Variation in the D-loop of Arctic Charr (*Salvelinus alpinus*) morphs from Lake Thingvallavatn

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Variation in the D-loop of Arctic Charr (*Salvelinus alpinus*) morphs from Lake Thingvallavatn.
Breytileiki í D-lykkju bleikju (*Salvelinus alpinus*) afbrigða úr Þingvallavatni.
The D-loop in Arctic charr morphs.

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Abstract

There are four morphs of arctic charr (*Salvelinus alpinus*) present in post-glacial formed Lake Thingvallavatn. The morphs can be classified according to phenotypical and ecotypical differences: small benthivorous, large benthivorous, planktivorous and piscivorous. Because of the morphological differences some genetical differences are expected that may explain the ecotypes.

To check for genetical differences I studied the *D-loop*, a mithocondrial DNA region, in 264 individuals of arctic charr, focusing in the differences between the small benthics versus the planktivorous and having as reference the large benthics. The results show that the morphological differences between the morphs doesn't correlate with the *D-loop* genotype by the genetics didn't appear because of the morphs, there is however weak genetic differentiation between sexes.

The study of the developmental gene *FGOP2* in 54 individuals does not indicate differences in allele frecuency between the three morph types in Lake Thingvallavatn.

Table of contents

Figures

Tables

1 Introduction

1.1 Introduction

Speciation is the proccess by which a population of species give rise to other populations, reproductively isolated from the other population and between them. This procces can be produced by cladogenesis, because of the differentiation of lineages, or anagenesis, in which the new species belong to the same lineage.

The way that the speciation happens can be by four different models according to a geographical clasification: allopatric, peripatric, parapatric and sympatric. The sympatric speciation occurs by the evolution of new species from a single ancestral species within the same geographic region.

This study is focused in a proccess of possibly sympatric speciation in Lake Thingvallavatn, due to the separation of the initial species in several ecotypes, and seemingly a reproductive isolation between the adapted morphs to their corresponding ecosystem, having the environmental conditions an important effect in the genetically based phenotypic variation exposed to natural selection, Parsons et al. (2010).

The different ecotypes are formed by the presence of physical or nonphysical (behavior, morphology, life history) barriers, that provoke reproductive isolation and limit the gene flow, as happens in several populations of seabirds (*Pterodroma phaeopygia*, *Synthiboramphus hypoleucus*), Friesen et al. (2007).

Lake Thingvallavatn (64º10’ N, 21º10’ W) is the biggest lake in Iceland, with a surface of 83 km^2 and an average volume of 2856 km^3 of water. The lake is located in the south-west part of the island, and it’s 100,5 meters above sea level. The average depth of the lake is 34 meters, with a maximum depth of 114 meters. The lake appeared at the end of last glaciation, approximately 10000 years ago, Adalsteinsson et al. (1992) (Figure 1).

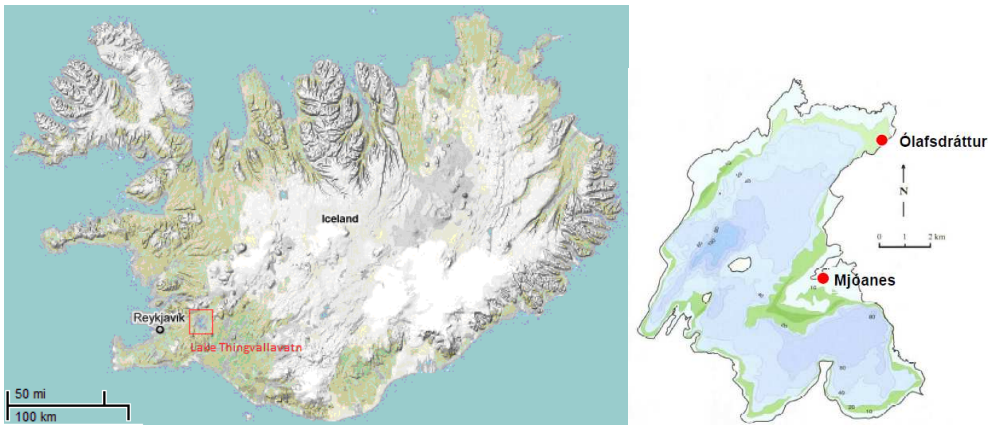

Figure 1. Location of Lake Thingvallavatn in Iceland and the two places where the arctic charr individuals were caught. The left figure is from Googlemaps, squared added in Paint, the right figure is provided by Kalina Kapralova.

Lake Thingvallavatn is populated by four morphs of Arctic charr: small benthic (dwarf), large benthic, planktivorous (murta) and piscivorous. They differ in morphology, behavior, diet and life history characteristics (Table 1) (Figure 2).

Table 1. Differences between the four morphs in Lake Thingvallavatn#.

