Marine Scotland Science Report

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AN OVERVIEW OF POPULATION GENETIC STRUCTURING IN THE ARGYLL FISHERIES TRUST

Prepared as part of the Focusing Atlantic Salmon Management on Populations (FASMOP) Project

Delivered in partnership with the Rivers and Fisheries Trusts of Scotland (RAFTS)

M W Coulson, L M I Webster, A Kettle-White, A Armstrong,

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An Overview of Population Genetic Structuring in the Argyll Fisheries Trust

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Executive Summary

This report describes how genetic information from juvenile Atlantic salmon sampled from nine river systems within the Argyll Fisheries Trust (Figure 1) have been analysed in order to help inform developing fisheries management activities. The key objective for the Trust was to define the genetic structure of the locations under investigation, in order to determine whether salmon in the area represent distinct breeding populations.

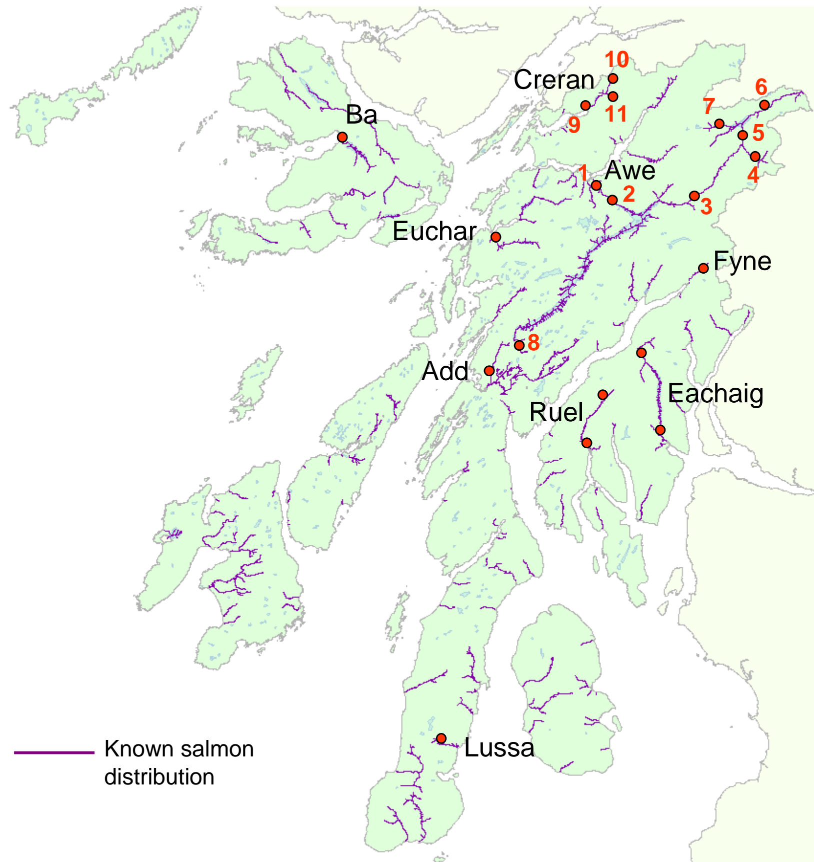


Figure 1. A map of the Argyll Fisheries Trust area, with sample sites that are involved in this report indicated in red with associated major river names. Red numbers adjacent to sites on the Awe system will be referred to as following in the text; 1. Awe A, 2. Awe B, 3. Orchy lower, 4. Kinglass, 5. Orchy upper, 6. Water of Tulla, 7. Abhainn Shira, 8. Clachan Dubh Burn, 9. Creran lower, 10. Creran upper, 11. Ure.

Summary of findings

The analysis showed that, in the majority of cases, there are significant but variable levels of genetic differences between the sites examined. The Awe in particular exhibits large differences to all other systems as well as among sites within the Awe system. This is particularly true above and below Loch Awe. Other sites around the Trust show lower levels of genetic differences among them, with the rivers Creran and Ba being the most distinct.

The differing scale of genetic differentiation observed within Argyll is largely reflected by the ability to predict where a sample is from using only genetic information (genetic assignment); where genetic signatures are strongly related to location, individuals are more likely to be assigned to the location from which they were originally sampled. The average value of correct assignment to system is 65%, which is much higher than you would expect if there was no genetic structure in the data. However, the ability to assign individuals correctly varied considerably across the geographic range studied.

Implications for management

The aim of the current FASMOP project was to identify distinct breeding populations of salmon within Argyll. The results to date suggest that each river system likely represents a distinct breeding population. However, there is also evidence for multiple breeding populations within several systems, particularly the Awe.

For the purposes of stocking programmes, these results encourage a continued caution with respect to sourcing brood stock. The genetic differentiation observed among sites is strongly indicative of distinct breeding populations, which could be associated with adaptations to local conditions. If this is the case, then sourcing brood stock locally should provide the best chance of maintaining any local adaptations that lead to increased survival.

The power to assign fish of unknown origin to their location of origin with high accuracy is possible where candidate locations show strong genetic differentiation. This could be useful, for instance, in assigning rod caught adults to their natal river allowing identification of the geographic origin of distinct components (e.g. spring salmon) of a river stock. The assignment to the Awe, Orchy, Clachen Dubh burn and the Creran are all approximately 80% or greater, but assignments to the other sites are much more variable (30% to 70% depending on the system). In order to improve assignments and gain better distinction for potential breeding populations, larger sample sizes and/or additional genetic markers will be required as well as a more complete baseline of potential populations sampled.

Introduction

Atlantic salmon (*Salmo salar* L.) are one of the world's most widely recognized and prized fish species. However, declines in numbers across much of the species' range have been cause for concern and the focus of intense management schemes and research efforts. Central to these efforts is the recognition of how the species is structured spatially across both broad and fine scales. Given the large native range encompassed by the species and their well-known ability to home to natal rivers, it is expected that Atlantic salmon will demonstrate a considerable degree of population structuring, representing discrete breeding units that are reproductively separated to varying degrees (Webb et al. 2007; King et al. 2007). This reproductive separation underlies the basis for locally adapted traits to establish across a widely variable environment. Indeed, ample evidence exists in favour of local adaptation in many salmonid species (Taylor 1991; Garcia de Leaniz et al. 2007; Fraser et al. 2011).

