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Selection of optimal spawner-pairs based on the polymorphism of microsatellite loci in a partially-tetraploid fish species (*Coregonus lavaretus*)

Dariusz Kaczmarczyk[1], Dorota Fopp-Bayat[2], Anna Wiśniewska[2]

[1] Department of Environmental Biotechnology, [2] Department of Ichthyology, University of Warmia and Mazury in Olsztyn, Poland

Corresponding author: Dariusz Kaczmarczyk, Department of Environmental Biotechnology, University of Warmia and Mazury in Olsztyn, Sloneczna 45G, 10-718 Olsztyn, Poland; Phone: +48 89 523 41 51; E-mail: d.kaczmarczyk@uwm.edu.pl

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ABSTRACT

Many species that are in danger of extinction require human support in the form of captive-breeding programs to help maintain their populations in the wild. When breeding in captivity, it is important to select breeding pairs that will create the most genetically diverse progeny. Based on the polymorphism of their microsatellite loci, breeding pairs of diploid land animals have been successfully selected. In this theoretical paper, we asked how to adapt this technique to the selection of spawner pairs for restocking populations of partially tetraploid fish species. To test our calculation techniques, we used actual data on the polymorphism of the loci of captured whitefish (*Coregonus lavaretus*). The data enabled calculations showing which spawner pairs would create the most genetically diverse cohort of offspring if they were bred. The method presented in the paper can be used for breeding fish in aquaculture conditions to help conserve species.

INTRODUCTION

Maximizing genetic diversity by selecting optimal breeding pairs helps to maintain populations of species that are human-dependant and are supported by juveniles bred in aquaculture conditions (Bartel 2000). Maintaining this variation of species helps to promote adaptability of populations to changes in environmental conditions (Fopp-Bayat and Luczynski 2006; Fraser 2008; Luczynski et al. 1992). In contrast, inbreeding can result in expression of deleterious traits and reduce the viability of future generations (Hallerman 2003). To reduce inbreeding depression, spawning pairs should be assembled with fish that genetically differ as much as possible (Kaczmarczyk and Fopp-Bayat 2013).

One technique for selecting optimal pairs of spawners is based on the polymorphism of their microsatellite loci (Brudford and Wayne 1993; Hansen et al. 2000; Henderson et al. 2004; McConnel et al. 1994). This method includes three measures of genetic variation that are known to be important for conservation of species (Stow and Briscoe 2005). These are: (1) potential heterozygosity, (2) the number of alleles inherited by the progeny cohort of each spawning pair (Kaczmarczyk and Fopp-Bayat 2013), and (3) the number of weak heterozygotes ("weak heterozygotes" are fish that are tetraploid at a given locus and have three alleles that are the same and only one allele that differs at that locus, for example *AAAB*).

The whitefish (*Coregonus lavaretus*) is an example of a partially tetraploid species (Le Comber and Smith 2004) that is often kept in existence by supporting its wild populations with juveniles bred in aquaculture conditions (Nynca et al. 2012). This means that in many lakes the population of this fish depends on human activity, and therefore, errors when assembling fish into spawning pairs (i.e. crossing genetically similar fish or closely related individuals) may significantly reduce the genetic variation of this species (Bryant et al. 1986) and make a conservation program unsuccessful (Hallerman 2003).

Accordingly, we asked how to use the data from microsatellite assays to select the best pairs of partially tetraploid spawners that would create the most genetically diverse offspring. We used whitefish as a model to simulate the procedure of selecting from a group of spawners the individuals that genetically differed the most to give the highest possible heterozygosity and allelic diversity in the next generation.

MATERIALS AND METHODS

Fish samples

Twenty-nine whitefish inhabiting Lake Łebsko (Poland) were sampled (9 females and 20 males). These fish were part of a large group of almost 600 whitefish that were taken from Łebsko Lake, Galadus Lake, Mamry Lake and the Pomorska Bay (Fopp-Bayat et al. 2015). Before sampling, all fish were tagged. Pelvic or pectoral fin clips (approximately 10mm^2) were taken from each spawner. The tissues collected were preserved in 70% ethanol and stored at -20°C until their examination. DNA was extracted using Chelex 100 chelating resin (Walsh et al. 1991).