Morph	Benthic		Pelagic	
	Small	Large	Planktivorous	Piscivorous
Lower jaw	Short		Long	
Snout	Blunt		Pointed	
Mouth	Subterminal		Terminal	
Body	Stocky		Fusiform	
Fins	Long pectoral		Small pectoral	
Gill rakers	Few and spaced-out		High and dense	
Sexual maturity	2 years (2 years males, females 4 years*)	6 years (8 years*)	4 years (2 years males, females 5 years*)	6 years
Fork lenght	7 cm	22 cm	15 cm	23 cm
Asymptotic lenght	13,3 cm	55,4 cm	20,5 cm	30,2 cm
Feeding	Gastropod *Lymnaea peregra*	Gastropod *Lymnaea peregra*	Crustacean zooplankton: (*Daphnia longispina* and *Cyclops abyssorum*¤)	Feeds on three-spined stickleback, *Gasterosteus aculeatus*
Spawning time*	August – November	July – August	September – November	September - November
Juvenile parr marks	Retained	Lost	Lost	Lost
Melanized under the lower jaw	Frequently	No	No	No

Most of the data are from Snorrason et al. (1994a).

* Sandlund et al. (1992). ¤ Snorrason et al. (1994b)

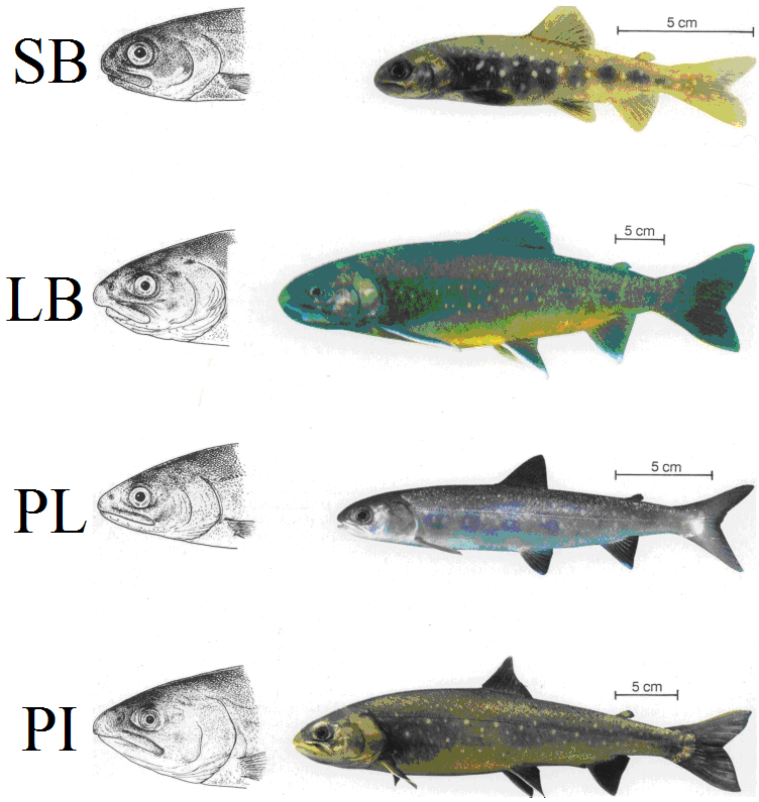

Figure 2. Aspect of the four morphs of arctic charr, from top: SB=Small benthic, LB=Large benthic, PL=Planktivorous, PI=Piscivorous, from Sandlund et al. (1992).

All morphs feed in the littoral habitat (0-20 meters depth), but pelagics also feed in the pelagic habitat (0-70 meters depth). The diet composition is somewhat similar between the morphs, but the diets aren't overlapped between the benthics and the pelagics. The size of the charrs is directly related between the size of snails eaten, Malmquist et al. (1992). Furthermore, there are several behavioural differences genetically based in consequence of adaptive trophic specializations, as planktivorous charrs reject to feed with low prey densities in the pelagic habitat because of the balance of energy earned – lost wouldn't be favorable, Skúlason et al. (1993).

About the reproduction, if small benthic males sneak into the nest of large benthic females, they will be attacked by large benthic males and females, which is a reason for the reproductive isolation between small and large benthic fishes, Sigurjónsdóttir & Gunnarsson (1989).

According to Skúlason et al. (1996), life-history differences among the morphs are partly genetically based. The small benthivorous charr is the morph that grow slowly, while the large benthivorous charr grow faster and the planktivorous and piscivorous charr have even higher initial growth rate, Jonsson et al. (1988).

The behaviour of all the morphs is flexible in early phases, having a morphological divergence in more advanced phases, knowing that morphological and genetic divergence of morphs is correlated, it could be possible signal the trophic adaption as the cause of the segregation of the morphs, Gislason et al. (1999).

