Present-Day Genetic Structure of Atlantic Salmon (Salmo salar) in Icelandic Rivers and Ice-Cap Retreat Models

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Abstract
Due to an improved understanding of past climatological conditions, it has now become possible to study the potential concordance between former climatological models and present-day genetic structure. Genetic variability was assessed in 26 samples from different rivers of Atlantic salmon in Iceland (total of 2,352 individuals), using 15 microsatellite loci. F-statistics revealed significant differences between the majority of the populations that were sampled. Bayesian cluster analyses using both prior information and no prior information on sampling location revealed the presence of two distinguishable genetic pools - namely, the Northern (Group 1) and Southern (Group 2) regions of Iceland. Furthermore, the random permutation of different allele sizes among allelic states revealed a significant mutational component to the genetic differentiation at four microsatellite loci (SsaD144, Ssa171, Ssp2201 and SsaF3), and supported the proposition of a historical origin behind the observed variation. The estimated time of divergence, using two different ABC methods, suggested that the observed genetic pattern originated from between the Last Glacial Maximum to the Younger Dryas, which serves as additional evidence of the relative immaturity of Icelandic fish populations, on account of the re-colonisation of this young environment following the Last Glacial Maximum. Additional analyses suggested the presence of several genetic entities which were likely to originate from the original groups detected.

Introduction
For decades, several types of genetic markers have been used to define populations’ boundaries in a multitude of species. An alternative approach has only been recently developed due to better understanding of past climatological conditions (i.e. the potential concordance between former climatological models and present-day genetic structure). Indeed, contemporary genetic patterns are known to be the result of both present-day and historical factors [1]. One of the most fundamental premises is that glaciation and inter-glaciation periods of the Pleistocene have shaped the genetic structure of many contemporary species. Present-day genetic structure of populations of a species inhabiting past refugia show a higher level of genetic diversity than those inhabiting formerly glaciated regions, due to range expansion and genetic drift following deglaciation [2,3]. In the last few decades, examples of concordance between climatological history and present-day genetic structure have been reliably noted within taxa inhabiting freshwater or land habitats [4–12], depending on the availability of climatological information and models. Accurate and precise models of environmental conditions and ice-sheet covers have only recently become available for the North Atlantic [13–17], and as a consequence the effect of glaciation on the genetic structure of marine species has only recently been investigated - especially so in the North Atlantic Ocean [13,18–24]. This is also true for the climatological history around Iceland, which has been only recently resolved [17,25–27].

Although phylogeography studies related to anadromous fish such as the Atlantic salmon [28,29] sometimes consider the effect of the Pleistocene on contemporary genetic patterns [30–32], so far no correlation has been made between the divergence time and climatological history of this species. The Atlantic salmon is a philopatric species exhibiting a complex biological cycle that includes spawning in rivers, a freshwater juvenile phase followed by subsequent oceanic feeding migrations, and a high fidelity to natal rivers [33]. This complex life-cycle is often considered the source of reproductive isolation, which facilitates the evolution and persistence of locally adapted populations [34]. In the last 20 years, genetic studies regarding the Atlantic salmon have flourished, in that they have confirmed that populations are highly structured both between and within rivers systems [35–38], and that the associated genetic pattern was usually temporally stable [39–41]. In Icelandic rivers, the only genetic study performed using allozyme data suggested restricted gene flow among rivers as well as within large river systems [35].

Although the complex biological cycle of the Atlantic salmon has been determined to be the primary source of potential genetic signal, another major factor that could have been the origin of the present-day genetic pattern in the Atlantic salmon is the geological
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Sampling and genotyping

A total of 2,352 salmon parr were sampled by electro fishing from 400–500 m stretches at 26 rivers in 2002 (Ellidaa, (25)) and 2004 [all others] (Figure 1, Table 1). Each sample consisted of 54–94 parr, comprising 3 or 4 year classes ranging from 1 to 5 years old. All samples were collected by the Institute of Freshwater Fisheries with full authority from the Directorate of Fisheries in accordance with Icelandic law on salmon and trout fishing number 61/2006, article 26, subparagraph two [45]. All fish were put to death in accordance with Icelandic law on “Animal Welfare number 15/1994, article 14” [46]. Samples were taken from across the species range in Iceland – the lack of samples obtained from the southern and eastern coast is due to the fact that the rivers in this region are mostly turbid, glacial-fed rivers, which are unsuitable salmon habitats [47]. Genotype information was retrieved from 15 microsatellite loci (Table S1), and both PCR condition and genotyping procedures were performed as described in Olafsson et al. [48].