PCR amplification

Six microsatellite loci: *Cocl-Lav8*, *Cocl-Lav18*, *Cocl-Lav28*, *Cocl-Lav80* (Rogers et al. 2004), *Sfo292lav* (Perry et al. 2005) and *Str73* (Estoup et al. 1993) were amplified using the polymerase chain reaction (PCR). The reaction mixtures were prepared in a total volume of 25μl with 40ng DNA template, 1×PCR reaction buffer (50mM KCl, pH 8.5, Triton X-100), 0.4mM of each primer, 0.25mM of each deoxynucleotide triphosphate (dNTP), 3.3mM $MgCl_2$ and 0.6 unit Go *Taq* Flexi DNA Polymerase (Promega, Madison, WI, USA). Re-distilled water was used to bring the reaction mixture to the desired final volume. Amplification was conducted with a Mastercycler gradient thermocycler (Eppendorf, Germany), with initial denaturation at 94°C for 5min, followed by 33 amplification cycles (94°C, 1min; 53-55°C, 30s; 72°C 30s), and final elongation at 72°C for 5min. In order to enable genotyping of PCR products with an Applied Biosystem 3130 Genetic Analyser, forward primers were 5'-labeled with different fluorescent reporter dyes (*Cocl-Lav8*-PET, *Cocl-Lav18*-VIC, *Cocl-Lav28-6*-FAM, *Cocl-Lav8*0–NED, *Str73*–NED, *Sfo292lav*-6 FAM).

Genotyping

The lengths of the amplified DNA fragments were determined using an Applied Biosystems 3130 Genetic Analyser sequencer against a GeneScan 500 [LIZ] size standard. Individual microsatellite loci, amplified using primers with different attached fluorescent dyes, were arranged into sets and analyzed in multiplex mode. Fragment size and allele determination were performed using GeneMapper 4.0 genetic analyser software (Applied Biosystems), following the manufacturer's recommendations. Genetic profiles for each fish were prepared containing the tag number, sex and list of alleles detected at the examined loci. To distinguish between alleles and stutter bands, the height of the peaks and their shape were used. For example, at tetrasomic *Cocl-Lav28* and *Sfo292lav*, if the 4 peaks in the sample that were visible had a similar height and a shape typical for those microsatellites, and the first peak was the highest, then this fish was identified as a heterozygote with 4 different alleles. In contrast, stutter bands were 5-10x lower and differed in shape, and thus were easy to exclude.

Mathematical analysis

At disomic loci, the heterozygosity and allelic diversity of the parental group were calculated with MSA software (Dieringer and Schlötterer 2003). At tetrasomic loci, heterozygotic genotypes were identified. We calculated the heterozygosity for each locus, then used these values to calculate the average observed heterozygosity (H_o) of the parental group across all di- and tetrasomic loci. With the genotyping results of the parental group, we identified weak heterozygotic genotypes and calculated their percentage for each given locus and for all tetrasomic loci.

Estimation of potential heterozygosity (h) in the cohort of progeny from each pair of spawners was based on the genetic differences between the spawners. The heterozygosity of these progeny was calculated according to Kaczmarczyk and Fopp-Bayat (2013). The proportion of homozygous and weak heterozygous genotypes in the potential progeny cohort of each male x female combination was estimated for each locus according to Mendelian Laws and then averaged (Kaczmarczyk and Fopp-Bayat 2013).

The number of different alleles at the investigated microsatellite loci that would be transferred to the progeny cohort was counted for each locus. The values obtained from all the loci were totalled. The suitability of spawning pairs for breeding was evaluated by taking into account the number of different alleles inherited by their progeny cohort (*ar*), the potential heterozygosity of this cohort (*h*), and the percentage of weak heterozygotes (*wh*) in the cohort. The importance of these three values was equally weighted when choosing the spawning pairs (Kaczmarczyk and Fopp-Bayat 2013). All values of genetic variation indicators expected in the progeny of each possible spawning pair were computed by Genassemblage 1.0 software (Kaczmarczyk 2015).