A principal tool used to resolve population structuring is the use of genetic markers. Such approaches have been used for decades, with most of the contributions towards salmonids occurring within the past 20 years (Verspoor, 2007). Initially, this work focused on range-wide patterns of differentiation (e.g. King et al. 2001; Verspoor et al. 2005) or documenting the expected structuring existing between different river systems (e.g. O'Reilly et al. 1996; Fontaine et al. 1997; McConnell et al. 1997; Spidle et al. 2003; Castric and Bernatchez 2004; Dillane et al. 2007). However, not as much is known about structuring within river systems and few examples exist for Scottish rivers (but see for example, Verspoor et al., 1991; Jordan et al., 2005).

Ecological studies have shown that different tributaries within a river may exhibit differences in traits such as run-timing (Stewart et al. 2002, 2006), variation in age at smolting (Englund et al. 1999) and sea-age at maturity (Niemela 2006), among others. When such differences are shown to have a genetic basis (e.g. Stewart et al. 2002, 2006), then salmon breeding in separate locations, for instance, above and below waterfalls or other natural features may often be heritably different in ways that affect their behaviour, survival and reproductive success. This may be true of neighbouring tributary populations and, since intermixing of these populations runs a risk of unknown magnitude, may not be desirable. Indeed, at its worst, mixing in vulnerable populations could have long term negative effects on population viability by reducing survival (McGinnity et al., 2003). Recent genetic analyses of Atlantic salmon have indicated that rivers may be structured on fine scales into multiple distinct breeding populations. Such studies have used both neutral genetic markers (Garant et al. 2000; Vaha et al. 2007; Dionne et al. 2008; Dillane et al. 2008) as well as markers for which there is an underlying basis for natural selection (Landry and Bernatchez 2001).

The suite of genetic markers used in the current survey are “neutral” (meaning they are not known to be linked to heritable characteristics that may differ among locations such as run timing, growth rate, etc.). They will therefore largely reflect the shared ancestry of salmon among sites rather than make a direct assessment of the heritable trait characteristics that differ between them. Differences at such markers may *imply* that groups are sufficiently distinct for heritable trait differences to have evolved, however, where no difference is observed, we cannot rule out the possibility that these sites differ in heritable traits.

Given the recognition of the ‘population’ as a focal unit for management, it follows that knowledge of the genetic structuring among sites is required for certain management and conservation schemes. The potential for multiple, distinct populations to become established within a single river means that detailed knowledge needs to be gained regarding the scale at which such populations occur. As a first step in this process, a baseline for systems needs to be constructed and built upon as more information becomes available.

In 2009, a partnership between the Rivers and Fisheries Trusts of Scotland (RAFTS), Marine Scotland Science (MSS), and the participating individual Fisheries Trusts and Boards was established. It set out to undertake a Scotland-wide survey of genetic structuring within all Scotland’s major salmon-producing rivers. This project, entitled **F**ocusing **A**tlantic **S**almon **M**anagement **O**n **P**opulations (FASMOP), had as its central aim to undertake a program of genetic sampling of Atlantic salmon stocks in river systems across Scotland. The purpose of this sampling scheme was to define the genetic structure among locations in order to determine whether salmon within and among the various systems in a given area represent distinct breeding populations This work, alongside the EU SALSEA-MERGE and other MSS projects, is creating a genetic map of salmon populations across Scotland, to help inform management and conservation efforts.

Summary of Methods

Juvenile salmon from various locations around Argyll systems were sampled for genetic material by the Trust in order to inform fisheries management following methods outlined by Verspoor and Laughton (2008). Figure 1 shows the locations of the 20 sites that have been included in the genetic analysis for the Argyll Fisheries Trust. Samples generally consisted of fry and/or parr (n= 12-61, depending on site) and for each individual, data from 17 genetic markers (microsatellites) were collected. The results from the microsatellite marker SsaF43 allowed us to identify any trout or trout/salmon hybrids that may be present among samples. These individuals were then removed prior to analysis.

It is possible that samples are more reflective of families rather than populations, given the life-history stage(s) targeted by sampling and the potentially fine-scale geographic coverage (Hansen et al. 1997). This occurrence can alter the genetic signature of the sample and obscure population level differences. Therefore, prior to population level analyses, each site was screened for the presence of full-siblings, representing family groups and when identified, all but one individual of a full-sib family were removed. Additionally, this analysis can estimate how many breeders contributed to producing each sample, which may include contributions from precocious parr. Initial sample sizes as well as sample sizes after full-siblings were removed are presented in Table 1.

Where there was more than one site sampled within a 5-km distance, two life-history stages (i.e. fry and parr) sampled at the same site and/or a site was sampled in different years, the data were initially tested for differences using the program CHIFISH (Ryman 2006). Where no significant differences were found, data from these sites or time points were combined; otherwise they were left separate for all further analyses. This resulted in 20 sites for subsequent analyses.

Data were then analysed using standard population genetic methods to evaluate the genetic relationships and groupings among the sample sites in order to obtain a general overview of population structure and address the objectives of the Trust.

A detailed methods and analysis section can be found in Appendix 1.

Results

Broadly speaking, most sites exhibit significant genetic differences from one another that are inconsistent with the fish at the sites belonging to a single genetic population. It is the interpretation of these differences in terms of the relationships among populations, combined with the known history and geographical proximity of sites that can be useful to inform fisheries management decisions. Here we discuss the results of the FASMOP project summarizing the main genetic findings in terms of population genetic structuring of the systems.

Family effects

A total of six hundred and ninety-one juvenile salmon from Argyll were involved in the genetic analysis. All sites were examined for family effects with relatively few samples being removed due to full-sibling relationships (Table 1). The level of family effects differed between samples with the largest family present in the individual samples ranging from 1 to 8 and the samples subsequently being reduced by 0-40%. Family

effects were controlled for at each site before all further analyses. There were no trout or salmon/trout hybrid samples identified at any location.

Table 1

Details of samples used for this analysis, including original sample size, and details of each site following COLONY analysis to re-construct family relationships.