Statistical analyses

Samples from early life stages contain only the genetic material of successful breeders – often of a single year- and may be biased towards particular families [49]. Full-sibling and half-sibling groups were identified within each sample using COLONY 1.2 [50]. After identifying any full sibling groups nestled within half-sibling families, we used a trial and error based method with HWE as a benchmark with which to assess the appropriate stringency level required to eliminate the sampling of siblings’ effect [49]. After repeated attempts of various combinations of tolerance level, we found that, by allowing only two full siblings and six half-siblings in each family, we were able to reduce the sib-ship effects sufficiently with reference to HWE. After the reduction of full-and half sibling groups in our samples, genetic variability was assessed as number of alleles, and the observed ($H_O$) and expected ($H_E$) heterozygosities and F-statistics [51] were calculated in GENETIX 4.05.2 [52] for each sample. The samples were also tested for conformation to Hardy-Weinberg expectations (HWE) with 4.05.2 [52] for each sample. The samples were tested for FST based on 100,000 simulated loci, providing an expected neutral distribution of $F_{ST}$ values and an estimate of p-value for each locus.

In order to infer genetic ancestry and to identify subgroups that have different genotype patterns, multi-locus genotypes were analysed by a model-based clustering algorithm with STRUCTURE 2.3 [58]. Three runs were performed for each K value, ranging from 1 to 26. We chose 500,000 iterations of the Gibbs sampler after a burn-in of 50,000 iterations, applying a model that allows for admixture whilst implementing a correlated allele frequency. To determine the most likely hierarchical level of genetic structure, we plotted values of $LnP(D)$ (the log probability of data) for each K and estimated $\Delta K$ statistics, which is based on the rate of change in $LnP(D)$ between successive K values [59]. We also analysed the data using the admixture model with the LOCPRIOR setting, which considers location information. STRUCTURE was also used to calculate the $F$ value for the implemented $F$-model for correlated allele frequencies which can be used in a historical inference [60]. The model for correlated allele frequencies developed by Falush et al. [60] assumes that, “$K$ populations represented in our sample have each undergone independent drift away from ancestral allele frequencies at rates parameterized by $F_1, F_2, F_3...F_K$ respectively”. These $F_1$ to $F_K$ values can be used to make evolutionary inferences (see [60], page 1570).

Evanno et al. [59] suggests that STRUCTURE tends to only capture the major structure in the data, although a subsequent STRUCTURE analysis performed on each identified cluster can potentially demonstrate a more intricate population structure within these clusters. This analysis was performed using a modified “hierarchical STRUCTURE analysis” as outlined by Vaha et al. [61] and Warnock et al. [62]. Calculations were made applying the same parameters for the Gibbs sampler and the burn-in iteration, but with $K$ ranging from one to ten; $\Delta K$ statistics were consequently calculated to decide the hierarchical structure at each level [59]. We therefore conducted a hierarchical analysis by performing similar Structure runs on each detected group (K) containing several samples. The estimated number of group (K) was based on $\Delta K$ statistics and changes in the pattern of $LnP(D)$ values.

A hierarchical analysis of molecular variance (AMOVA) was performed using Arlequin [55] to quantify the degree of differentiation between the post-hoc defined regions according to the Bayesian approach in STRUCTURE, i.e. for $K = 2$. A consensus neighbour joining tree of pairwise $D_A$ [63] between all samples was calculated and constructed using the software POPULATIONS 1.2.29 [64]. Genetic diversity indices, such as $H_S$ and Allelic richness, were then compared between groups of the uppermost hierarchical structure based on $\Delta K$ from STRUCTURE [58] using FSTAT [54].

To assess the potential impact of mutation vs. drift on the genetic pattern detected, we performed the allele-size randomization test [65] implemented in SPAgeDi 1.3 [66]. The objective of this analysis was to test whether $F_{ST} = R_{ST}$, where $R_{ST}$ is a stepwise mutation model (SMM)-based measure of genetic differentiation informed by microsatellite allele size variance. This is analogous to $F_{ST}$, and unbiased with respect to any differences in sample size between populations or any differences in variance between loci. When mutations contribute little to genetic differentiation, estimates of differentiation using $F_{ST}$ and $R_{ST}$ should show similar results. On the other hand, if stepwise-like mutations have contributed significantly to divergence, $R_{ST}$ should demonstrate a larger differentiation than that of $F_{ST}$. Potential historical signatures in the genetic data were therefore assessed by permutating allele sizes at each microsatellite locus among allelic states (max 20,000 replicates) to simulate distribution of $R_{ST}$ values ($pR_{ST}$) with 95% confidence intervals (CI).

and climatological history of the North Atlantic Ocean. Past geological and climatological history has, indeed, been shown to be at the origin of genetic structure and patterns of several land or marine organisms [3,10,42–44].  

