Genetic Sampling of Salmon on Eight Rivers in Lochaber

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Introduction

Atlantic salmon (*Salmo salar*) were genetically sampled across eight rivers in Lochaber (the Cona and Scaddle, the Finnan, the Loy, the Lundy, the Moidart, the Nevis, the Roy and the Shiel). Genetic sampling of juvenile salmon shows relatedness and thus allows an estimate of numbers of breeding adults in the populations of the eight rivers.

Sampling was undertaken by Lochaber Fisheries Trust and the samples were then sent to the University of the Highlands and Islands (UHI) for genetic analyses (see Appendix A).

Methodology

Sites along the Cona and Scaddle, Finnan, Loy, Lundy, Moidart, Nevis, Roy and Shiel were sampled (see Table 1 and Figures 1 - 8 for details of sites). Sampling was by electrofishing carried out according to Scottish Fisheries Coordination Centre (SFCC) protocol(s).

Following electrofishing fish were lightly anaesthetised for safe handling during processing. Genetic samples were taken from salmon parr, and also from fry where parr numbers were low. All fish were allowed to recover from the anaesthetic before being returned to the river.

Genetic samples were then taken to UHI for genetic analyses (see Appendix A).

Table 1. Electrofishing site locations and numbers of samples.

River	Site Code	Easting	Northing	Date Sampled	Number of Samples Collected
Cona and Scaddle	Con1	200337	770072	16/10/2021	15
Cona and Scaddle	Con2	197774	771636	16/10/2021	6
Cona and Scaddle	Con3	193695	772430	16/10/2021	7
Cona and Scaddle	Sca2	200041	768693	16/10/2021	18
Finnan	SHI12aa	191114	783767	22/09/2023	14
Finnan	SHI13	191326	782027	22/09/2023	16
Finnan	SHI12c	191256	782902	22/09/2023	15
Finnan	SHI12b	191124	783352	22/09/2023	14
Loy	Loy1	214748	781871	07/09/2021	11
Loy	Loy2	213397	782916	09/09/2021	8
Loy	Loy3	213222	783043	09/09/2021	12
Loy	Loy Bridge	212616	783157	15/10/2021	12
Loy	Loy4	212116	783169	09/09/2021	16
Loy	Loy6	209254	784679	09/09/2021	14
Lundy	Lun1	212933	776477	11/10/2021	10
Lundy	Lun3	213332	776698	11/10/2021	22
Lundy	Lun4	214612	777101	13/09/2021	12
Lundy	Lunfall	215015	776941	11/10/2021	10
Lundy	Happy Valley	214138	777237	08/09/2021	11
Moidart	MoiFordLow	172187	771840	16/09/2021	21
Moidart	MoiMid	173551	772013	16/09/2021	22
Moidart	MoiTop	174014	772230	16/09/2021	23

Nevis	Nev1	212323	772992	19/07/2024	14
Nevis	Nev2	213073	771293	19/07/2024	14
Nevis	Nev3	213509	770234	19/07/2024	14
Nevis	Nev4	214006	769080	19/07/2024	15
Roy	Loc35	231349	789364	01/08/2024	12
Roy	Loc36	233313	791025	01/08/2024	12
Roy	Loc37b	235370	792431	31/07/2024	15
Roy	Loc38a	238365	792947	31/07/2024	12
Roy	Loc40a	239641	793494	31/07/2024	5
Shiel	Shielgate	166558	770247	15/09/2021	20
Shiel	ShiUp	167517	768991	15/09/2021	20
Shiel	Shi1	165996	770585	15/09/2021	20

