs that deviated from Hardy–Weinberg equilibrium ($P \leq 0.05$) after Bonferroni correction for multiple comparisons (Table 1).

Population-specific F_{IS} estimates were non-significant in all studied populations ($P \leq 0.05$), whereas pairwise F_{ST} values were significant before and after Bonferroni correction ($P \leq 0.05$) for all tests, with higher values between present-day Slupia (SSP) salmon and the extinct Vistula (SVex) salmon (Table 2). By contrast, F_{ST}

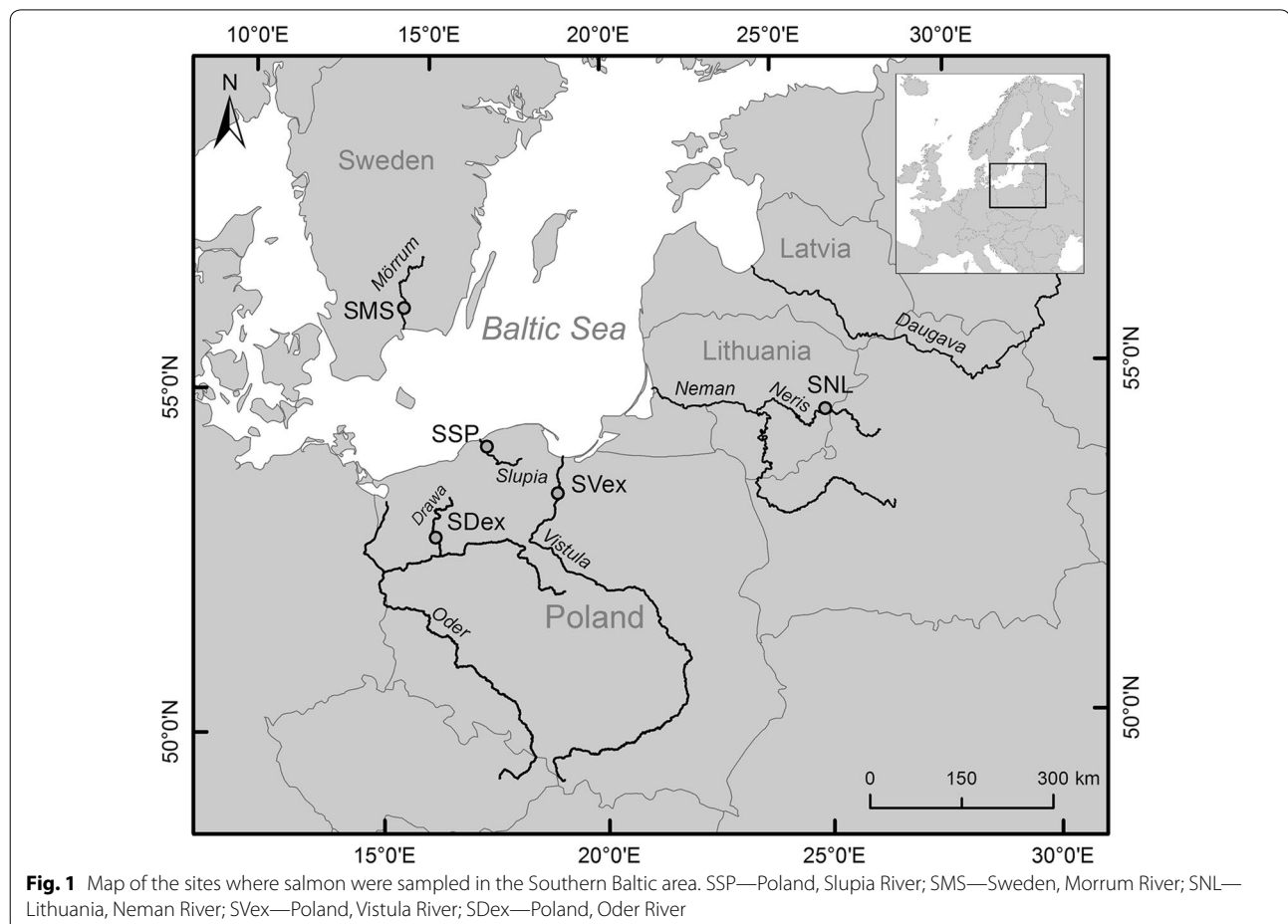


Table 1 Basic statistics of five salmon populations from the southern Baltic Sea

Population acronyms	NI	NPL	MNA	H _O	H _E	A _R	P _{AR}	DHWE*	F _{IS}
SSP	28	1291	1.63	0.33	0.32	1.20	0.01	1	-0.03
SNL	28	1168	1.57	0.33	0.32	1.18	0.02	0	-0.05
SMS	28	1608	1.78	0.29	0.29	1.23	0.05	0	0
SVex	24	874	1.67	0.23	0.24	1.16	0.04	7	-0.03
SDex	21	904	1.75	0.28	0.29	1.22	0.03	9	-0.03

