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RESEARCH ARTICLE

Genetic characterization of Polish cultured brook trout, *Salvelinus fontinalis* (Mitchill), based on microsatellite DNA analysis

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Dorota Fopp-Bayat, Małgorzata Jankun, Henryk Kuźmiński

Abstract. The genetic variability of two farmed strains of brook trout, *Salvelinus fontinalis* (Mitchill), was examined using six microsatellite DNA loci. The objective of the present study was to evaluate locus-specific genetic markers in brook trout strains cultured in Poland and to assess their levels of genetic diversity. The average number of alleles at all loci in the studied stocks differed between the two groups: 2.20 in the Rutki strain and 7.20 in the Canada strain. For the microsatellites examined, the number of alleles per locus ranged from 1 (locus Ssa-171 in the Canada strain) to 24 (locus Sfo-292 in the Canada strain). The results suggested that the Canada strain had higher gene diversity than the Rutki strain. The molecular analysis described in the present study will be useful for monitoring long-term genetic variation and for the identification of suitable parents for the development of stocks with suitable gene diversity in cultured Polish brook trout.

Keywords: allelic diversity, brook trout, heterozygosity, microsatellites, *Salvelinus fontinalis*

D. Fopp-Bayat [✉], M. Jankun
Departament of Ichthyology
Faculty of Environmental Sciences and Fisheries
University of Warmia and Mazury in Olsztyn
Oczapowskiego str. 5, 10-718 Olsztyn-Kortowo, Poland
Tel./Fax: +48 89 5234772, +48 89 5233754, e-mail:
foppik@uwm.edu.pl

H. Kuźmiński
Department of Salmonid Research Rutki
The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland

Introduction

The brook trout, *Salvelinus fontinalis* (Mitchill), is a northwestern American species. It was introduced to Poland at the end of the nineteenth century. The Rutki stock of brook trout was established in the 1960s. About 18000 fertilized eggs from the Stara Krasnica hatchery were transferred to the Department of River Fisheries (Inland Fisheries Institute, Olsztyn, Poland) fish farm in Oliwa near Gdansk, Poland. In 1984 the population was transferred to the experimental fish farm at Rutki near Gdansk, Poland. This stock shows relatively high mortality in the early stages of development. It was suspected that this was due to inbreeding depression caused by a population bottleneck that resulted from the founder effect. The second strain of brook trout studied was purchased in Canada. This one was established from a population obtained from Quebec in 1973. About 22000 eyed eggs were imported from this population to the Rutki facility in Poland in November 2002. This strain was extremely sensitive to bacterial infections, and only a small number of adult breeders was finally obtained. The fish used for the current study were from the F1 generation of these breeders. There have been no further introductions of brook trout to Poland since these two events.

Almost nothing is known about the genetic diversity of the brook trout breed in Poland. This

information is important for establishing rational management plans and to assess the value of these stocks as genetic resources. The gene pool of fish brood stocks in aquaculture is sometimes characterized by reduced diversity. Studies of fish indicate that small founder populations quickly lose the rarest alleles (Allendorf 1986). Careful genetic management of founder populations can reduce the rate of these losses (Lacy 1989), and multi-locus molecular markers (e.g., microsatellite DNA) can be used to monitor reductions in diversity (Brudford and Wayne 1993). Breeding systems (e.g., small broodstocks, sex-ratio biases, harem formation, inbreeding, high reproductive variance among individuals, etc.) and fluctuations in population size over time can also reduce effective population size (Ne) and result in the loss of allelic diversity in fish stocks. The proper detection and analysis of recent dramatic changes in population size (population bottlenecks) is an important aspect of any population monitoring program. In recently bottlenecked populations, the observed heterozygosity is higher than the expected equilibrium heterozygosity when it is calculated from the number of alleles observed (Luikart and Cornuet 1998).

The first objective of the present study was to evaluate the locus-specific genetic markers in brook trout strains and assess their level of genetic diversity. The second aim was to compare the genetic variability of both brook trout strains in Poland and try to determine if crossing the strains to produce one stock of *S. fontinalis* in Rutki, Poland would be a useful strategy for improving broodstock quality.