There are genetic evidences that suggest that the differents morphs of Lake Thingvallavatn haven't appeared only because of phenotypic plasticity, there is a background in genetics. There are four morphs, but according to laboratory-rearing experiments there are only three populations, such experiments also show that there is a genetic basis with a significant maternal effect that provokes the differences between the morphs. There are also differences in the ontogeny explained as developmental heterochrony, because in the embryo firstly appears a subterminal mouth and a blunt snout, and the pelagic morphs have more differences with the embryonic phenotype than the benthic ones, Skúlason et al. (1989).

The choice of the *D-loop* is because of the maternal inheritance of this gene and the assumed lack of selection. Preliminary studies with the *D-loop*, or mitochondrial DNA control region, lead by Sigrún Reynisdóttir and Arnar Pálsson, found weak evidence of genetic differentiation between the different morphs (Sigrún Reynisdóttir and Arnar Pálsson – unpublished results), consistent with the patterns observed by Kapralova et al. (2011), in which the level of differentiation is consistent with strong reproductive isolation.

In the study of the gene *FGOP2* (*FGFR1OP2*/*wit3.0*) (fibroblast growth factor receptor 1 oncogene partner 2), Lin et al. (2010), found it related to the formation of the skeleton of the head. Preliminary studies by Arnar Palsson that indicated allele frequency differences in the different populations. As it's a developmental gene that take part in the differents head morphs it could be under selection.

1.2 AIM

The goals of this study are to check the sympatric model, that predicts that all the morphs have the same genetic background, but currently present some genetic differences between the three morphs (small benthic, large benthic and planktivorous) because of a continuos process of sympatric speciation that is happening, due to previous works show some slighty signs of these genetical differences.

Restriction analysis on mtDNA show that the morphs are very closely related (Danzmann et al. 1991). Some microsatellite markers of Kapralova et al. (2011) show that morphs are genetically differentiated. Nevertheless, others studies can not find any differences, as the work by Volpe and Ferguson (1996) that didn`t have to many individuals (117) or data (48 restriction endonucleases for the *D-loop*, 5 that detected polymorphism; synthetic array of 16 bp tandem in multilocus minisatellite DNA), as they worked with a shorter region (1 kb) of the *D-loop*; a direct sequencing of 275 bp of the *D-loop* and single locus minisatellite analysis detected insufficient variation.

2 Materials and Methods

2.1 Sampling

The 264 individuals of arctic charr (small benthic, planktivorous and large benthic) were caught by gill netting between September and October of the year 2010 from two different places in Lake Thingvallavatn, Mjóanes and Ólafsdráttur. The morphs were classified according to the criteria used to Snorrason et al. (1989), having similar number of planktivorous (murtas) and small benthics (dwarfs), and a small quantity of large benthic individuals.

Murtas were caught in Mjóanes and Ólafsdráttur in similar proportions, while dwarfs were caught mainly only in Ólafsdráttur. The large benthic charr was used as reference population, they were caught from Ólafsdráttur, place where is the main spawning territory. The caught fishes were separated and frozen for the posterior processing. In the laboratory the right pectoral fin was removed and frozen for the DNA extraction.

2.2 DNA & PCR

The genomic DNA of 262 samples (2 specimens were missing) was made from a piece of the fin stored before using a standard phenol chloroform procedure, Conejeros et al. (2008), 6% Chelex 100 (Bio-Rad) or CTAB extraction, Weetman (from protocols of Samadi & Shaw) by Sigrún Reynisdóttir and Jetty Ramadevi.

The *D-loop* region were amplified by polymerase chain reaction (PCR) using the specific reactions (Table 2).

Table 2. Amount of the reactives used for the PCR.

DNA [50 ng/µl]	1 µl
dNTPs [2,5 µg each]	2 µl
TEQ [5 units]	0,4 µl
10x Buffer [10 ng/µl]	2 µl
F Primer [10 ng/µl] (5'TTG TTC GTT ACC CAC CAA GC3')	0,4 µl
R Primer [10 ng/µl] (5'TGC CTG TGG GAC TTT TTA GG3')	0,4 µl
ddH_2O	13,8 µl
Total volume	20 µl

The protocol was changed to use 0,2 µl of TAQ [5 units] instead the TEQ, because of the better results, and the correspondly amount of water (14 µl).

FGOP2 reactions were the same as the *D-loop* amplified with TAQ, but with the procol adjusted to other quantity of DNA and with their respective pair primers:

3 µl DNA [50 ng/µl] for FGOP2-F (5'GGA GAT TGA ATC GCT GAA CC3') / Del-R (5'CAC ACA TTA TAC TCA CTT CTG TGG AT3').

4 µl DNA [50 ng/µl] for AC-F (5'AAT CCA CAG AAG TGT GAG TAT AAT GTG3') / FGOP2-R (5'TCT ATG TGC GCT TGC ATT TC3').

The PCR programs used for the *D-loop* were 94 ºC during 5 minutes for initial denaturation, 33 cycles of 57 ºC for 45 seconds as annealing temperature, 1 minute at 70 ºC and 45 seconds at 94 ºC; and finally a step of 10 minutes at 72 ºC (Figure 3).