RESULTS

Within the investigated group of spawners, all the examined microsatellites were polymorphic (Table 1). The genotyping of the fish in this study, as well as that of all samples investigated by Fopp-Bayat et al. (2015), have shown that the *Cocl-Lav28* and *Sfo292lav* loci are tetrasomic. We found homozygous fish, and heterozygous fish with 2, 3 or 4 different alleles. Of the investigated loci, the highest polymorphism was observed at locus *Cocl-Lav80* (14 alleles) and the lowest at loci *Cocl-Lav18* (2 alleles) and *Str73* (monomorphic). At locus *Cocl-Lav8*, 8 alleles were detected; at locus *Cocl-Lav28*, 7 alleles; and at locus *Sfo292lav*, 5 alleles. Calculated across loci, the average

heterozygosity of the broodstock was 0.65. Within the analysed stock, the average percentage of weak heterozygotes was 0.24. Those genotypes were detected mostly at locus *Sfo292lav*, where their frequency reached 0.31.

Table 1. The alleles (expressed in bp) at studied loci detected in genomes of investigated spawners of *Coregonus lavaretus*. Locus *Str73* was monomorphic for the allele *131*.

Fish		Locus													
No.	Sex	*Cocl-Lav8*		*Cocl-Lav28*				*Cocl-Lav80*		*Cocl-Lav18*		*Sfo292lav*			
103	**F**	258	258	166	166	170	198	250	298	149	149	209	209	209	209
107	**F**	218	218	166	166	192	198	252	258	149	155	209	209	209	209
108	**F**	254	254	170	170	176	176	292	292	149	149	203	209	209	209
110	**F**	248	258	166	166	170	170	256	314	149	149	199	209	209	209
111	**F**	254	258	166	176	176	176	326	326	149	149	203	205	209	209
113	**F**	242	246	166	176	176	198	250	258	149	155	203	203	209	209
115	**F**	242	246	176	176	176	176	252	302	149	155	209	209	209	209
116	**F**	246	246	166	166	176	176	252	316	149	155	203	209	209	209
119	**F**	218	248	166	170	176	192	314	314	149	149	203	205	209	209
210	**M**	242	246	166	166	166	176	252	326	149	149	203	209	209	209
211	**M**	242	242	166	170	176	192	252	256	149	149	199	203	205	209
212	**M**	246	248	166	166	166	198	324	324	149	149	209	209	209	209
213	**M**	248	258	166	166	170	170	252	304	149	149	199	203	209	209
214	**M**	242	258	166	176	176	176	252	324	149	155	203	209	209	209
217	**M**	242	254	166	170	170	198	250	304	149	155	203	209	209	209
218	**M**	254	258	166	176	176	176	256	256	149	149	203	205	209	209
220	**M**	246	254	170	176	176	198	266	266	149	155	203	203	209	209
221	**M**	246	254	168	170	176	176	324	324	149	155	203	209	209	209
222	**M**	242	254	168	170	170	176	266	324	149	155	203	203	209	209
224	**M**	246	254	166	166	176	198	256	316	149	155	209	209	209	209
225	**M**	246	258	166	166	168	176	324	324	155	155	209	209	209	209
228	**M**	242	258	166	168	176	176	252	324	149	155	203	209	209	209
229	**M**	246	254	166	176	176	182	258	324	149	155	199	203	209	209
231	**M**	246	254	166	170	176	176	252	260	149	149	203	205	209	209
232	**M**	218	254	176	176	176	198	250	314	149	155	207	209	209	209
233	**M**	246	254	170	170	176	176	298	324	149	155	205	209	209	209
236	**M**	240	258	166	166	170	182	252	324	149	155	209	209	209	209
237	**M**	242	258	166	168	170	176	252	260	149	155	203	203	209	209
240	**M**	242	246	166	176	176	176	252	324	149	155	209	209	209	209

The average heterozygosity within the progeny groups varied between 0.38 and 0.81 when calculated across loci (Table 2). Out of 180 possible combinations of spawning pairs, 89 (49%) would create progeny with heterozygosity equal to or higher than that observed in the parental generation. The average heterozygosity calculated across progeny cohorts was 0.64.

The average fraction of weak heterozygotes in all progeny cohorts would be 0.35, but this fraction would range from 0.00 to 0.67. Of the spawning pairs studied, only 28 (16%) could create progeny with a fraction of weak heterozygotes equal to, or lower than, that in the parental generation (Table 2).