Site	Site ID	Original sample size	Sample size analysed (sibs removed)	Number of breeders contributing to sample	Largest single family	Year sampled
Ba	BAM	31	23	34	3	2008
Awe A	AWA	57	48	63	3	2007
Awe B	AWB	34	33	55	2	2007
Orchy lower	ORL	30	30	43	1	2009
Kinglass	KIN	30	28	30	2	2009
Orchy upper	ORU	30	28	37	2	2009
Abhainn Shira	SHI	22	21	35	2	2004
Water of Tulla	TUL	23	20	27	2	2004
Clachan Dubh	CLA	30	18	25	6	2007
Euchar	EUC	60	60	87	1	2005/2008
Add	ADD	53	47	74	5	2007
Lussa	LUS	50	38	58	4	2007
Ruel lower	RUL	17	17	27	1	2008
Ruel upper	RUU	22	19	33	2	2007/2008
Eachaig lower	EAL	37	33	46	2	2008
Eachaig upper	EAU	12	11	20	2	2008
Fyne	FYN	61	44	47	8	2002/2010
Creran lower	CRL	29	25	35	2	2009
Creran upper	CRU	28	19	29	4	2009
Ure	URE	35	24	28	5	2009

Population structuring

The genetic differences among sites are variable in magnitude but 98% (186 out of 190) of pairwise comparisons were significantly different (Appendix 2). Some sites show substantial differences, whereas others show a closer genetic relationship – perhaps sharing more recent ancestry or even some exchange of individuals (migration) among breeding locations. A visual representation of these relationships among locations can be found in Figure 2, which uses multi-dimensional scaling to represent pairwise estimates of genetic differentiation among sites (Appendix 2). Points which are closer together on the plot have a more similar genetic makeup while points further apart are more genetically discrete.

The largest differences are seen to the sites within the Awe system, which are all on the right-hand side of the plot in Figure 2, with the exception of the Clachan Dubh burn. This latter site is a tributary in the south of the catchment and does not group with the other sites in the north of the Awe-Orchy system. Furthermore, within the Awe-Orchy system, there are two distinct groups. The lower Awe sites A and B are close together towards the top-right of the plot, whereas sites from the Orchy are all to the bottom right (Figure 2).

The sites to the left of the plot in Figure 2 contain all other systems than the Awe-Orchy. Within this group, the Ba and the Clachan Dubh Burn sites are most different followed by the upper Eachaig and a couple of sites on the Creran. From the geographical positions of these locations, one on an island (Ba), two above freshwater lochs (Clachan Dubh Burn, upper Eachaig), and one contained within a narrow sea loch (River Creran), one might expect these to be distinct. The remaining locations on the MDS plot, however, are also quite geographically separate, yet the magnitude of differences among them is smaller, and without any obvious geographic pattern; sites further apart geographically are not necessarily further apart on the plot.

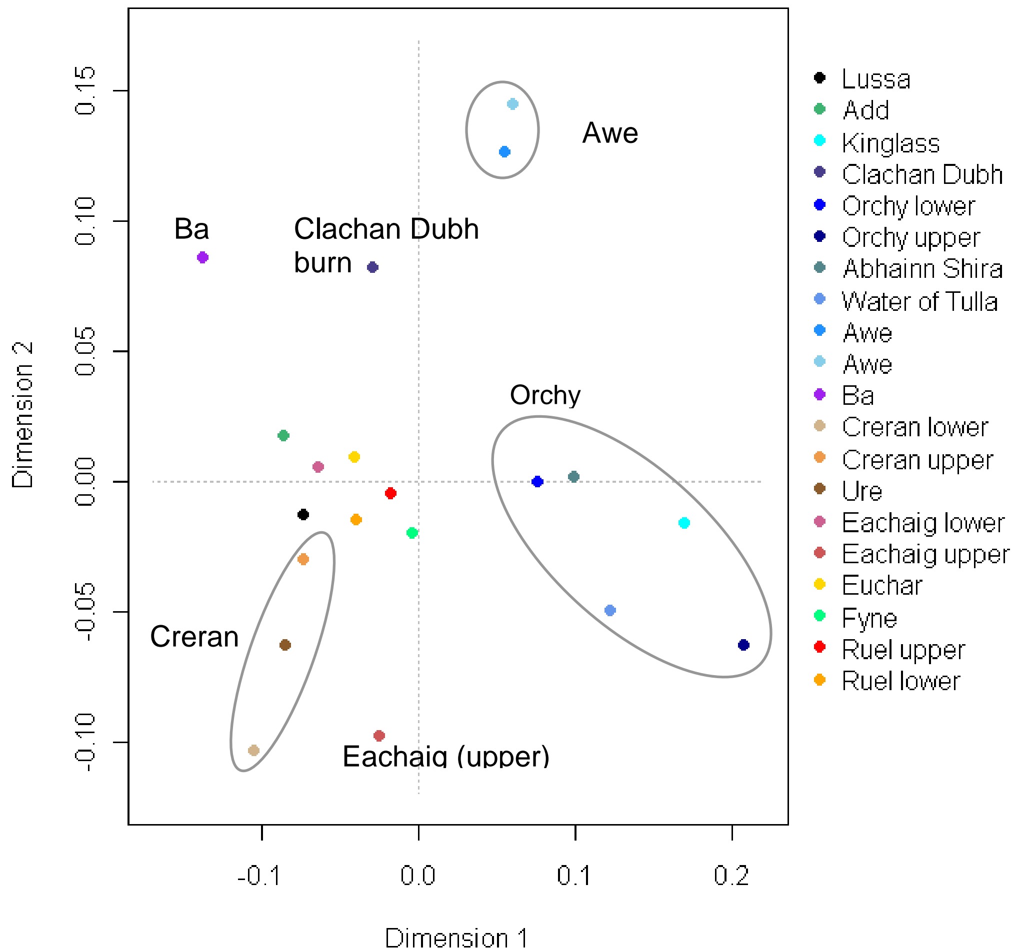


Figure 2

Multi-dimensional scaling (MDS) plot of genetic relationships among all sites based on pairwise estimates of genetic differentiation (Jost's D; see the appendix for details). Points which are closer together on the plot have a more similar genetic makeup while points further apart are more genetically discrete.

Four of the pair-wise comparisons show no significant differences (Appendix, Table 2), and most of these involve the Ruel sites and others situated in the Firth of Clyde. This could be, in part, due to the small sample sizes at these two locations (n=17 & 19 after family effects removed). Finally, the comparison between the Abhainn Shira and Water of Tulla is also not significant. This could also be due to relatively small sample sizes, however, these two sites are located relatively close together on the same system and therefore it is not surprising that they are more genetically similar.