Materials and methods

DNA from tissue samples collected from 206 brook trout individuals (106 individuals from the Rutki strain and 100 individuals from the Canada strain) and fixed in 96% ethanol was extracted with the Chelex resin technique described by Walsh et al. (1991). Six polymorphic microsatellite loci were amplified: Sfo-262, Sfo-292 (Perry et al. 2005), Ssa-85, Ssa-171, Ssa-197 (O'Reilly et al. 1996), and Str 73 (Estoup et al. 1993) using the polymerase chain reaction (PCR) conditions described by Fopp-Bayat et al. (2007). Aliquots containing PCR products and reaction buffer were electrophoresed using 6% polyacrylamide gel, and DNA bands were then visualized with the silver staining method (Tegelström 1986). Electrophoresis was conducted on the Bio-Rad SequiGen Sequencing Cell-system, and gel size was 38 x 30 cm. Amplified fragments were sized by comparing them with two DNA standards: ϕX 174 DNA/Hinf *I* DNA Step Ladder (Promega, Madison, WI, USA) and 25bp DNA Step Ladder (Promega, Madison, WI, USA). Allele frequencies, observed heterozygosity (H_o), and expected heterozygosity (H_e) for each locus were computed using the microsatellite toolkit macro for Microsoft Excel^{TM} (Park 2001). The studied stocks were tested to determine whether they had gone through a bottleneck during their history using Bottleneck 1.2 software (Cornuet and Luikart 1996). The software tests for departure from mutation drift equilibrium based on heterozygosity excess or deficiency. Recent population bottlenecks assuming a Stepwise Mutation Model (SMM) and Infinite Allele Model (IAM) for two strains of brook trout were tested. The genetic differences between the sampled fish groups were tested using Fstat software (Goduet 1999), and Fst was calculated according to Weir and Cockerham (1984). Critical significance levels for multiple testing were corrected following the sequential Bonferroni procedure (Rice 1989).

Results

High levels of polymorphism were observed in the Canada population of brook trout established using eggs purchased in Quebec in 1973, while the Rutki population established in the 1960s was characterized by lower levels of variability.

A number of loci showed quite different allele frequency distributions between the two populations studied. Two microsatellites, Ssa-85 and Ssa-171, were monomorphic in the Rutki and Canada populations, respectively. Three multiallelic microsatellite loci, Sfo-292, Sfo-262, and Ssa-85, were highly polymorphic in the groups of fish studied (Table 1). The

Table 1
The range of allele size in base pairs (bp), the polymorphic information content (PIC) value, and the private alleles for studied loci of studied brook trout strains

Stock/Locus	Size of alleles (bp)	PIC value	Private alleles
Rutki			
Sfo-262	275-303	0.510	298
Sfo-292	169-217	0.374	-
Str-73	134-138	0.375	-
Ssa-85	114-116	0.00	-
Ssa-171	93-101	0.197	93, 101
Ssa-197	142-158	0.532	-
Canada			
Sfo-262	259-303	0.789	259, 263, 267, 271, 279, 283, 303
Sfo-292	169-237	0.869	175, 179, 181, 183, 185, 189, 191, 193, 195, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 233, 237
Str-73	134-138	0.365	-
Ssa-85	98-120	0.719	98, 100, 108, 110, 112, 118, 120
Ssa-171	97	0.000	-
Ssa-197	130-158	0.609	130, 134, 150

Table 2
Comparison of observed (H_o) and expected (H_e) heterozygosity in studied brook trout. Expected gene diversities were calculated assuming a Stepwise Mutation Model (SMM) and Infinite Allele Model (IAM); k_o – number of alleles, P – level of significance