Deglaciation following the Last glacial maximum (LGM) may, therefore, illustrate the process of river colonization, which would in turn partly explain the genetic structure of later generations of Atlantic salmon in Iceland. LGM is usually reported between 21–17 cal. kyr BP (calibrated years before the present) [17,25–27].

The objective of the present study was, therefore, to investigate the genetic structure of Atlantic salmon collected from 26 Icelandic rivers using 15 neutral markers, and to assess whether the genetic pattern observed could be attributed to the climatological history of Iceland.
To further clarify evolutionary history, the time of divergence was approximated using two different ABC approaches. First, the time of divergence was approximated using the Approximate Bayesian Computation (ABC) implemented in DIYABC v1.0.4.46 [67]. ABC is a Bayesian-based approach where the posterior distributions of the parameters in the model are predicted by replacing the calculations of the likelihood (probability of observed data given the values of the model parameters) by a measure of similarity between observed and simulated data [67]. The ABC method can be described as three sequential steps - the first step (simulation) simulates many multilocus data sets with characteristics similar to the observed data set; the second step (rejection) compares the simulated data set to the observed data set and retains simulations that are arbitrarily close to the observations whilst rejecting others; the final step (estimation) then estimates posterior distributions of parameters through locally weighted linear regression on the summary statistics associated with the retained simulations [68]. The tested scenario was that two populations of size $N_1$ and $N_2$ have diverged $t$ generations in the past from an ancestral population of size $N_1 + N_2$, assuming a stepwise mutation model with no indel mutations and a mutation rate $\mu = 10^{-4}$. In the first step, 1,000,000 datasets were simulated, and 1% of the simulated data sets that most closely fit the observed data were used to inform the posterior distribution of parameter values. The uniform prior distributions used in the DIYABC analyses ranged from 0–20,000 for both $N_1$ and $N_2$ and 0–50,000 for $t$. Secondly, the time of divergence was approximated using the popABC software [69] where we evaluate a model of an ancestral population of size $N_A$ that splits at time $T$ in the past to give two populations of size $N_1$ and $N_2$. Since the time of splitting, immigration occurs at rates $m_1$ and $m_2$, where immigrants into one population are drawn from the other population. The mean of mean mutation rate among loci was assumed to be $5 \times 10^{-4}$, normally distributed with a standard deviation of $5 \times 10^{-5}$. A mean mutation rate of $5 \times 10^{-4}$ is commonly assumed in demographic models [70], but is a little lower than pedigree-based estimates for autosomal microsatellites in humans [71,72]. Five million data points from the joint distribution of parameters and summary statistics were simulated, and the 50,000 points closest to each target set of summary statistics were used for regression-adjustment [73].

Results

Genetic variability and siblings

A total of 324 individuals (i.e. 13.78% of the total database) were discarded due to sibling effects within the sampling, ranging from 40 in Líta Laxa (12) to none in Laxa Dolum (10) (table 1). A total of 205 alleles were observed across the 15 loci, ranging from two alleles in Ssa14 and SsaD486 to 32 in SsaD144. The locus SsaD486 is usually considered a continental analysis marker, and one that is therefore poorly suited to small-scale genetic analysis,
and not polymorphic within eastern populations of Atlantic salmon [74]. However, it has been suggested to be particularly useful in discriminating between brown trout (Salmo trutta), salmon, and hybrids thereof [73]. Hybridization between the two taxa has been widely documented [76–78]. Based on this locus, two individuals were discarded from our samples as hybrids of salmon and trout, showing the exact allele sizes for SsaD486 as described in Perrier et al. [75]. This locus was monomorphic in 18 of the 26 populations, and the frequency of the second allele was 0.0029 overall, and thus we chose to discard it from subsequent analyses.

Only one sample deviated from HWE (i.e. the river Hvita Efst (6) (Table 1)); this was mainly due to locus Ssa2216 being out of HWE in that sample. The overall genetic variability of samples, as measured by gene diversity ($H_S$) and allelic richness ($A_e$), ranged from 0.630–0.730 and 6.78–9.23, respectively (Table 1).