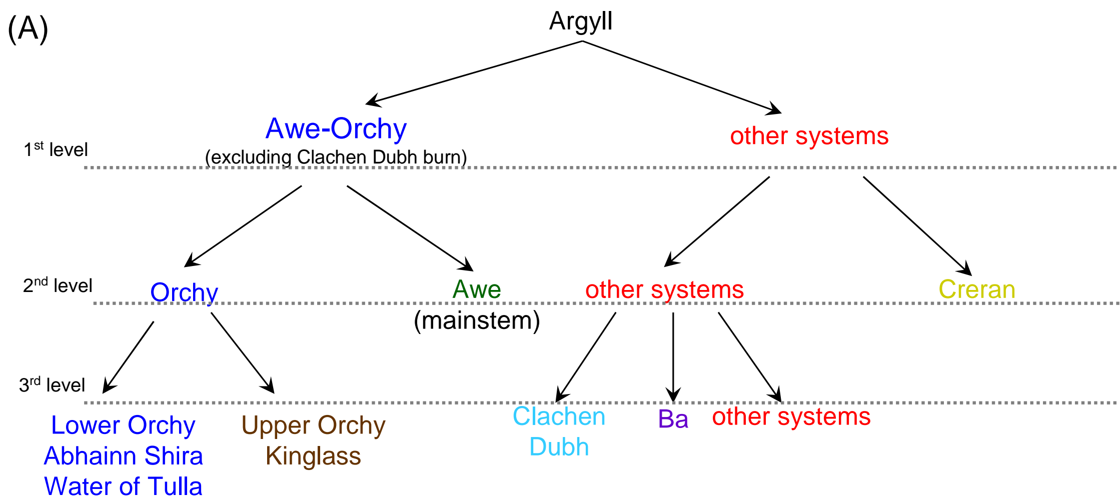
A clustering analysis that explores possible groupings of individuals other than the defined sampling sites, generally confirmed the differences observed in the MDS plot in

Figure 2. This analysis was done in a hierarchical fashion, as larger genetic differences among groups may obscure weaker differences at smaller spatial scales. For each level, the analysis aims to determine the most likely number of groups and the membership of each individual into those groups. Each of the groupings identified by the first round, are then separately analyzed in a second round and this process is repeated until further identification of groupings is not possible. At the broadest level, the analysis revealed two most likely groups which were (1) the Awe-Orchy system versus (2) all other sites (Figure 3A). As noted earlier, the Clachen Dubh burn grouped with all other sites at this broadest level rather than with Awe-Orchy sites. Within just the Awe-Orchy sites (blue, Figure 3A), a second round of clustering analysis revealed a further two groups, which were (1) the two Awe sites (mainstem) versus (2) the rest of the sites further up the Orchy system (5 sites). Among these latter five sites, these could be further divided into two groups: (1) Kinglass and upper Orchy versus (2) Lower Orchy, Water of Tulla and Abhainn Shira (Figure 3A).

Among the non-Awe-Orchy sites (red, Figure 3A), a separate clustering round of analysis revealed two distinct groups: (1) the three sites on the Creran vs. (2) all other sites. When the Creran sites were removed and the analysis repeated, three subsequent groupings were evident: (1) Clachen Dubh burn, (2) Ba system and (3) the remaining sites (Lussa, Add, Eachaig, Euchar, Fyne and Ruel). Below this level of analysis, clusters showed less confidence indicating a lesser degree of genetic differentiation. This does not mean, however, that there are not significant genetic differences below this level, but that using this clustering approach, these smaller differences are more difficult to tease apart and the distinction for choosing a particular number of groups is less obvious.

The results of the successive rounds of the clustering analysis are summarized in Figure 3B. In total, there appear to be at least seven distinct groupings, reflected by the various colours (Figure 3B). These results suggest that in spite of some substantial differences among sites within Argyll, the pattern of differences is not always easy to interpret. On the one hand, some of the largest differences seen are within a single system (the Awe, containing four of the seven clusters), and yet other sites which are more geographically separate than those within the Awe system (e.g. Eachaig lower and Lussa) appear to have a closer relationship to one another (Figure 2, Figure 3B).

(A)



(B)

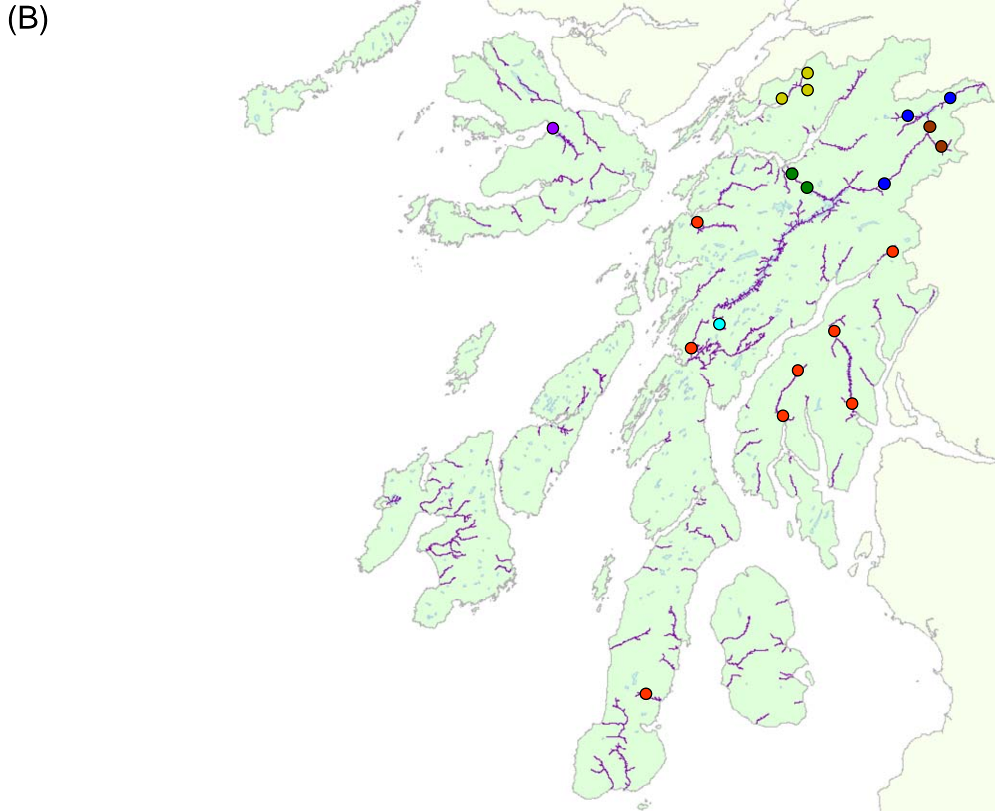


Figure 3

(A) Schematic showing the order in which the different clusters or groupings were resolved according to the hierarchical analysis. (B) Geographic representation of the relationships among sites, following a cluster analysis (STRUCTURE; see appendix). Locations with the same colour are more similar to one another and belong in the same cluster.