	Observed			IAM		SMM	
Fish stock/ locus	N	k_o	H_o	H_e	P	H_e	P
Rutki							
Sfo-262	106	4	0.602	0.419	0.156	0.579	0.486
Sfo-292	106	2	0.392	0.185	0.179	0.222	0.230
Str-73	106	2	0.504	0.184	0.021	0.225	0.030
Ssa-85	106	2	0.000	Monomorphic locus			
Ssa-171	106	3	0.224	0.179	0.351	0.226	0.463
Ssa-197	106	4	0.612	0.325	0.04	0.454	0.127
Canada							
Sfo-262	100	10	0.819	0.736	0.176	0.844	0.180
Sfo-292	100	24	0.899	0.869	0.125	0.924	0.209
Str-73	100	2	0.485	0.186	0.083	0.223	0.092
Ssa-85	100	9	0.767	0.530	0.029	0.676	0.234
Ssa-171	100	1	0.000	Monomorphic locus			
Ssa-197	100	7	0.675	0.469	0.075	0.627	0.383

generally high degree of polymorphism implies that each marker locus is informative and could be used in population studies. The Canada strain exhibits widely different allele frequencies at the studied loci compared to the Rutki strain (Figure 1). Allelic richness per locus and population was statistically different (P = 0.006), and the allele size ranged from 93 to 303 base pairs (bp) in the fish groups studied (Table 1).