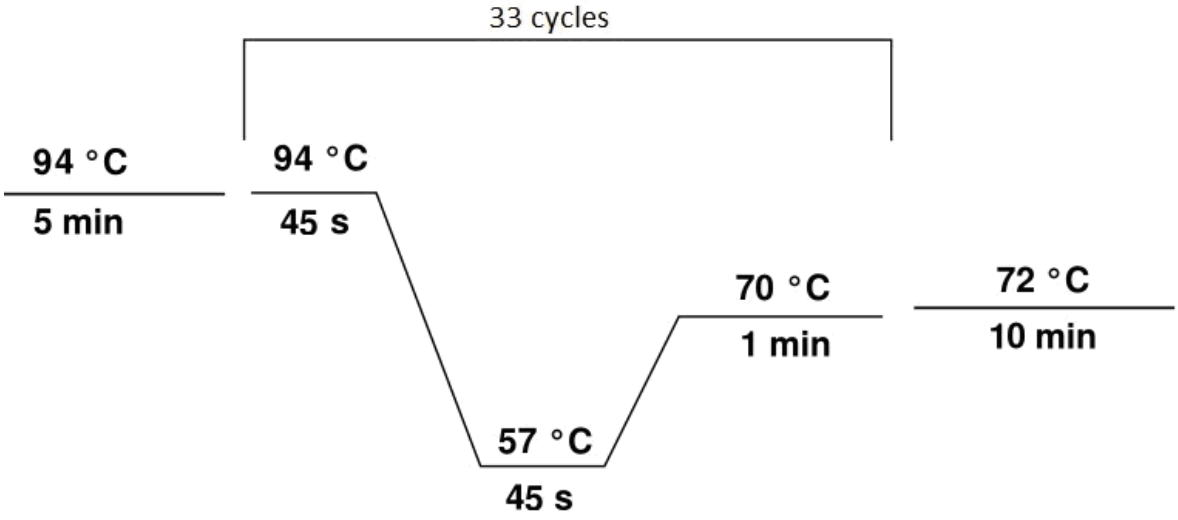

Figure 3. Diagram of the temperatures used in the PCR.

For *FGOP2* the annealing temperature was decreased to 53 ºC. The obtained PCR products were separated by electrophoresis in different gels.

The *D-loop* was surveyed on 1% agarose + ethidium bromide gel during 15 minutes at 90 volts to check the quality of the PCR (gel sample in the appendix). Similarly, the *FGOP2* was surveyed on 2% agarose + ethidium bromide gel during 50 minutes at 60 volts to differentiate between the size of the two amplified fragments (gel sample in the appendix). The gels were visualized using the fluorescence imagining system PSRemote v1.5.2 (Breeze Systems) for Canon Powershot G10.

2.3 PCR Cleanup

Once the *D-loop* DNA was correctly amplified, the purification of the PCR products was made using ExoSAP protocol (Table 3).

Table 3. Amount of the reactives used for the purification of the PCR product.

(Exo1) Fosfatase buffer 10x	1 µl
Antatric phosphatease [5 units]	0,2 µl
Exo 1 [2 units]	0,1 µl
ddH_2O	3,7 µl
PCR product	5 µl
Total volume	10 µl

The reaction was run on thermocycler at 38 ºC during 35 minutes and 80 ºC during 20 minutes, and stored at -20 ºC until sequencing.

2.4 DNA sequencing

Afterwards was made the reaction for the purified DNA sequencing (Table 4).

Table 4. Amount of the reactives used for the sequencing.

VII 5x buffer	2,756 µl
TRR BigDye [5 units]	0,487 µl
D-loop F Primer [1 pm/µl]	1,5 µl
ddH_2O	5,25 µl
ExoSAP product	5 µl
Total volume	15 µl

The temperature regime for DNA sequencing (Figure 4).