Genetic assignment of individuals

The assignment analysis shows how useful this baseline genetic information is to identify which of the sampled sites a fish of unknown origin is from (Figure 4). Each individual fish is taken in turn and it is assessed from which of the sampling locations provided in the baseline, is that individual most likely to have originated. Assignment of fish back to their specific site of collection was, on average, correct 54% of the time. However, this assignment improves to over 65% on average if we group locations within a system and assign individuals to river (still allowing for the big split in the Awe system, Figure 4). Accuracy of assignment to some sites needs to be taken with caution as they become a "catch-all" site for mis-assigned samples. For instance the assignment accuracy of ~60% to the Euchar is misleading as a disproportionate number of samples from across the Trust get assigned to this site. In fact of all the fish assigned to the Euchar, only ~25% were actually sampled there. Each of these averages is much better than would be expected if assignments were purely random (11 groups, random = ~9%). This reflects the population genetic structure underlying the data, but the magnitude of differences observed with the current markers among sites is not large enough to assign fish to location of sampling with higher accuracy for all cases. However, relatively high assignment (>80%) is seen for the Orchy, Awe, Clachen Dubh and the Creran, reflecting the fact that these sites were most differentiated based on the MDS plot (Figure 2) and by the clustering analysis (Figure 3).

It may be possible to improve accuracy by implementing a cut-off rule for the probability that an individual gets assigned or by allowing individuals to not be assigned to any of the sites in the baseline (i.e. came from an unsampled population). For example, if we assign only fish that have a minimum of 70% assignment probability, then we can improve the accuracy of assignment to system by ~20% percent (data not shown). However, this increased assignment comes at a cost, as now only 30% of all fish are assigned anywhere if this cut-off criterion is used.

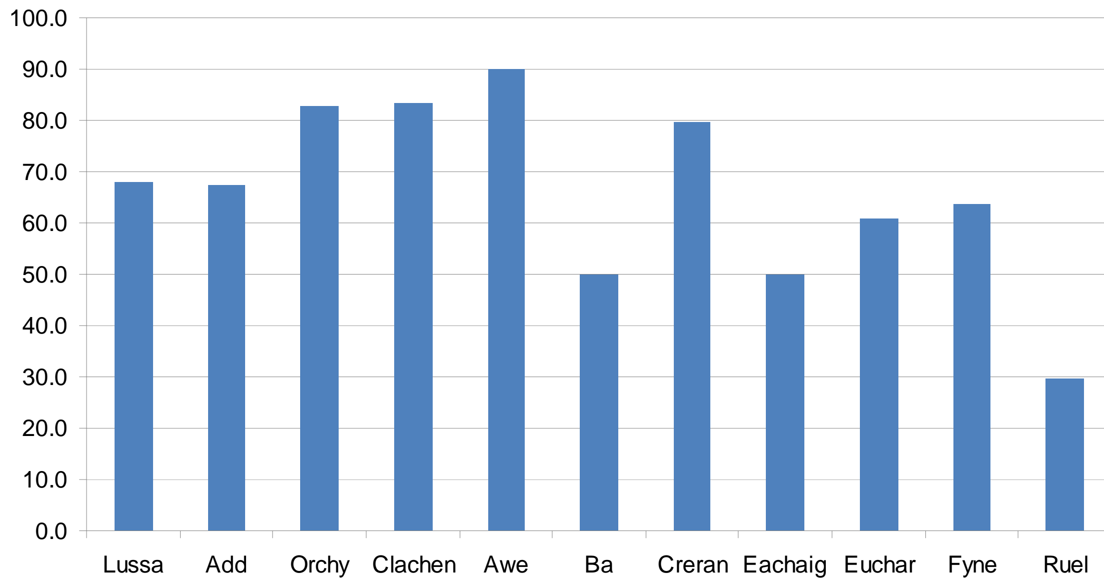


Figure 4
Percentage of fish sampled from each site that correctly assign back to that site.

Discussion

Population structure

The aim of the FASMOP project for the Trust was to identify distinct breeding populations of salmon within Argyll. The results to date strongly indicate that each river system likely represents a distinct breeding population. However, there is also evidence for multiple breeding populations within a system. This is most noticeable in the Awe system, which contained four of the seven groupings identified by the clustering analysis. The separation of the Awe from the Orchy sites to the north is likely due to the spawning and juvenile habitat discontinuity presented by the large loch (Awe) separating the two parts of the river.

The presence of lochs appears as an influential boundary for separating salmon populations and has been seen in other systems throughout Scotland (unpublished data). At the finest scale within the Orchy groupings, it is curious that the lower Orchy groups more closely with the Water of Tulla and Abhainn Shira sites, than with the Upper Orchy and Kinglass sites. There has been some stocking (2000-2006) in the Tulla tributaries and Kinglass sites using broodstock sourced from below Loch Tulla (AKW, personal communication). This may, in part, explain the closer relationship between the

Tulla and lower Orchy. However, a more targeted approach would be necessary to properly address this issue.

Beyond the major split between the Awe and Orchy groupings, the Creran and the Ba were the next most distinct systems. The grouping of the Clachan Dubh burn outwith the Awe-Orchy system at the broadest level may reflect historical changes in catchment boundaries, possibly associated with previous periods of glaciation. The remaining systems, while all significantly different from one another, show a smaller degree of differentiation between them than to the Awe-Orchy, Creran and Ba systems (Figure 2; Appendix 2). Overall, this suggests that while there is genetic structuring within Argyll, the scale of the structuring is quite variable.

For the purposes of stocking programmes, these results encourage a continued caution with respect to sourcing brood stock. The genetic differentiation observed between sites is inconsistent with a single genetic population of salmon within Argyll. Therefore, this suggests that there are distinct breeding populations, which could be underpinned by adaptations to local conditions. If this is the case, then locally sourced brood stock should reduce the risk of disrupting any local adaptations that lead to increased survival.