* After Bonferroni correction ($P \leq 0.05$)

NI number of individuals, NPL number of polymorphic loci, MNA mean number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, A_R allelic richness, P_{AR} private allelic richness, DHWE number of loci that deviate from Hardy-Weinberg equilibrium, F_{IS} population-specific inbreeding coefficient

SSP = Poland, Slupia River; SMS = Sweden, Morrum River; SNL = Lithuania, Neman River; SVex = Poland, Vistula River; SDex = Poland, Oder River

Table 2 Genetic diversity indices for the investigated salmon populations

	SSP	SNL	SMS	SVex	SDex
SSP	<i>408.305</i>	119.976	118.100	188.257	98.740
SNL	0.236	<i>370.559</i>	155.020	125.223	58.974
SMS	0.212	0.269	<i>471.783</i>	200.768	113.329
SVex	0.349	0.275	0.343	<i>282.548</i>	96.021
SDex	0.200	0.136	0.208	0.227	<i>377.460</i>

Below the diagonal are given the F_{ST} values for pairwise comparisons of five salmon populations, which were all significant ($P = 0.05$); on the diagonal in italic characters are given the average numbers of within-population pairwise differences; above the diagonal are given Nei's genetic distances (D_A)

SSP = Poland, Slupia River; SMS = Sweden, Morrum River; SNL = Lithuania, Neman River; SVex = Poland, Vistula River; SDex = Poland, Oder River

was lowest for the Oder River (SDex) pair. Estimation of global F_{ST} by AMOVA for all pairs of loci was equal to 0.2515, which indicates a high level of genetic divergence among the analyzed populations. Overall F_{IS} reached -0.0065.

The STRUCTURE software was used to examine the relationships among the salmon populations. The maximum value of ΔK was found for $K = 3$ and the mean log likelihood against K showed a clear plateau at $K = 3$. Bayesian analysis revealed the existence of substructures in the extirpated populations SDex and SVex (Fig. 2). These sub-clustering phenomena were further investigated by assignment tests, which revealed the presence of potential migrants. Populations from Slupia, Morrum and Neman matched with a 100 % score to their own sample group. Six Vistula specimens from the preserved collection and five specimens from the Drawa River had a genetic makeup that was similar to that found for the current Neman River salmon population; in addition, one SDex fish from the archive collection was genetically related with fish collected in the Morrum River. Results from principal coordinate analysis showed large distances between investigated populations. However, the

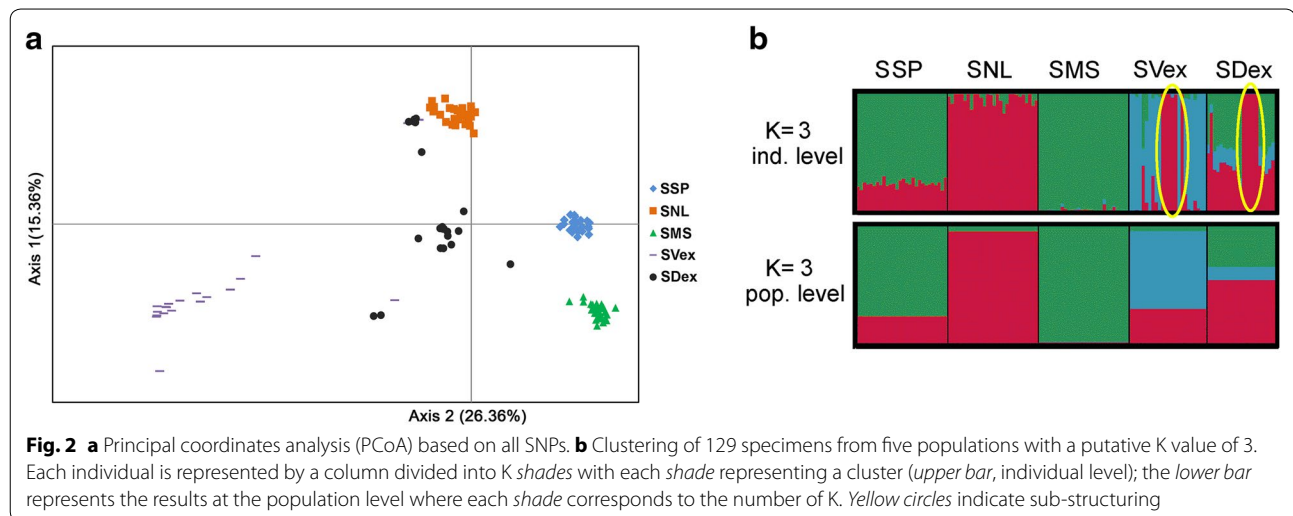
SNL population was more closely related to SDex than to SVex (Fig. 2).