### Genetic differentiation

An overall $F_{ST}$ value of 0.057 was observed on a sample level. The degree of genetic differentiation between sampling locations, as estimated by $F_{ST}$, ranged between 0.006 and 0.140 (Table S2). Highly significant genetic differentiations (Fisher’s exact test $P<0.01$) were observed between all samples except: Olfusa (20), Sog (16), Blanda (1), and Svarta (18). A PCA based on the $F_{ST}$ is presented in Figure S1. No signs of directional or balancing selection were identified at the 14 loci among the two populations (Figure S2).

The Bayesian cluster analysis and subsequent calculations of $\Delta K$ showed that the most likely number of genetically distinguishable groups ($K$) was two, both with $\text{Lnp}(D)^2\pm S.D. = -95402\pm 462$ and without location information $\text{Lnp}(D)^2\pm S.D. = -95725\pm 922$ (Figure 2). The first group (Group 1) was composed of samples from the northwest, north, and east rivers: 1; 3–5; 8–11; 13–15; 17–19; 23; 25–26 (Figure 1 green). The second (Group 2) was composed of samples from the southwest and south of Iceland: 2; 6–7; 12; 16; 20–22; 24 (Table 1, Figure 1 yellow). The average F-values as outputted by STRUCTURE for the $K=2$ runs were 0.032 and 0.060 for the two groups, Group 1 and Group 2 respectively. The population structure revealed by the hierarchical clustering method is outlined in Figure 3. Initial partitioning of the dataset at the 3rd level using $K=2$ separated the data into the two previously described groups, Group 1 and Group 2. Further levels of analysis within these and subsequent hierarchical groupings resulted in geographically comprehensive groupings at each level, leading to a total of eight major regional groupings at the 2nd and 3rd levels.
3rd level of analysis (Figure 3). Branches on the NJ-plot tree of the genetic distance $D_A$ [63] corresponded to the colours of the eight groups identified with hierarchical structure analysis (Figure 3). The clustering assignment results for $K = 2, 3, \text{ and } 4$, with and without location information, are presented in Figure S3. The AMOVA between groups detected by the Bayesian clustering method corresponded to the historical origin in the data. The observed level of genetic differentiation ($F_{ST}$) was 0.072 between the two groups (Table S3).

In addition, when the genetic diversity of Group 1 and Group 2 were compared, no significant trend was observed, although the gene diversity ($H_s$) and allelic richness ($A_r$) tended to be slightly higher in Group 1 than in Group 2, ($A_r = 8.436$ and 8.094, $H_s = 0.699$ and 0.685, respectively).

Historical origin

The random permutation of different allele sizes among allelic states at each locus revealed that estimates of $R_{ST}$ were significantly larger than the 95% CI range of the $R_{ST}$ values at four microsatellite loci (SsaD144, Ssa171, SSsp2201 and SsaF3), suggesting a mutational component to genetic differentiation. $R_{ST}$ was also larger than the $pR_{ST}$ values at SSsp2210, but not significantly. When considering the two groups detected with the Bayesian method, the overall estimate of $R_{ST}$ was also significantly larger than the 95% CI range of the $pR_{ST}$ values (Table 2).

In the case of the DIY ABC approach, the simulations were based on the scenario of two populations that were diverged $t$ generations ago from a shared ancestral population. The two populations seem to have diverged from 24.9 kyr BP (seven years generation time) to 17.8 kyr BP (five years generation time). In the popABC approach the time of divergence was estimated as 17,454 (8,485–26,323). Other parameters estimated by the popABC are available in Table S4. Both of these methods suggest that the genetic structure may have originated in the late Pleistocene, and that the two gene pools could have segregated before the last glacial maximum (LGM) in Icelandic waters.