Genetic assignment

The power to assign fish of unknown origin to their location of origin with high accuracy is possible where candidate locations show strong genetic differentiation. This allows one to calculate the probability that a given fish originated from a particular location. Then the location with the highest probability is usually taken as the site from where that individual originated. This is done for each individual and Figure 3 shows the proportion of individuals from a given site, which was assigned back to that site based on their genetic profile. The differing scale of genetic differentiation observed within Argyll is largely reflected by this assignment accuracy. If each location exhibits large differences from everywhere else, one would expect the accuracy of assigning individuals to the location from which they were sampled to be high (e.g. 90-100%). The average value of correct assignment to system is 65% (Figure 4) which is much higher than one would expect if there was no genetic structure in the data. The assignment to the Awe, Orchy, Clachen Dubh burn and the Creran are all approximately 80% or greater, but assignments to the other sites are much more variable (30% to 70% depending on the system). While this supports the conclusion that there is genetic differentiation among locations, indicative of separate breeding populations, the data do not at present have the power to assign fish of unknown origin (e.g. rod caught adults) to their location of origin with high accuracy for each system.

Assignment to the Euchar is slightly misleading. The Euchar has a larger sample size than other systems (Table 1), and is also close to the centre of genetic diversity for Argyll sites (Figure 2). As such, it has become a "catch-all" site for incorrect assignments. Many of the fish that are not correctly assigned to site of collection from any location are assigned instead to the Euchar. Therefore many of the fish that have here been assigned to the Euchar are simply falling into the "catch-all" category, and inflating the proportion of Euchar fish being assigned to the Euchar. To a lesser extent, this is also true for the Add and the Fyne. This is likely to be, in part, due to the lower differentiation among some systems and possibly the lack of a more complete baseline.

The genetic assignment of fish could be useful, for instance, in assigning rod caught adults to their natal river (or possibly tributary) allowing for identification of the geographic origin of distinct components of a river stock. For example, it may be possible to use genetic assignments to determine whether salmon returning to a river at different time points are destined for different parts of the catchment if there is well defined structuring between these components. In some cases, a stringent cut-off may be needed to identify, with reasonable confidence, the river or site of origin of an individual. In such cases, when individuals are assigned there will be much greater certainty, however this will come at the cost of the proportion of a sample that would get assigned anywhere. As mentioned earlier, if we apply a 70% cut-off we can improve the accuracy of assignment to system in Argyll by ~20% percent. However, this increased assignment comes at a cost, as now only 30% of all fish are assigned anywhere if this cut-off criterion is used. Depending upon the particular application for using assignment and the level of confidence deemed appropriate by managers, this could present a valuable approach.

In order to improve assignments and gain better distinction for potential breeding populations, larger sample sizes and/or additional genetic markers may be required as well as a more complete baseline of potential populations sampled. As many assignments will try to assign individuals to sites represented in the baseline, if the 'true' site has not been sampled, fish from these missing sites will be forced to be incorrectly assigned. At present, these assignments represent our best estimates since all fish assigned were known to originate from sites in the baseline. For the most accurate and complete picture of assignments within a system, detailed knowledge of *all* breeding populations is required. However, the results presented here demonstrate the potential for assigning individuals in some systems using microsatellite markers. It should be noted, however, that at certain geographical scales, assignment may not be possible with high accuracy, regardless of the samples and markers employed. If there is exchange of even modest amounts of spawning individuals over time between sites, then the genetic make-up of these sites will look relatively similar and prevent

assignment with high accuracy due to the biological and demographic mixing of the stock components.

Future work

While a number of factors may underlie population genetic structuring, there are at least two more, which were not directly addressed here, that may be of potential importance in contributing to the levels of differentiation observed. Firstly, stocking in many areas has been common for Atlantic salmon both within and between systems. For instance, within Argyll, the Orchy has a history of stocking, often with progeny of salmon sourced from the Shin (AKW personal communication). Therefore, it could be possible that large differences seen to the Orchy could be due the mixing of local and Shin genetic stock over time. Detailed knowledge of stocking history along with a representative baseline from the Shin and any other donor systems would allow for such an issue to be addressed in the future. The availability of any historical samples that pre-date the stocking era would be of particular value in addressing such an issue. However, it may be that this stocking has not had a lasting impact and therefore the differences observed reflect the natural history of colonisation and adaptation among these sites.

A second unexplored contribution to the patterns of population structure observed may be aquaculture escapes. Intermixing of farm fish with native strains may profoundly affect the genetic structuring of wild populations (Ferguson et al, 2007). It may also potentially cause reduced differentiation among wild strains where there is significant introgression into wild populations from the same farm strains. However, it is important to remember that there will be a lot of genetic variation among Scottish fish farm strains, which underscores the importance of a broad sample of baseline farm strains for comparison with wild populations. The collection of such a farm baseline as well as new markers that have been developed specifically to address such issues (Karlsson et al. 2011) will allow for a comprehensive survey of the potential impacts of farm escapes on wild populations. This should be addressed in the future for the Argyll Fisheries Trust, given the long history of fish farming in the area.

Summary

This analysis demonstrated significant levels of population structuring across systems within the Argyll Fisheries Trust. The results clearly are inconsistent with salmon in the area representing a single genetic stock. Samples from each river are consistent with being separate breeding populations and there are varying levels of differentiation observed within systems, suggesting more fine-scale separation of breeding populations. This is most notable in the Awe-Orchy system. The overall levels of differentiation allowed an average of 65% of individuals to be correctly assigned back to the locations

from which they were originally sampled, although this percentage various among sites. The high assignment values shown at some sites suggest that in certain areas it may be feasible to use genetic assignments to answer suitable management questions. Extending baseline genetic surveys as well as addressing outstanding issues, such as the effects of stocking and aquaculture, will contribute to the overall understanding of the underlying salmon population structure assisting the efficient management and conservation of this valuable resource.

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Appendix 1

Laboratory Procedures

DNA was extracted from individual fin clips using a standard proteinase K digestion (Fisher Scientific UK). The crude DNA extract was diluted (1 in 10) in 1xTE (Tris-EDTA) buffer for all further work. Seventeen microsatellite markers that have previously been developed for Atlantic salmon were amplified from each DNA extract by polymerase chain reaction (PCR) using fluorescently-labelled primers. The microsatellite markers used were: SP2201, Sp2210, SPG7, SP1605, SP1608, SP2216, SP3016 (Paterson et al., 2004), SsaD144, SsaD157, SsaD48, SsaD71 (King et al., 2005), Ssa14, Ssa289 (McConnell et al., 1995), Ssa202, Ssa171, Ssa197 (O'Reilly et al., 1996), SsaF43 (Sánchez et al., 1996). These 17 markers were amplified in three multiplex reactions according to the mixtures in Table 1 of this appendix. PCR reactions were conducted using the Type-it Microsatellite PCR kit (Qiagen). Cycle conditions were as follows : an initial denaturation at 95°C for 5 min followed by 32 cycles of 94°C for 30 s, annealing at either 58°C (mix A and C) or 55°C (mix B) for 90 s and extension at 72°C for 60 s. After cycling, a final extension was completed at 60°C for 30 min.