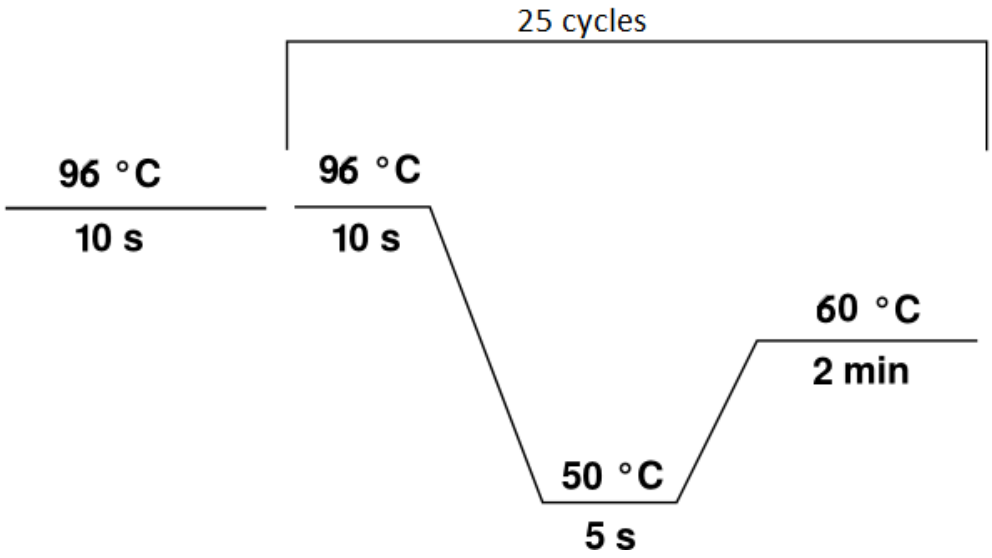

Figure 4. Diagram of the temperatures used in the sequencing.

2.5 Sequencing cleanup

The last step was a standard ethanol precipitation (Table 5).

Table 5. Amount of the reactives used for the standard ethanol precipitation.

ddH2O	40,297 µl
NaOAc [3 M]	4,479 µl
Glycogen [20mg/ml]	0,224 µl

The protocol was as follows:

1. Add 45 µl of the mixture in each well.
2. Add 125 µl of 96% ethanol at -20 ºC.
3. Centrifugate at 4000 rpm during 30 minutes.
4. Invert inmediatly in the sink to keep the precipitate.
5. Centrifugate at 300 rpm during 2 minutes the plates face down to dry the precipitate.
6. Add 250 µl of 70% ethanol.
7. Centrifugate at 4000 rpm during 5 minutes.
8. Invert inmediatly in the sink.
9. Centrifugate at 300 rpm during 5 minutes the plates face down.
10. Let the plates dry in darkness while the sequencing covers of the plates are under the UV light.
11. Add 9,9 µl HiDi.
12. Shake during 1 minute.
13. Centrifugate at 1500 rpm for a moment.
14. Store at 4 ºC

The final products were run on Applied Biosystems 3500xL Genetic Analyzer (Hitachi).

2.6 Data analyses

The base calling was done with Sequencing Analysis Software v5.4 with KB^{TM}Basecaller v1.41 (Applied Biosystems), used for sequence base call editing, re-basecalling, trim, display, edit and export the data in .ab1 extention.

Software phredPhrap, polyphred and consed, Gordon (2003), was used to edit the sequences, discriminating between correct and incorrect base-calls produced in the previous step of the sequencing and to find polymorphic sites.

Once the sequences were verified, all sequences were aligned in ClustalX v2.1, Thompson et al. (1997), through sequence weighting, position-specific gap penalties and weight matrix choice.

Program GeneDoc v2.7, Nicholas et al. (1997), was used to convert the files to the extension .phy and divide the different sequences into subsets according to location, sex and morph criteria.

The sequence analysis and some results were done with DnaSP v5.1, Rozas & Rozas (1995). The AMOVA results were calculated with Arlequin v3.5.1.2, Excoffier et al. (2005).

3 Results

3.1 The D-loop

The objetive of the study was to study the genetic variation in three morphs from Lake Thingvallavatn. For that, the amplification of the *D-loop* region from genomic DNA (from 262 samples) was done by PCRs. A a result 226 sequences were obtained.

The first objetive was to evaluate the polymorphism in the *D-loop*, for that 226 sequences each around 456 base pairs were sequenced. Three mutations were found, at locations 121(GA), 181(CT) and 190(TG). The distribution of the mutations according to morph and sex is shown below (Table 6) (results were obtained with DnaSP v5.1).

Table 6. Distribution of the mutations according to morph and sex.

Morph	Mutation in 121	Mutation in 181	Mutation in 190
Murta	5 Males, 5 Females	2 Males	0
Dwarf	2 Males, 3 Females	0	1 Female
Large Benthic	1 Male	0	0

According to this table it is possible remark the mutation 121(GA) as the most common one, but without differences between morphs or sexes. To describe the nucleotide diversity in the *D-loop*, population statistics were calculated in DnaSP v5.1 (Table 7) (raw output in the appendix).

Table 7. Population statistics for the D-loop.

Morph	Sex	# Individuals	# Segregations	# Haplotypes	Haplotypes diversity	Variation	Pi	Theta
Murta	M	61	2	3	0,212	0,00447	0,00106	0,00208
	F	41	1	2	0,322	0,00582	0,00110	0,00080
Dwarf	M	27	1	2	0,142	0,00743	0,00045	0,00082
	F	78	3	4	0,169	0,00311	0,00069	0,00216
Large Bent.	M	18	1	2	0,111	0,00928	0,00037	0,00096
	F	1						

The lenght of the region was 456 base pairs for all morphs.