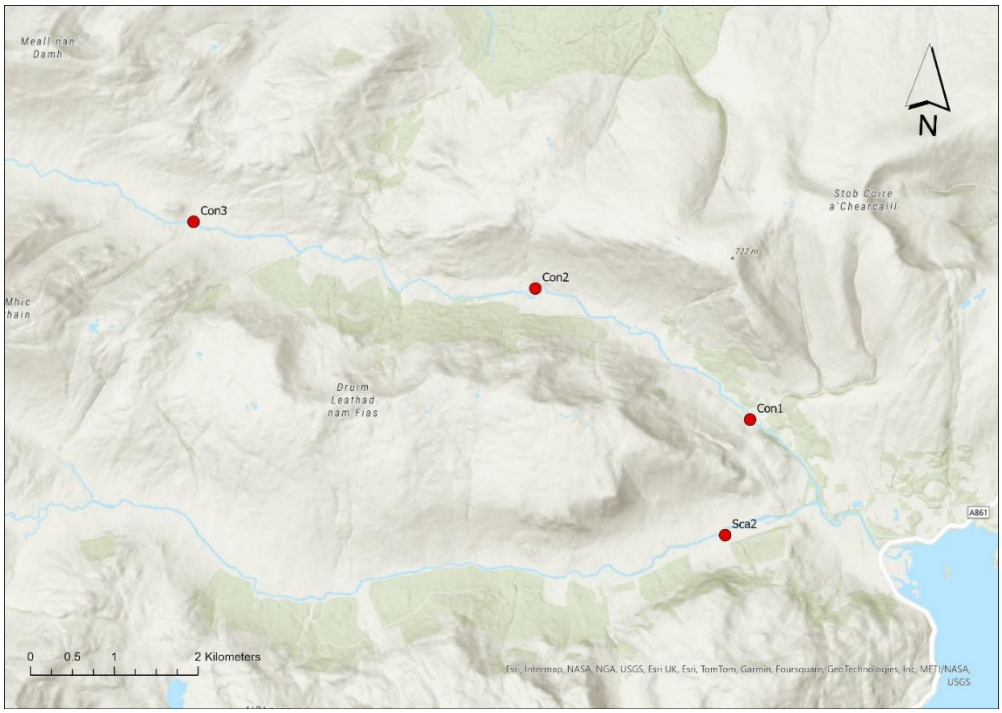

Figure 1. Sites on the Cona and Scaddle, marked by red dots.

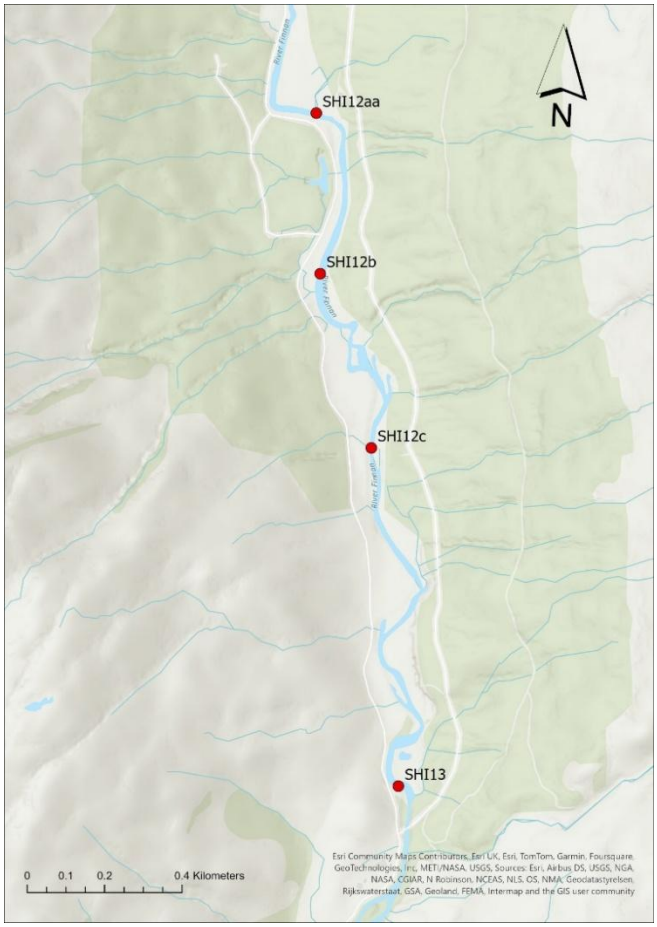

Figure 2. Sites on the Finnan, marked by red dots.