Discussion

Sampling of siblings’ effect

An accurate result from population genetic analyses requires a random, independent sample from each population of interest. However, within a population the genetic patterns can differ among demographic groups, and attention to this factor is important in order to attain an accurate conclusion regarding population genetic structure [79]. Parr are often sampled for population genetic research as they are more accessible and abundant than adult salmon, but parr samples may lead to false conclusions concerning population genetic structure due to the small numbers of successful breeders and biased breeding success [80]. If differences in allele frequencies between adult salmon and parr samples are solely due to skewed sampling of full-siblings, then removing the majority of full-siblings from the sample database should in turn remove these differences. The program STRUCTURE has been shown to be sensitive to samples with related individuals and can in such cases detect genetic structure within a sample where there is none [81]. Therefore it is important to take the sampling of sibling effect into account, and the stringency applied in this study was in accord with the suggestions of Anderson & Dunham [91]. For our dataset, the effect of removing siblings is most apparent in the case of Líta Laxa (12), where the average $F_{ST}$ compared to other samples is 0.027 lower. Removing siblings also caused the genetic distance between the two samples from the Blanda river system to drop from significant to non-significant.

Genetic pattern, restricted gene flow, and discordance allozyme-microsatellite loci

Understanding the distribution and connectivity of populations has been one of the major challenges of recent decades, and has been recognized as a necessity for biodiversity conservation [82]. As such, indirect estimates of gene flow (i.e. the exchange of genes among populations due to the successful migrations of individuals) have received considerable attention in recent years. In marine organisms, populations that have been thought to be homogenous owing to a lack of obvious barriers have been shown to exhibit a clear genetic structure (i.e. restricted gene flow among populations on a small or large geographical scale [21,24,83,84]).

The Atlantic salmon exhibits a complex biological cycle involving stages that inhabit both marine and freshwater environments. Several genetic studies have demonstrated its propensity to form distinct and reproducitively isolated populations [37,38,85–87]. In the present study, substantial genetic differentiation was found between all collected samples except Olífsa (20) – Sog (16), and Svarta (18) – Blanda (1) (Table S2). Olífsa and Sog are indeed located in the Ölfus river system - the river Sog is a tributary to Olífsa, with 13 km between sampling sites. Svarta is a tributary of Blanda, with only six kilometres between these sampling sites. The geographical proximity of these areas might have precluded any genetic differentiation among the samples collected in these rivers.

One noteworthy result from the present study was the high divergence between almost all samples collected, among all river systems, whereas a previous genetic study using allozyme loci and 22 sampling locations identical to the present research found that a total of 52 out of 231 possible pair-wise comparisons were non-significant [35]. This is in concordance with the assumption that microsatellite markers yield a higher resolution power than allozymes when identifying populations, due to the presence of many more alleles [88]. A study by King et al. [37] found that Icelandic salmon populations seem to be less diverse than other salmon populations originating from Europe, but more diverse than salmon populations in the USA and Canada (the average gene diversity for the Atlantic salmon populations in Iceland in this study was 0.69, compared to 0.60 in the USA and Canada and 0.74 for the rest of Europe [37]). Although there are only five out of twelve comparable microsatellite markers between the two studies, the same pattern was observed looking only at samples included in King et al. [37]. The highest pairwise $F_{ST}$ value between salmon populations in Iceland was 0.14 found between Kálfa (7) and Laxa Adaldal (9). Kálfa appeared to be a very unique population genetic research as they are more accessible and abundant than adult salmon, but parr samples may lead to false conclusions concerning population genetic structure due to the small numbers of successful breeders and biased breeding success [90]. If differences in allele frequencies between adult salmon and parr samples are solely due to skewed sampling of full-siblings, then removing the majority of full-siblings from the sample database should in turn remove these differences. The program STRUCTURE has been shown to be sensitive to samples with related individuals and can in such cases detect genetic structure within a sample where there is none [91]. Therefore it is important to take the sampling of sibling effect into account, and the stringency applied in this study was in accord with the suggestions of Anderson & Dunham [91]. For our dataset, the effect of removing siblings is most apparent in the case of Líta Laxa (12), where the average $F_{ST}$ compared to other samples is 0.027 lower. Removing siblings also caused the genetic distance between the two samples from the Blanda river system to drop from significant to non-significant.
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The diagram shows two plots. The upper plot is a scatter plot with a logarithmic scale on the y-axis, labeled \( \ln p(D) \) vs. \( K \). The lower plot is a line graph with \( \Delta K \) vs. \( K \). The data points are represented by red and blue markers, and the lines connect the points to illustrate the trend.
using the pairwise FST comparison (Table S2). It should be noted that although we reduced the likelihood of false discovery by removing sibling groups from our samples, caution should be applied when interpreting FST values, as they can be inflated by the effect of family structures between streams [89] or the possibility of low intra-stream heterozygosity [90].