Dwarf females have the higher number of segregation sites, due to the fact that the only individual having a mutation in 190 belongs to this group. Large benthics are the population with fewest segregating sites, only one. The highest haplotype diversity appears in murtas, above all in the females. Furthermore, the Pi value, nucleotide diversity, is also higher in murtas than in other morphs. Theta values, number of mutations per site, are similar in the sets of murtas and dwarfs, but the values differ between the sexes in each group. Overall, murtas appear to be more polymorphic than dwarfs and large benthics because all of them have similar number of mutations, but murtas have higher Pi and haplotype diversity, which means that the mutations are more common (at higher frequency) in this group.

The haplotypes found in the three morphs are shown below (Table 8).

Table 8. Haplotype distribution for the D-loop.

	Murta		Dwarf		Large benthic		Total
Haplotype	Male	Female	Male	Female	Male	Female	
1: GCT	54	33	25	71	17	1	201
2: ACT	5	8	2	5	1		21
3: GCG				1			1
4: GTT	2						2
5: ATT				1			1

5 haplotypes, haplotype diversity = 0,2003.

With the purpose to see whether morph, sex or location explained the DNA variation in arctic charr, an AMOVA analysis was done (with Arlequin v.3.5.1.2) (raw output in the appendix).

First I looked at the relation between morph and sex (Table 9).

Table 9. AMOVA for morph and sex for the D-loop.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices	P value
Among morphs	2	0,146	-0,00477 Va	-4,44	-0,04441	0,79374
Among sexes within morphs	2	0,818	0,00681 Vb	6,34	0,06072	0,00684
Within individuals	221	23,276	0,10532 Vc	98,1	0,01901	0,02737
Total	225	24,239	0,10736			

The results show that the variation in the *D-loop* is better explained by sex (6,34 %) than morph.

Second, I checked the relation between location and morph (Table 10).

Table 10. AMOVA for location and morph for the D-loop.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices	P value
Among locations	1	0,751	0,00679 Va	6.00	0,06001	0,00000
Among morphs within locations	3	0,297	-0,00025 Vb	-0,22	-0,00233	0,46921
Within individuals	221	23,545	0,10654 Vc	94,22	0,05782	0,02444
Total	225	24,593	0,11307			

These results show that the variation is better explained by location (6 %) than by morph. These results might be distorted because of the fewer dwarfs sampled in Mjóanes (6) compared to Ólafsdráttur (99). Therefore, there are two variables (location and sex) that explain the genetic variation better than morph.

Third, I studied the relation between location and sex only in the morph murta, because of the similar distribution of the samples in relation with the spawning location (Table 11).

Table 11. AMOVA for location and sex for the D-loop in murta.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices	P value
Among locations	1	0,025	-0,00733 Va	-5,63	-0,05633	0,64614
Among sexes within locations	2	0,297	0,00748 Vb	5,74	0,05438	0,07527
Within individuals	98	23,545	0,13002 Vc	99,89	0,00111	0,17791
Total	101	24,593	0,13016			

Eventhough the variation appears to be better explained by sex (5,74 % of the variation) than by location (0 % of the variation), none of the results of this test are statisticly significant (P > 0,05).

3.2 FGOP2

Previous work by Ragnar Óli Vilmundarson had indicated genetic differentiation between murtas and dwarfs at *FGOP2*. To check that I genotyped 28 murtas, 28 dwarfs dwarfs and 8 large benthics by allele specific PCR in the *FGOP2* region. The results were checked visually on agarose gels, the statistics are shown below (Table 12 & 13).

Table 12. FGOP2 genotypes for the different morphs.

		Morph		
	Genotype	Murta	Dwarf	Large Benthic
All data	Del / Del	0	1	0
	AC / Del	9	10	3
	AC / AC	19	17	5
Good data	AC / Del	5	7	3
	AC / AC	16	12	2

The distinction between good, medium and poor data was made by subjective criteria of strenght of the bands visualized in the gel (see appendix A).

Table 13. Frecuency of AC allele.

	All data	Good data
Murta	0,8392857	0,8809524
Dwarf	0,7857143	0,8157895
Large Benthic	0,8125	0,7

These results do not show a significant difference in allele frecuency between the three morphs (All data: $X^2 = 1.4786$, df = 4, p = 0.8304. Good data: $X^2 = 2.5624$, df = 2, p = 0.2777). There are traces showing that individuals homozygous for the deletion are infrequent in the population studied.

4 Discussion

I studied the *D-loop*, a mtDNA region, a gene that doesn't determine the morphology of the fish, and it is a very small part of the genome of *S. alpinus*. I didn't find any differences between the three morphs. That doesn't mean that there aren't differences in other genes, specially in genes responsible for determining the head morphology, for example *FGOP2*.

The results of the study of the *D-loop* show the presence of three polymorphisms in the group of arctic charrs studied. These polymorphisms don't show any distiction between the three morphs studied (similar results as Volpe & Ferguson 1996), according to this data it won't be possible to distinguish three different populations of arctic charr in relation with the *D-loop* genotyping. The most variable morph appears to be murta: mutations are more frequent in this morph than in the others.