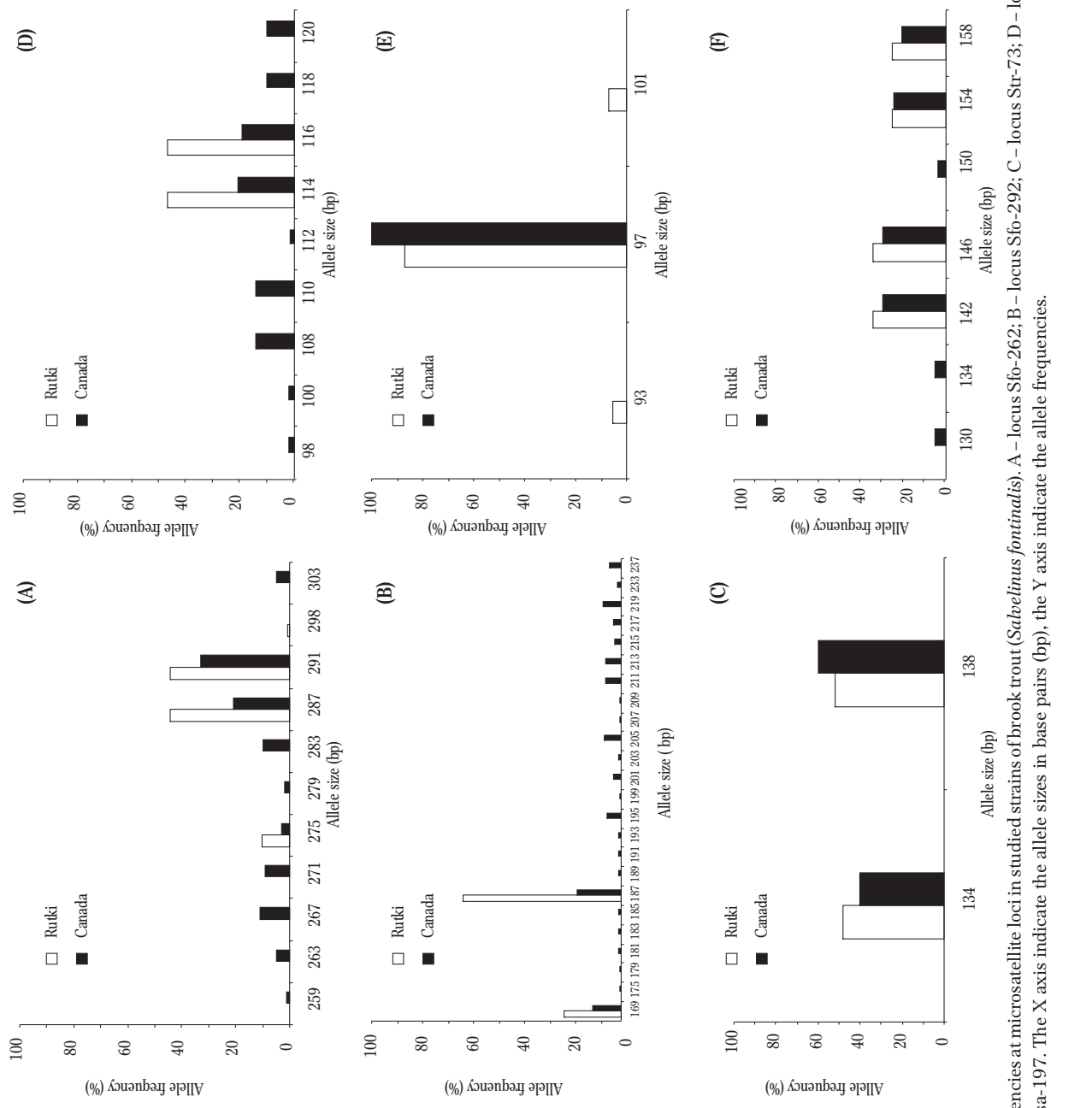

Figure 1. Allele frequencies at microsatellite loci in studied strains of brook trout (*Salvelinus fontinalis*). A – locus Sfo-262; B – locus Sfo-292; C – locus Str-73; D – locus Ssa-85; E – locus Ssa-171; F – locus Ssa-197. The X axis indicate the allele sizes in base pairs (bp), the Y axis indicate the allele frequencies.

Some population-specific alleles were found in both groups of fish studied (Table 1), and some alleles were observed that displayed a high frequency of between 0.39-0.65. In the Canada strain of brook trout, 39 private alleles were observed, while in the Rutki strain there were three private alleles in the microstellite loci studied (Table 1). The average number of alleles at all loci was significantly different between the two groups at 2.20 in the Rutki strain and 7.20 in the Canada strain. The number of alleles per locus ranged from 1 (locus Ssa-171 in the Canada strain) to 24 (locus Sfo-292 in the Canada strain) in the microsatellites that were examined (Table 2).

The observed (Ho) and expected heterozygosities (He) and their probability for the studied strains of brook trout are presented in Table 2. The Canada strain had the highest heterozygosity in four studied loci (Sfo-262, Sfo-292, Ssa-85, Ssa-197). Table 2 also shows the ratio of loci showing heterozygosity excess to those showing heterozygosity deficiency with the two models. The heterozygosity excess test revealed that both populations had a higher observed gene diversity than expected with the SMM and IAM in several of the microsatellite loci studied (Table 2).

The two-sided P-values obtained after 2000 permutations for Ho and gene diversity were 0.006 and 0.005, respectively. The pairwise tests of differentiation yielded significant differences between the two strains of brook trout studied at a 5% level of significance. The Fst value over all loci and fish groups was 0.227 (95% confidence interval 0.072-0.374).

Discussion

The molecular screening of cultured populations is useful for understanding population genetic differentiation among cultured stocks within species, inferring parentage in mixed-family assemblages, maintaining genetic variability in populations, estimating the effective size of populations, and inferring the effects of selection within cultured stocks (Hallerman et al. 2007). Allelic diversity, for example the number of alleles displayed per locus, is a useful measure of genetic variability within populations. Brook trout from the Rutki strain show a reduction in the number of alleles when compared to the more recently established Canada strain.

Genetic diversity, which can be evaluated as the allele number by locus and the average number on all loci, varied among the studied fish groups. It seems that the Rutki strain has suffered a reduction in allelic diversity. Heterozygosity is another way to assess genetic diversity. There was quite a high value in the Canada brook trout (0.485-0.899), while in the Rutki population the value of this parameter was lower (0.224-0.602). Similarly low values were reported for Atlantic salmon, *Salmo salar* L., from the sub-Antarctic Kerguelen Islands (Ayllon et al. 2004) in which heterozygosity varied from 0.000 to 0.645 in different loci. In contrast, high heterozygosity levels (H_e) were found in populations of Arctic charr, *Salvelinus alpinus* L., with values ranging from 0.435 to 0.898 (Ditlecadet et al. 2006). The high heterozygosity levels might result from the low rates of selection pressure stemming from a lack of improvement programs and the existence of several genetic lineages.