In all, the present study corroborated previous genetic studies performed on Atlantic salmon by suggesting high levels of genetic differentiation between populations both within river systems [35,38] and on a larger geographical scale [35,37,85–87]. Although historical climatological conditions in Iceland could explain the observed genetic pattern (see below), the present results suggest that gene flow tends to be restricted among Icelandic populations of Atlantic salmon.

When climatology meets genetics

The glaciation and interglaciation periods of the Pleistocene have considerably shaped the distribution and connectivity of contemporary populations [10–12,91–94]. Indeed, one of the major concerns about the genetic structure detected with microsatellite loci is that contemporary levels of differentiation might reflect a historical restriction of gene flow, rather than the current isolation of populations. Emerging evidence has shown historical signals embedded in microsatellite loci [4–6,10,11,20], especially in Icelandic waters [22–24,95] in which populations of marine organisms have colonized the ice-free environments following the LGM, examples of which are composed of relatively young populations on an evolutionary time scale.

Although the complex biological cycle of the Atlantic salmon may be the primary source of potential genetic signals, the past geological and climatological history of Iceland may in part have been the origin of the contemporary genetic structure of salmon populations. In Iceland, most of the shelf water was covered by an ice-cap during the LGM; although scientists do not fully agree on the exact dates that the coastline of Iceland was deglaciated, they generally do agree upon the order of deglaciation in different

**Figure 2.** Uppermost hierarchical structure based on ΔK. (A) Estimated likelihood, LnP(D) for values of K ranging from 1 to 26. The mean LnP(D) for each K over 3 runs are represented by red solid dots (●) and a blue diamond when considering location information (○). (B) ΔK calculated according to Evanno et al. [59]. The modal value of this distribution corresponds to the true K(*) or the uppermost level of structure, here two clusters. doi:10.1371/journal.pone.0086809.g002

**Figure 3.** Population structure as estimated from 3 levels of hierarchical STRUCTURE analysis (Pritchard et al. [58]). Populations are represented by vertical lines which are partitioned into shaded segments representing the populations estimated membership of the two or three possible clusters defined in the STRUCTURE run. Rows represent the hierarchical approach with subsets of populations separated and re-analysed. The subsets used are delineated below each plot. A consensus neighbour joining tree of pairwise D_A [63] between all samples is also presented; colors of the branches/groups correspond to the different groups detected in the hierarchical structure analysis. doi:10.1371/journal.pone.0086809.g003
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to increase the population
(HWE) exists within populations, such that genetic drift is the
distribution within Iceland. This pattern are still visible when examining the modern-day glacial
Iceland is unusual as it follows a north to south pattern dissimilar
southwest and south of Iceland (Figure 4). Although Iceland is
north and east of Iceland, before the final retreat occurred in the
(Figure 1). Thereafter, the ice-cap progressively retreated from the
Dryas. Based on these particular studies performed in Iceland, the
in Figure 4, on the time scale from 25 ka BP to the Younger
events during the ice cap deglaciation of Iceland is demonstrated
subsequently retreated well inside the coastline [27]. The order of
areas. It has been suggested that this event started circa 17.5–15.4
cal. kyr BP and that by 15.4–14.6 cal. kyr BP the glaciers
subsequently retreated well inside the coastline [27]. The order of
events during the ice cap deglaciation of Iceland is demonstrated
in Figure 4, on the time scale from 25 ka BP to the Younger Dryas.
Based on these particular studies performed in Iceland, the
first viable and ice-free environment for migrating salmon was
likely to be located at Breiðafjörður in the northwest of Iceland
(Figure 1). Thereafter, the ice-cap progressively retreated from the
north and east of Iceland, before the final retreat occurred in the
southwest and south of Iceland (Figure 4). Although Iceland is
small compared to other areas where a latitudinal gradient in
colonization would be expected, the icecap retreat model in
Iceland is unusual as it follows a north to south pattern dissimilar
to that of most other deglaciating areas [25], and the residues of
this pattern are still visible when examining the modern-day glacial
distribution within Iceland.

When considering neutral genetic markers the F-model assumes
that a linkage equilibrium and Hardy-Weinberg equilibrium
(HWE) exists within populations, such that genetic drift is the
only force acting upon the population. With time, genetic drift will
increase the population F-value, as was demonstrated by Group 2
having a higher F-value than Group 1. One possible interpretation
of these results is that Group 2 was possibly colonized from Group
1 [60], or alternatively represents a second colonization event from
another “source” population. However, other alternative processes
might explain these results such as Holocene bottleneck and a
smaller effective population size in the southern region (Group 2).