The study shows that the DNA variation is mostly strongly associated with the sex of the individuals, not morphotype. These results appear to refute the hyphotesis of the presence of genetic differences between the different morphs. The results that show the sex as main cause of the DNA variation could be explained because Salmonids have a homing behavior, demonstrated for instance by Nordeng (2009), where the females come back to the same place to spawn while the males not. Nevertheless, these results should be used as an initial point for futures studies, not as a irrefutable truth.

The results of the study of *FGOP2*, a developmental gene, don't show the expected effect of natural selection in the distribution of this gene. This can be explained by (i) the low number of individuals studied (56), thus the result can not be extrapolated to the entire population of arctic charr in Lake Thingvallavatn; (ii) the ambiguity of the results, because some individuals showed different patterns of homozygous or heterozygous for *FGOP2* depending on the PCR; or (iii) the validity of the test.

To know whether *FGOP2* is under selection it will be necessary to increase the sample size (study 262 samples) and sequence the whole gene instead of using allele specific primers (with or without the deletion).

In conclusion, it is not possible differentiate between the three morphs studiying the *D-loop*, and in consequence, distinghish between the three expected populations that is supposed that they are forming because of a process of sympatric speciation. The study of *FGOP2* could in principle be more useful for this purpose, as it is a developmental gene involved in the head morphology. However the data do not show significant differences between the morphs, in harmony with the results from the *D-loop*.

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6 Apendices

6.1 Appendix A.

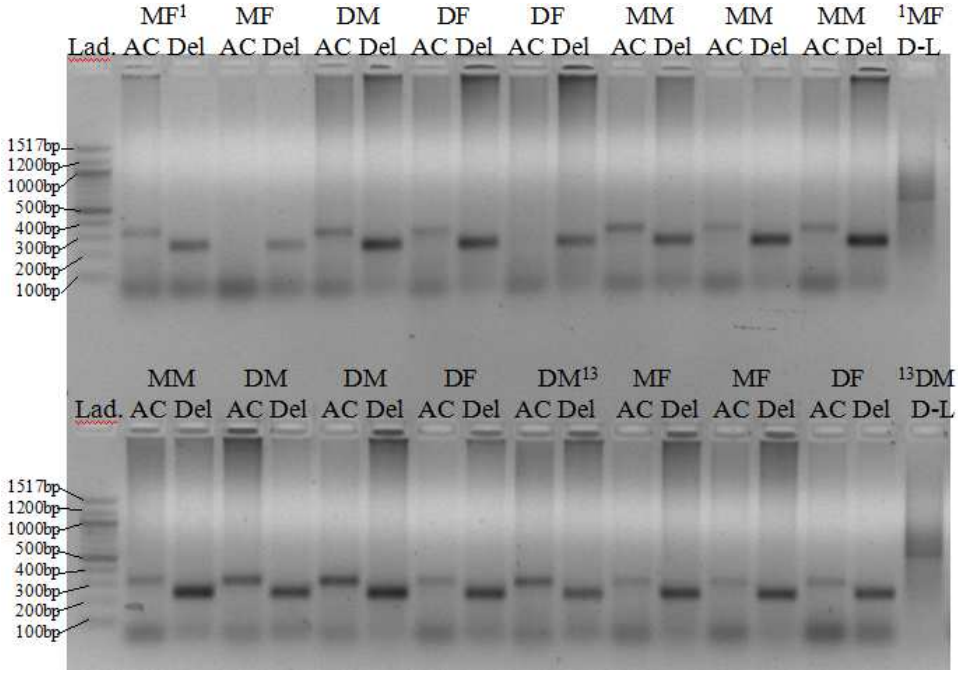

Figure A1. 2% Agarose gel of Allele specific PCR. AC (primer AC-front/FGOP2-reverse), Del (primers FGOP2-front/Del-reverse).

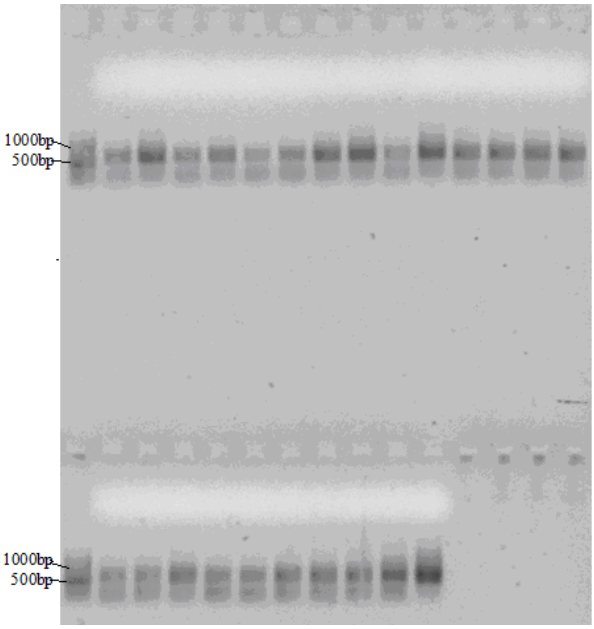

Figure A2. 1% Agarose gel of PCR for the D-loop. D-loop primers. Band of the D-loop aproximatly 600 base pairs.