The progeny cohort of each spawning pair would inherit between 11 and 19 (average 15) alleles at the investigated loci. 15 spawner pairs would create progeny cohorts with the lowest values of allelic diversity (11-12 alleles); of these spawner pairs 5 would create cohorts with only 11 alleles. 14 spawner pairs would create progeny cohorts with the highest allelic diversity (18-19 alelles); although only one of these pairs would create a cohort inheriting 19 alleles (Table 2).

Table 2. Indicators of genetic diversity of offspring cohorts of *Coregonus lavaretus* spawner pairs: potential heterozygosity (*h*), percentage of weak heterozygotes (*wh*) and number of alleles (allelic richness) (*ar*).

Male No.	Indicator	Female No.								
		103	**107**	**108**	**110**	**111**	**113**	**115**	**116**	**119**
210	***h***	0.57	0.61	0.63	0.61	0.57	0.65	0.54	0.57	0.65
	wh	0.47	0.47	0.29	0.47	0.47	0.42	0.50	0.51	0.35
	ar	15	15	13	16	13	14	12	12	16
211	***h***	0.67	0.71	0.67	0.63	0.67	**0.67**	0.63	0.63	**0.67**
	wh	0.29	0.29	0.21	0.20	0.17	**0.13**	0.50	0.21	**0.04**
	ar	18	18	16	17	17	**18**	16	16	**17**
212	***h***	0.49	0.57	0.58	0.53	0.64	0.68	0.54	0.57	0.60
	wh	0.22	0.22	0.31	0.53	0.50	0.50	0.00	0.53	0.50
	ar	12	13	13	13	14	14	13	13	15
213	***h***	0.55	0.68	0.65	0.56	0.62	**0.75**	0.68	0.69	0.62
	wh	0.49	0.44	0.33	0.43	0.15	**0.19**	0.33	0.32	0.19
	ar	14	16	14	13	15	**18**	16	15	16
214	***h***	0.58	0.63	0.69	0.67	0.65	0.68	0.50	0.65	0.74
	wh	0.29	0.29	0.47	0.29	0.47	0.48	0.50	0.51	0.35
	ar	15	15	14	16	14	15	13	13	17
217	***h***	0.63	0.67	0.62	0.70	0.69	0.65	0.63	0.71	0.74
	wh	0.33	0.29	0.37	0.40	0.22	0.26	0.25	0.29	0.26
	ar	14	16	14	17	16	15	16	16	18
218	***h***	0.56	0.72	0.56	0.53	0.54	0.73	0.64	0.72	0.66
	wh	0.38	0.38	0.43	0.25	0.36	0.38	0.58	0.47	0.24
	ar	14	16	12	14	11	16	15	14	15
220	***h***	0.72	0.72	0.65	0.74	0.69	0.69	0.65	0.65	0.75
	wh	0.33	0.33	0.40	0.25	0.36	0.33	0.67	0.37	0.19
	ar	15	16	12	17	15	15	14	14	17
221	***h***	0.67	0.67	0.62	0.71	0.68	0.69	0.60	0.62	0.74
	wh	0.25	0.25	0.40	0.29	0.43	0.38	0.58	0.36	0.26
	ar	16	17	12	17	15	16	14	14	17
222	***h***	0.72	0.72	0.65	0.73	0.70	0.70	0.68	0.74	0.75
	wh	0.38	0.33	0.40	0.33	0.28	0.26	0.58	0.29	0.19
	ar	17	18	13	18	16	17	15	16	18
224	***h***	0.58	0.58	0.58	0.62	0.68	0.68	0.54	0.54	0.72
	wh	0.11	0.11	0.35	0.42	0.54	0.46	0.25	0.47	0.42
	ar	15	15	14	16	15	15	14	13	18