PCR products were run on a MegaBACE capillary sequencer (Amersham Biosciences) and compared against a size standard of Et Rox 550 (GE Healthcare) run along with each sample. Fragment sizes were scored with Fragment Profiler version 1.2 software (GE Healthcare). For data quality control, all results were independently checked by two people and in addition one in five results were scored “double-blind” and the results compared to calculate error rates.

Data Analysis

The results from the microsatellite marker SsaF43 allowed us to identify any trout/salmon hybrids that may be present among samples, and also any mis-identified trout. The genetic information from these individuals was then removed from further analysis.

In order to remove bias in the data due to over-representation of family groups, an analysis of family relationships was performed using the software COLONY (Wang & Santure, 2009, Jones & Wang 2010) to identify full-sibling individuals. Furthermore, this analysis allowed for a prediction as to the number of breeders that contributed to each sample. For each location sampled, all but one member of a full-sibling group were removed from analysis.

Table 1

List of microsatellites used in the genetic survey with primer sequences, multiplex mixture, final primer concentration in the PCR and the reference reporting the microsatellite locus.

Microsatellite marker	Sequence forward primers 5'-3'	Sequence reverse primers 5'-3'	Multiplex mixture	Final primer concentration (μM)	reference
Sp2201	TTTAGATGGTGGGATACTGGGAGGC	CGGGAGCCCCATAACCCTACTAATAAC	A	0.02	Paterson et al., 2004
Sp2210	AAGTATTCATGCACACACATTCACTGC	CAAGACCCTTTTTCCAATGGGATTC	A	0.02	Paterson et al., 2004
SPG7	CTTGGTCCCGTTCTTACGACAACC	TGCACGCTGCTTGGTCCTTG	A	0.02	Paterson et al., 2004
Ssa 202	CTTGGAATATCTAGAATATGGC	TTCATGTGTTAATGTTGCGTG	A	0.02	O'Reilly et al., 1996
SsaD144	TTGTGAAGGGGCTGACTAAC	TCAATTGTTGGGTGCACATAG	A	0.03	King et al., 2005
SsaD157	ATCGAAATGGAACTTTTGAATG	GCTTAGGGCTGAGAGAGGATTAC	A	0.03	King et al., 2005
Sp1605	CGCAATGGAAGTCAGTGGACTGG	CTGATTTAGCTTTTTAGTGCCCAATGC	B	0.015	Paterson et al., 2004
Sp1608	AGCACACTCATCATCTTACCTAGAG	ATGGACAGAAAGATAATGAGGG	B	0.015	Paterson et al., 2004
Sp2216	GGCCCAGACAGATAAACAAACACGC	GCCAACAGCAGCATCTACACCCAG	B	0.015	Paterson et al., 2004
Ssa171	TTATTATCCAAAGGGGTCAAAA	GAGGTCGCTGGGGTTTACTAT	B	0.015	O'Reilly et al., 1996
Ssa14	CCTTTTGACAGATTTAGGATTTC	CAAACCAAACATACCTAAAGCC	B	0.02	McConnell et al., 1995
Ssa289	GTTTCTTTACAAATAGACAGACT	TCATACAGTCACTATCATC	B	0.02	McConnell et al., 1995
Sp3016	GACAGGGCTAAGTCAGGTCA	GATTCTTATATACTCTTATCCCCAT	C	0.02	Paterson et al., 2004
Ssa197	GGGTTGAGTAGGGAGGCTTG	TGGCAGGGATTTGACATAAC	C	0.02	O'Reilly et al., 1996
SsaF43	AGCGGCATAACGTGCTGTGT	GAGTCACTCAAAGTGAGGCC	C	0.02	Sánchez et al., 1996
SsaD48	GAGCCTGTTCAGAGAAATGAG	CAGAGGTGTTGAGTCAGAGAAG	C	0.03	King et al., 2005
SsaD71	AACGTGAAACATAAATCGATGG	TTAAGAATGGGTTGCCTATGAG	C	0.03	King et al., 2005

Where there was more than one site sampled within a 5-km distance, two life-history stages (i.e. fry and parr) sampled at the same site and/or a site was sampled in different years, the data were initially tested for differences using the program CHIFISH (Ryman 2006). Where no significant differences were found, data from these sites or time points were combined; otherwise they were left separate for all further analyses.

The program MICROCHECKER (Van Oosterhout et al. 2004) was used to screen for genotyping errors and non-amplifying variants (null alleles) in the raw data. In addition markers were checked for conformity to linkage equilibrium (probability test) and Hardy-Weinberg equilibrium (exact test), as implemented by GENEPOP version 4 (Rousset, 2008). In all cases, inference of significance was corrected for multiple-testing using the false discovery rate (FDR) method (Narum, 2006). Allelic richness is an estimate of the number of genetic variants found in a sample after controlling for sample size. This was calculated using the program HP-Rare (Kalinowski, 2005), and allows an assessment of differences in genetic diversity among samples standardized to a common sample size.

The genetic structure between groups was examined using two measures of genetic differentiation - pairwise F_{ST} (calculated as θ; Weir & Cockerham 1984) calculated in the program GENETIX (Belkhir et al. 2004) and assessed for significance with permutation tests using 500 randomizations. The second measure of differentiation, pairwise Jost's D (Jost, 2008) was calculated with the program SMOGD (Crawford 2010). A pairwise matrix of both distance measures is presented in Table 2 of this appendix. A multi-dimensional scaling (MDS) plot was drawn to illustrate the relationships among sites using the Jost's D measure of differentiation.

Clustering of individuals among potential distinct groups was undertaken with STRUCTURE 2.3.3, using the admixture model with correlated alleles (Pritchard et al., 2000). Briefly, this method assumes the number of distinct groups (*K*) in turn to be from 1 to some defined upper limit (i.e. the number of sites sampled). The analysis then determines which *K* is most consistent with the observed data,and assigns each individual to one of the defined groups. Furthermore, prior information on sampling sites was used to initiate the analysis with the LOCPRIOR option available in STRUCTURE 2.3.3 (Hubisz et al. 2009). A burn-in phase of 100,000 iterations was followed by a run phase of 200,000, using a minimum of five independent runs for each number of groups (*K*) being tested. Both the log-likelihood probabilities and the delta *K* method (Evanno et al., 2005) were examined to find the most likely *K*.