The genetic analyses, i.e. the existence of two main genetic
clusters and the higher F-value in the south, do not provide any
direct support for an initial colonisation in the north but are
nevertheless consistent with the ice-cap retreat model in Iceland
(and that the northern region was deglaciated first). When
considering the deglaciation model of Iceland it is highly probable
that pioneer salmon colonized the northern region before the
southern region. The first available habitat for salmon in Iceland
after the LGM is located in the Northwest region (Breiðafjörður)
approximately 17.5 ka BP. From there suitable habitats were
consecutively formed as the progression of deglaciation went north
and east. The estimated time of divergence (DIYABC and
popABC) demonstrated the existence and timing of subdivision
between the northern and southern populations, and suggested
that pioneer salmon were arriving in Iceland at a time when the
only viable habitats were in the northern region (Breiðafjörður).
Therefore, when we considered the available deglaciation model
for Iceland, the presented genetic results seemed to be consistent
with an initial settlement of Atlantic salmon in the northern region
of Iceland.

The proposed order of settlement offers the possibility of three
settlement scenarios: First, that the colonizing salmon inhabit the
northern region and subsequently colonize the south. Second, that
the colonizing salmon inhabit the north of Iceland and a second
colonization event from the same population of origin occurs in the
southern area. Third, that the colonizing salmon inhabit the
north of Iceland and a second colonization event from a different
refugium occurred in the southern area. Although we cannot
distinguish between the suggested three settlement scenarios, our
results suggest that the glaciation event in Iceland has imprinted
the present day genetic structure of Atlantic salmon.

Although a three cluster scenario suggesting a colonization
process consisting of multiple events cannot be completely ruled
out, the current analysis better support the two cluster scenario.
The additional within-river groups detected within each original
group (Group 1 and Group 2) during our three levels of
hierarchical structure analysis, and confirmed by the consensus
neighbour joining tree of pairwise D_{A} [63] which is expected to
produce the best branching tree pattern [96], might reflect more
recent historical colonisation of river systems and current drift
events due to recent isolation of populations (well after the
colonisation). It might therefore represent the step-by-step
colonisation of the different river systems, and more recent
isolation events. Although Icelandic salmon populations are highly
reproductively isolated, there is a possibility of gene flow between
the two population groups - the DIYABC method does not take
this into account, but this limitation is met by adding an analysis
using the popABC program which allows for the modelling of gene
flow between groups. Furthermore, minor inter-group gene flow
might affect the estimates by underestimating the divergence time,
but neither of these properties affect the glacial period in question.

When history explains the exceptions

The rivers Ellidaa (25), Leirvogsa (11), and Langa (23) seemed
to be misplaced in these ice-free environment re-colonisation events;
however, a closer inspection of Icelandic volcanic history and of
contemporary human activity offered explanations for these
anomalies.

The lava field, Leitárhraun, was formed in a volcanic eruption
5,200 years ago [97], and stretches from the Bláfjöll down to
Ellívatn lake, where it formed the rootless volcanic cones,
Rauðhólar, and from there it ran down to what has become the
present river channel of Ellidaa (25) [98] (see insert on Figure 1).
No pioneer salmon in Ellidaa (25) could have survived this natural
event, therefore we suggest that a re-colonization event occurred,
possibly shifting the river from its presumed geographical origin.
Unknown human activity that might have caused this seems very
unlikely but cannot be completely ruled out.