225	***h***	0.58	0.58	0.75	0.70	0.76	0.68	0.54	0.58	0.81
	wh	0.11	0.11	0.35	0.42	0.54	0.46	0.25	0.47	0.42
	ar	14	15	14	15	14	15	13	13	17
228	***h***	0.58	0.63	0.70	0.67	0.68	0.69	0.56	0.66	0.74
	wh	0.29	0.29	0.36	0.29	0.43	0.38	0.58	0.40	0.26
	ar	16	16	15	17	15	16	14	14	18
229	***h***	0.72	0.68	0.65	0.74	0.69	0.66	0.65	0.65	**0.75**
	wh	0.38	0.38	0.33	0.25	0.32	0.26	0.67	0.36	**0.15**
	ar	18	17	15	18	16	16	16	15	**19**
231	***h***	0.64	0.68	0.56	0.65	0.61	0.70	0.61	0.61	0.66
	wh	0.38	0.38	0.36	0.28	0.32	0.27	0.67	0.36	0.15
	ar	16	17	13	17	14	17	15	14	16
232	***h***	0.63	0.58	0.61	0.67	0.65	0.68	0.58	0.69	0.61
	wh	0.25	0.25	0.49	0.25	0.51	0.44	0.50	0.49	0.35
	ar	15	15	14	17	16	16	15	16	17
233	***h***	0.63	0.67	0.62	0.70	0.68	0.69	0.60	0.62	0.74
	wh	0.29	0.25	0.47	0.38	0.42	0.34	0.58	0.36	0.30
	ar	15	17	13	17	15	17	14	15	17
236	***h***	0.50	0.54	0.67	0.62	0.68	0.72	0.54	0.62	0.72
	wh	0.11	0.11	0.35	0.47	0.42	0.42	0.00	0.42	0.42
	ar	14	15	15	15	16	18	15	15	18
237	***h***	0.64	0.68	0.74	0.69	0.70	0.70	0.64	0.69	**0.75**
	wh	0.38	0.38	0.33	0.29	0.28	0.26	0.58	0.33	**0.15**
	ar	16	17	15	17	16	17	15	15	**18**
240	***h***	0.58	0.54	0.65	0.67	0.68	0.63	0.38	0.53	0.72
	wh	0.04	0.04	0.53	0.35	0.63	0.60	0.25	0.57	0.50
	ar	15	14	14	16	15	14	11	12	17

*Best combinations of spawners are marked in bold, the combination that should be avoided is underlined.

When all three indicators were taken into account (potential heterozygosity, fraction of weak heterozygotes and allelic diversity expected in a progeny cohort), the best combination was male 229 x female 119. This pair would create a progeny cohort that would be highly heterozygous (0.75) with few "weak heterozygotes" (0.15), and transfer the highest (19) number of different alleles to the next generation. Combinations of male 213 and female 113, male 237 and female 119, and male 211 and female 119 are expected to produce highly heterozygous progeny with diverse alleles and a low share percentage of weak heterozygotes. Therefore, they could be used to replace or supplement the best spawning pair.

DISCUSSION

This paper has presented a calculation technique that can be a useful tool for maintaining genetic diversity in fish species by assembling the best possible spawning pairs. By identifying genetic differences between spawners, and then assembling them into the most favourable pairs, highly diverse and heterozygous progeny cohorts can be produced.

The method presented in this paper shows how it is possible to assemble pairs of spawners from partially tetraploid fish species to maximize potential heterozygosity, percentage of weak heterozygotes and allelic diversity in their progeny cohorts. These values are calculated according to patterns of inheritance of microsatellite loci. Using only a few loci, it was possible to identify the genetic differences between the spawners and select the most favourable pairings. However, when applying this method, it may be desirable to use more microsatellite markers to increase the accuracy of detection of genetic differences between spawners (Ruzzante 1998).

A similar method has been used with diploid land animals to select breeding pairs based on genetic differences revealed in their microsatellite DNA, for example, in the restoration of the Mexican wolf (Hedrick et al. 1997; Parsons 1998). For fish, a similar approach has also been proposed for managing genetic variation in sturgeons, a fully tetraploid organism (Kaczmarczyk and Fopp-Bayat 2013). To the best of our knowledge, this study is the first attempt to use this method with a partially tetraploid organism.

The method presented in this paper can also be used for breeding other salmonids that depend on humans to produce juveniles in aquaculture conditions in order to restock their populations or prevent their extinction. It will help to maintain the genetic diversity of such populations, thus increasing their fitness in the wild.

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