Discussion

During the last decade, many new fish passes have been constructed on Pomeranian rivers in the southern Baltic area (e.g. seven in the Slupia River basin) and a previous fish pass on the Włocławek dam in the Vistula River was rebuilt in 2015. These systems allow the passage of Atlantic salmon and have increased their migration but the recovery program that still continues is only moderately successful [11]. In the Baltic Sea area, the percentage of wild stray salmon is relatively low and they only occasionally contribute to spawning nonnative rivers. However due to stocking activity and especially to the release of juveniles in river-mouth areas, the percentage of strayers can increase by up to dozens of percent [12].

Our findings demonstrate that preserved fish scales can be a very valuable source of genomic DNA in population genetic studies. However, analyses based on markers from non-coding regions such as microsatellites often lead to a large amount of allele dropout [13], whereas with SNPs this phenomenon is much less common [14] although archival samples can be affected by a reduction in the number of heterozygous alleles. Johnston et al. [15] reported that genotyping DNA from preserved Atlantic salmon scales was highly successful for samples up to 24 years old. Although the degree of DNA degradation increases with age, we were able to successfully genotype DNA from scales that were older than 50 years. Furthermore, as Johnston et al. [15] indicated, we observed that, with such samples, incorrectly aligned reads occur mainly in the case of multi-site variants, thus analysis of most of this type of genetic variation was not possible.

In our study, the lowest level of heterozygosity, most departures from HWE and the lowest allelic richness were found for the salmon individuals that were sampled from the Vistula River. These results can be explained by a reduction in population size that was inferred from a



drop in commercial and research catches prior to complete extinction in the 1960 s [3].

Säisä et al. [9] suggested that the southern Baltic Sea was colonized by salmon from a southern refugium during the Baltic Ice Lake stage since deglaciation occurred very early in the Baltic Sea history. Pre-glacial ice-dammed lakes are also known to have existed in the Neman, Vistula and Oder basins and they may have served as glacial salmon refugia [16, 17]. Our results partially support this theory. Among the rivers studied here, the Oder River was the first deglaciated area about 14.5 ka BP, followed by the Vistula and Neman Rivers about 13.8 to 13.2 ka BP, and finally the Murrum and Daugava rivers between 12.8 and 12.5 ka BP [18]. The Bayesian analysis showed that the populations from the Oder, Vistula and Neman Rivers were separated by relatively large genetic distances, in particular, between those from the Vistula and Oder Rivers, which supports the hypothesis that they originated from geographically close but separated refugia.

Our data showed that the historical salmon population from the Oder River was genetically closer to salmon from the Neman River than from the Vistula River. Salmon from the Vistula River were clearly separated from all other analyzed stocks. Although a mitochondrial DNA study reported that salmon populations from the Neman River clustered with populations from the eastern Baltic Sea including the Daugava River [10], our data did not confirm this result, which may be due to mitochondrial and nuclear markers having different discriminating potential [19].

Results from the STRUCTURE analysis provided evidence of population substructures in the samples from the Vistula and Oder Rivers, which originate from the

presence of migrant fish since among the samples from both of these extinct populations, potential migrant fish from the Neman River were detected (PCoA and assignment test). This may be explained by the presence of strayers from the Neman River that could have been caught in Polish rivers or the effect of stocking activities. In our analysis, the salmon population from the Murrum River and the Polish present-day line introduced from the Daugava River stock each formed a cluster, which indicates that the post-glacial origin of the Atlantic salmon from southern Sweden and the origins of the Polish populations from the Vistula and Oder Rivers differ. Since the formation of the southern Baltic rivers occurred much earlier than that of rivers from the Baltic Main Basin, this may explain the differences among Polish stocks and populations from the south Scandinavian Peninsula.

Conclusions

Native Atlantic salmon populations from the southern Baltic Sea originated from diverse sources after the retreat of the glacial ice sheet. Although the Polish historical salmon populations from the Oder and Vistula Rivers clustered together, they were separated by a significant genetic distance. The population from the Oder River was more closely related to the present-day Neman River stock than that from the Vistula River and genetic substructure was revealed in these historical populations. The Polish native populations had a different origin and clustered separately from the Murrum and Daugava River salmon.

Additional file

Additional file 1. Methodology used in this study. This file describes the methods applied in this study [20–29].

Authors' contributions

RB collected fish samples. RB and APK conducted the molecular work. RB and PD participated in design of the study. RW conceived the study, and participated in its design and coordination. RB and APK analyzed the data. RB, RW and PD wrote the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

This study was partially funded by the Ministry of Agricultural and Rural Development Board, Poland, operating under the "Stocking of Polish Marine Areas" program, statutory topic S-25 in the IFI Olsztyn and by statutory topic IV.1 in the IO PAS. Bayesian computations were performed on clusters from PL-GRID framework. Final Illumina scoring for historical samples was carried out in the Laboratory of High Throughput Technologies, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan.

Competing interests

The authors declare that they have no competing interests.

Received: 11 December 2015 Accepted: 22 March 2016

Published online: 01 April 2016

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