Small effective population sizes and poorly managed breeding programs are generally blamed for the loss of genetic diversity in farmed stocks (Norris et al. 1999). Cross et al. (1993), demonstrated that when measures to avoid inbreeding are implemented in breeding programs, genetic variation is not reduced despite small effective population sizes. This can be achieved by maintaining pedigree information on all individuals in the breeding program and using it to plan matings that will ensure only minimum increases in inbreeding every generation.

A population bottleneck of short duration, as would have been the case during the foundation of farmed strains, could severely reduce the number of alleles by eliminating rare alleles without having much effect on heterozygosity (Nei et al. 1975, Allendorf 1986). The bottleneck test applied in the present study was based on the principle that populations with a recent reduction in effective population size typically exhibit a reduction in both allele number and heterozygosity, but with faster reduction of

allele number compared with genetic diversity (Maruyama and Fuerst 1985). If a recent population bottleneck has occurred, the observed gene diversity (H_o) should be larger than expected gene diversity (H_e) based on the number of haplotypes present in the population. Using the methodology outlined above, the Rutki strain cultured in Poland showed indications of a bottleneck under the infinite allele model but not under the stepwise mutation model (Table 2). The heterozygosity excess at two studied loci (Str-73 and Ssa-197) in the Rutki strain might be indicative of a small founding population size.

This general observation suggests that natural populations display higher levels of variability than do artificial populations bred in fish farms, which are often affected by bottleneck and founder effects (Gum et al. 2003). The optimal proportions of the intra- and interpopulation components of gene diversity are disturbed not only through fishing, but also from artificial reproduction and breeding of stocks in hatcheries or fish farms. In all cases, the disproportional elimination of some genotypes and the underuse or irregular reproduction of other genotypes create negative consequences resulting in low population survival and reductions in productivity (Altukhov 2006).

This study demonstrates that artificial breeding practices result in a decrease in genetic variability in terms of allelic diversity, but this is not necessarily detectable from levels of heterozygosity. Presumably, Rutki represents a truly bottlenecked strain given the fact that the small number of alleles in the studied loci in comparison to those of the Canada population was detected and some rare alleles exist at high frequency in this strain (Table 1, Figure 1). This illustrates that founder effects and artificial selection have altered the genetic composition of the farmed strains. Despite high mortality during rearing, the Canada strain is not affected by the bottleneck effect and it preserved relatively high levels of either heterozygosity or allelic diversity (Table 1, Figure 1).

Conclusions

1. The studied microsatellite DNA loci showed different allele frequency distributions between the two studied strains of brook trout.
2. The Rutki strain of brook trout was characterized by lower gene diversity than the Canada strain.
3. The results indicate that the small group of breeders from the Canada stock should be used to increase the genetic variability in the Rutki strain. This would be a useful strategy for improving the broodstock quality.
4. The analyses used in the present study can be applied for the genetic monitoring of the brook trout strains during fishery management.

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Streszczenie

Charakterystyka genetyczna pstrąga źródlanego (*Salvelinus fontinalis* Mitchill) hodowanego w Polsce przy zastosowaniu mikrosatelitarnych fragmentów DNA

Przedmiotem opisywanych badań była analiza genetyczna dwóch stad pstrąga źródlanego *Salvelinus fontinalis*. Głównym celem badań było zidentyfikowanie alleli w wybranych loci mikrosatelitarnego DNA, charakterystycznych dla dwóch stad pstrąga źródlanego, pochodzących z Zakładu Hodowli Ryb Łososiowatych w Rutkach Instytutu Rybactwa Śródlądowego w Olsztynie. Średnia liczba alleli w analizowanych loci u badanych ryb wynosiła od 2,20 (stado Rutki) do 7,20 (stado Canada). Liczba alleli w badanych loci wynosiła od 1 (locus Ssa-171 dla stada Canada) do 24 (locus Sfo-292 dla stada Canada). W wyniku przeprowadzonych analiz stwierdzono, iż stado Canada charakteryzowało się wyższą zmiennością genetyczną w porównaniu do stada Rutki. Opisaną metodę przy zastosowaniu markerów mikrosatelitarnego DNA można wykorzystać do monitoringu genetycznego w trakcie prowadzenia prac hodowlanych.