The utility of the data to assign fish of unknown origin to river was examined by running individual assignment tests using the program ONCOR (Kalinowski et al. 2007). Assignments were conducted using the method of Rannala & Mountain (1997), testing assignment success to both site and to reporting groups (generally, river-level). Location

of assignment was taken as the site (or reporting group) with the highest probability. Only individuals with a complete multi-locus genotype (i.e. all 17 microsatellites) were chosen for assignment as comparing criterion values for individuals with differing number of markers typed is difficult (Piry et al 2004). Caution should be used when interpreting these results as the locations used may not represent the full spread of genetic diversity or populations present within the catchment, as well as differences in sample size, may affect the results.

Appendix 2

Pairwise estimates of genetic differentiation among groups as defined in Table 1 (main text). Jost's D above diagonal, F_{ST} below diagonal. Significant pairwise F_{ST} values are indicated in italics and shaded in gray.

ID	LUS	ADD	KIN	CLA	ORL	ORU	SHI	TUL	AWA	AWB	BAM	CRL	CRU	URE	EAL	EAU	EUC	FYN	RUL	RUU
LUS	-	0.063	0.263	0.159	0.157	0.290	0.161	0.217	0.176	0.227	0.153	0.178	0.100	0.133	0.082	0.081	0.083	0.087	0.019	0.074
ADD	*0.011*	-	0.262	0.114	0.122	0.291	0.170	0.244	0.168	0.183	0.083	0.129	0.071	0.151	0.041	0.104	0.041	0.053	0.002	0.065
KIN	*0.053*	*0.055*	-	0.238	0.078	0.068	0.074	0.088	0.203	0.196	0.321	0.318	0.239	0.281	0.275	0.218	0.231	0.145	0.164	0.208
CLA	*0.029*	*0.023*	*0.056*	-	0.114	0.278	0.209	0.246	0.148	0.162	0.181	0.260	0.146	0.197	0.112	0.216	0.114	0.132	0.122	0.152
ORL	*0.028*	*0.023*	*0.021*	*0.027*	-	0.064	0.020	0.057	0.098	0.128	0.230	0.197	0.170	0.197	0.125	0.141	0.113	0.092	0.091	0.103
ORU	*0.062*	*0.064*	*0.030*	*0.073*	*0.022*	-	0.034	0.047	0.230	0.238	0.387	0.318	0.279	0.301	0.265	0.229	0.244	0.190	0.194	0.258
SHI	*0.035*	*0.036*	*0.022*	*0.053*	*0.012*	*0.017*	-	0.013	0.079	0.100	0.251	0.230	0.177	0.204	0.143	0.175	0.117	0.103	0.072	0.129
TUL	*0.041*	*0.044*	*0.035*	*0.054*	*0.016*	*0.019*	0.010	-	0.196	0.237	0.278	0.239	0.186	0.256	0.210	0.242	0.182	0.134	0.134	0.187
AWA	*0.034*	*0.030*	*0.049*	*0.034*	*0.024*	*0.060*	*0.034*	*0.048*	-	0.014	0.211	0.303	0.225	0.229	0.170	0.271	0.141	0.123	0.119	0.179
AWB	*0.044*	*0.040*	*0.055*	*0.044*	*0.032*	*0.064*	*0.042*	*0.058*	*0.009*	-	0.208	0.308	0.212	0.263	0.201	0.283	0.180	0.168	0.139	0.203
BAM	*0.032*	*0.019*	*0.077*	*0.039*	*0.048*	*0.094*	*0.065*	*0.061*	*0.047*	*0.061*	-	0.205	0.118	0.222	0.134	0.257	0.119	0.150	0.061	0.146
CRL	*0.029*	*0.023*	*0.078*	*0.051*	*0.042*	*0.085*	*0.053*	*0.056*	*0.059*	*0.070*	*0.037*	-	0.076	0.125	0.177	0.232	0.172	0.150	0.131	0.192
CRU	*0.019*	*0.013*	*0.056*	*0.030*	*0.030*	*0.069*	*0.046*	*0.041*	*0.040*	*0.050*	*0.025*	*0.015*	-	0.092	0.103	0.167	0.092	0.082	0.076	0.114
URE	*0.032*	*0.030*	*0.068*	*0.045*	*0.044*	*0.076*	*0.048*	*0.060*	*0.046*	*0.060*	*0.050*	*0.030*	*0.025*	-	0.152	0.155	0.131	0.089	0.086	0.104
EAL	*0.017*	*0.008*	*0.057*	*0.024*	*0.024*	*0.063*	*0.038*	*0.038*	*0.031*	*0.043*	*0.024*	*0.031*	*0.016*	*0.034*	-	0.093	0.083	0.065	0.031	0.054
EAU	*0.022*	*0.025*	*0.056*	*0.055*	*0.035*	*0.060*	*0.034*	*0.052*	*0.058*	*0.067*	*0.055*	*0.041*	*0.039*	*0.039*	*0.029*	-	0.108	0.049	0.023	0.072
EUC	*0.014*	*0.008*	*0.047*	*0.028*	*0.023*	*0.055*	*0.030*	*0.032*	*0.031*	*0.040*	*0.028*	*0.031*	*0.018*	*0.029*	*0.014*	*0.024*	-	0.042	0.001	0.049
FYN	*0.017*	*0.012*	*0.038*	*0.028*	*0.018*	*0.045*	*0.023*	*0.028*	*0.029*	*0.039*	*0.032*	*0.028*	*0.017*	*0.023*	*0.013*	*0.017*	*0.007*	-	0.005	0.056
RUL	0.008	*0.002*	*0.047*	*0.025*	*0.023*	*0.057*	*0.029*	*0.035*	*0.028*	*0.036*	*0.017*	*0.023*	*0.014*	*0.020*	0.009	*0.018*	*0.001*	*0.003*	-	0.000
RUU	*0.018*	*0.013*	*0.055*	*0.031*	*0.025*	*0.064*	*0.037*	*0.037*	*0.034*	*0.044*	*0.029*	*0.035*	*0.023*	*0.028*	0.011	*0.025*	*0.011*	*0.010*	0.001	-

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