6.2 Appendix B.

Raw output for AMOVA analysis for morph and sex.

Genetic structure to test :

No. of Groups = 3

[[Structure]]

StructureName = "Dloop"

NbGroups = 3

#Group1

Group={

"MM"

"MF"

}

#Group2

Group={

"DM"

"DF"

}

#Group3

Group={

"LB"

}

Distance method: Pairwise difference

AMOVA design and results :

Weir, B.S. and Cockerham, C.C. 1984.
Excoffier, L., Smouse, P., and Quattro, J. 1992.
Weir, B. S., 1996.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	2	0.146	-0.00477 Va	-4.44
Among populations within groups	2	0.818	0.00681 Vb	6.34
Within populations	221	23.276	0.10532 Vc	98.10
Total	225	24.239	0.10736	

Fixation Indices

FSC : 0.06072

FST : 0.01901

FCT : -0.04441

Significance tests (1023 permutations)

Vc and FST : P(rand. value < obs. value) = 0.02737

P(rand. value = obs. value) = 0.00000

P-value = 0.02737+-0.00494

Vb and FSC : P(rand. value > obs. value) = 0.00684

P(rand. value = obs. value) = 0.00000

P-value = 0.00684+-0.00231

Va and FCT : P(rand. value > obs. value) = 0.79374

P(rand. value = obs. value) = 0.06354

P-value = 0.85728+-0.00976

//

END OF RUN NUMBER 1 (06/05/11 at 16:01:07))

Total computing time for this run : 0h 0m 0s 30 ms

//

Raw output for AMOVA analysis for morph and sex.

Genetic structure to test :

No. of Groups = 2

[[Structure]]

StructureName = "Loc"

NbGroups = 2

#Group1

Group={

"OlM"

"OlD"

"OlLB"

}

#Group2

Group={

"MjM"

"MjD"

}

Distance method: Pairwise difference

AMOVA design and results :

Weir, B.S. and Cockerham, C.C. 1984. Excoffier, L., Smouse, P., and Quattro, J. 1992. Weir, B. S., 1996.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	0.751	0.00679 Va	6.00
Among populations within groups	3	0.297	-0.00025 Vb	-0.22
Within populations	221	23.545	0.10654 Vc	94.22
Total	225	24.593	0.11307	

Fixation Indices

FSC : -0.00233

FST : 0.05782

FCT : 0.06001

Significance tests (1023 permutations)

Vc and FST : P(rand. value < obs. value) = 0.02444

P(rand. value = obs. value) = 0.00000

P-value = 0.02444+-0.00534

Vb and FSC : P(rand. value > obs. value) = 0.46921

P(rand. value = obs. value) = 0.00000

P-value = 0.46921+-0.01782

Va and FCT : P(rand. value > obs. value) = 0.00000

P(rand. value = obs. value) = 0.12121

P-value = 0.12121+-0.01192

//

END OF RUN NUMBER 1 (06/05/11 at 16:46:11))

Total computing time for this run : 0h 0m 0s 15 ms

//

Raw output for AMOVA analysis for morph and sex.

Genetic structure to test :

No. of Groups = 2

[[Structure]]

StructureName = "Dloop"

NbGroups = 2

#Group1

Group={

"MjMM"

"MjMF"

}

#Group2

Group={

"OlMM"

"OlMF"

}

Distance method: Pairwise difference

AMOVA design and results :

Weir, B.S. and Cockerham, C.C. 1984. Excoffier, L., Smouse, P., and Quattro, J. 1992. Weir, B. S., 1996.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	0.025	-0.00733 Va	-5.63
Among populations within groups	2	0.537	0.00748 Vb	5.74
Within populations	98	12.742	0.13002 Vc	99.89
Total	101	13.304	0.13016	

Fixation Indices

FSC : 0.05438

FST : 0.00111

FCT : -0.05633

Significance tests (1023 permutations)

Vc and FST : P(rand. value < obs. value) = 0.17791

P(rand. value = obs. value) = 0.00000

P-value = 0.17791+-0.01148

Vb and FSC : P(rand. value > obs. value) = 0.07527

P(rand. value = obs. value) = 0.01271

P-value = 0.08798+-0.00568

Va and FCT : P(rand. value > obs. value) = 0.64614

P(rand. value = obs. value) = 0.35386

P-value = 1.00000+-0.00000

//

END OF RUN NUMBER 1 (06/05/11 at 17:20:51))

Total computing time for this run : 0h 0m 0s 15 ms

//