| Table 2. Mean single locus and multilocus estimates of $R_{ST}$, $\theta_{ST}$ and $p_{R_{ST}}$ (95% distribution of central values in parentheses) between 26 samples of Atlantic salmon following 20 000 allele permutations (Hardy et al. [65]). |
|-----------------|-------|-------|-----------------|
|                | $\theta_{ST}$ | $R_{ST}$ | $p_{R_{ST}}$ (95% range) |
| Ssa14           | 0.054 | 0.054 | 0.0541 (0.0541–0.0541) |
| Ssa171*         | 0.073 | 0.138 | 0.0716 (0.0323–0.1446) |
| Ssa197          | 0.052 | 0.046 | 0.0526 (0.0178–0.1231) |
| Ssa202          | 0.045 | 0.042 | 0.0453 (0.0191–0.0752) |
| Ssa289          | 0.075 | 0.047 | 0.0673 (0.0319–0.0855) |
| Sp1605          | 0.066 | 0.023 | 0.0636 (0.0298–0.1007) |
| Sp2201*         | 0.036 | 0.065 | 0.0360 (0.0137–0.0675) |
| Sssp2210        | 0.081 | 0.094 | 0.0822 (0.0304–0.1546) |
| Sp2216          | 0.057 | 0.020 | 0.0554 (0.0253–0.0881) |
| Sssp3016        | 0.046 | 0.038 | 0.0473 (0.0201–0.0885) |
| Ssa144**        | 0.053 | 0.132 | 0.0521 (0.0148–0.1044) |
| Ssa157          | 0.032 | 0.002 | 0.0311 (0.0125–0.0597) |
| Ssa143**        | 0.079 | 0.109 | 0.0720 (0.0231–0.1114) |
| SsspG7          | 0.085 | 0.075 | 0.0829 (0.0271–0.1550) |
| Multilocus      | 0.058 | 0.035 | 0.0312 (0.0128–0.0596) |
| Group 1 vs Group 2* | 0.034 | 0.091 | 0.0356 (0.0073–0.0847) |

*P<0.05.
**P<0.01.
doi:10.1371/journal.pone.0086809.t002
Regarding Leirvogsa (11), the river was probably inaccessible for salmon in its original state as there were cascades near the estuary. However, after the cascades were artificially made accessible to enhance rod fishery, the river became known for its sea-run brown trout. Today, Leirvogsa (11) is a river with a healthy salmon population since the river became stocked with parr and smolts originating from Ellidaa (25).

In Langa, (23) there are two waterfalls at the estuary - Sjávarfoss and Skuggafoss - that were possibly impassable for salmon. Skuggafoss waterfall proved especially difficult for salmon to pass until a fishway was built in the 1950s [99]. In the 1960’s and 1970’s the river was stocked with salmon parr and smolt, which originated from stocks of various origins. Four additional fishways were installed in waterfalls further upstream, thereby increasing the salmon habitat from 13 km to 26 km. This high level of human intervention could easily have altered the genetic signature of this river from its logical position in the gene pool of Icelandic salmon.

Conclusion
The present study has yielded two important findings. Initially, that microsatellite loci revealed a significant genetic signal amongst the populations collected at 26 rivers distributed across the country, a result which suggested a restricted gene flow common to the sampled populations. Secondly, that the time of divergence between the two primary genetic groups detected was highly consistent with the ice-cap retreat model in Iceland, and that further analyses provide overwhelming evidence of a historical component to the observed genetic differentiation. The individual analysis of population structure strongly supports the presence of two genetically distinct groups, with a general trend of higher genetic variability in the northern areas in relation to that of the southern regions, thus substantiating the expectation of the existence of higher genetic variability in the potential initial area of colonization. The rivers Ellidaa (25), Leirvogsa (11) and Langa (23), however, do not seem to fit this re-colonisation hypothesis, although the volcanic history of Iceland and the history of human activity for the previous 60 years could be attributed responsibility for the displacement of these rivers in the presented salmon histories. In addition, the additional variation observed within each predominant group (Group 1 and Group 2) was likely due to more recent events of colonisation or population isolation, subsequent to the recolonisation event after the LGM. We therefore conclude that the observed genetic pattern at microsatellite loci for the Atlantic salmon in Icelandic rivers serves as additional evidence of the relative immaturity of Icelandic fish populations, on account of the re-colonisation of this relatively young environment following the Last Glacial Maximum.
**Supporting Information**

**Table S1** Microsatellite loci characteristics assessed as repeat motif (RM), number of alleles (n), allelic richness (Ar), allelic size range (size range), gene diversity (Measured as expected heterozygosity, He) and overall \( F_{ST} \) and \( R_{ST} \).

**Table S2** Pairwise \( F_{ST} \) values (below diagonal) and their corresponding p-values for 0.01 significance level (above diagonal) for all sample pairs, as calculated in Arlequin.

**Table S3** Hierarchical analysis of variance (AMOVA) among samples of Atlantic salmon grouped into the two \textit{a priori} groups detected with STRUCTURE. The variance among groups relative to the total variance, the variance among samples within groups and the variance among samples relative to the total variance are presented. The source of variation from among groups, among samples within groups, and within samples is given as a percentage for each comparison. All F-values were highly significant (P < 0.0001).

**Table S4** The estimation from popABC with 95% confidence interval in parenthesis for splitting time (T), effective population sizes (N1, N2, NA) and migration rates (m1, m2).

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