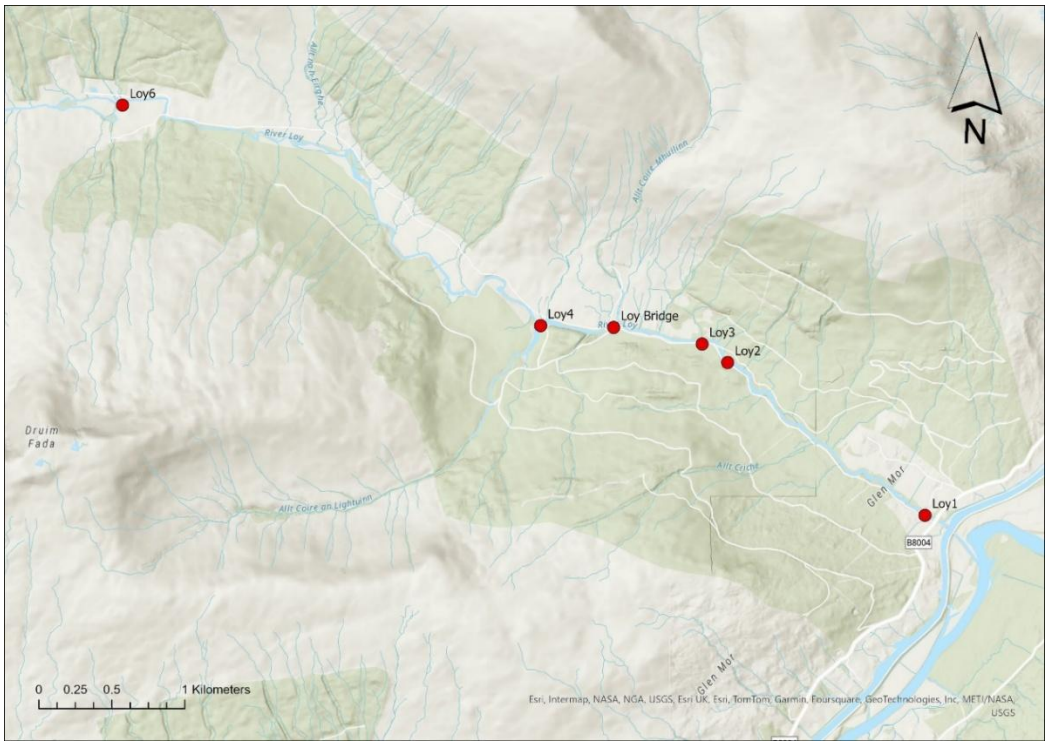

Figure 3. Sites on the Loy, marked by red dots.

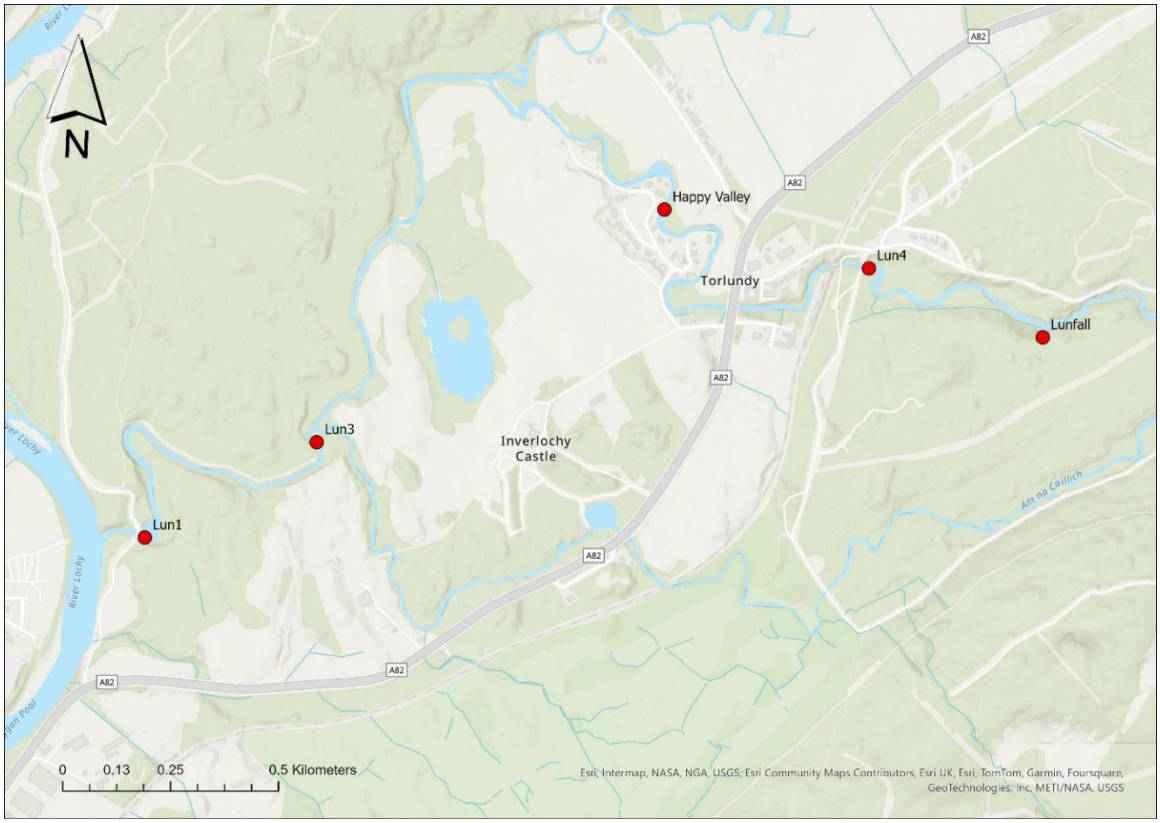

Figure 4. Sites on the Lundy, marked by red dots.

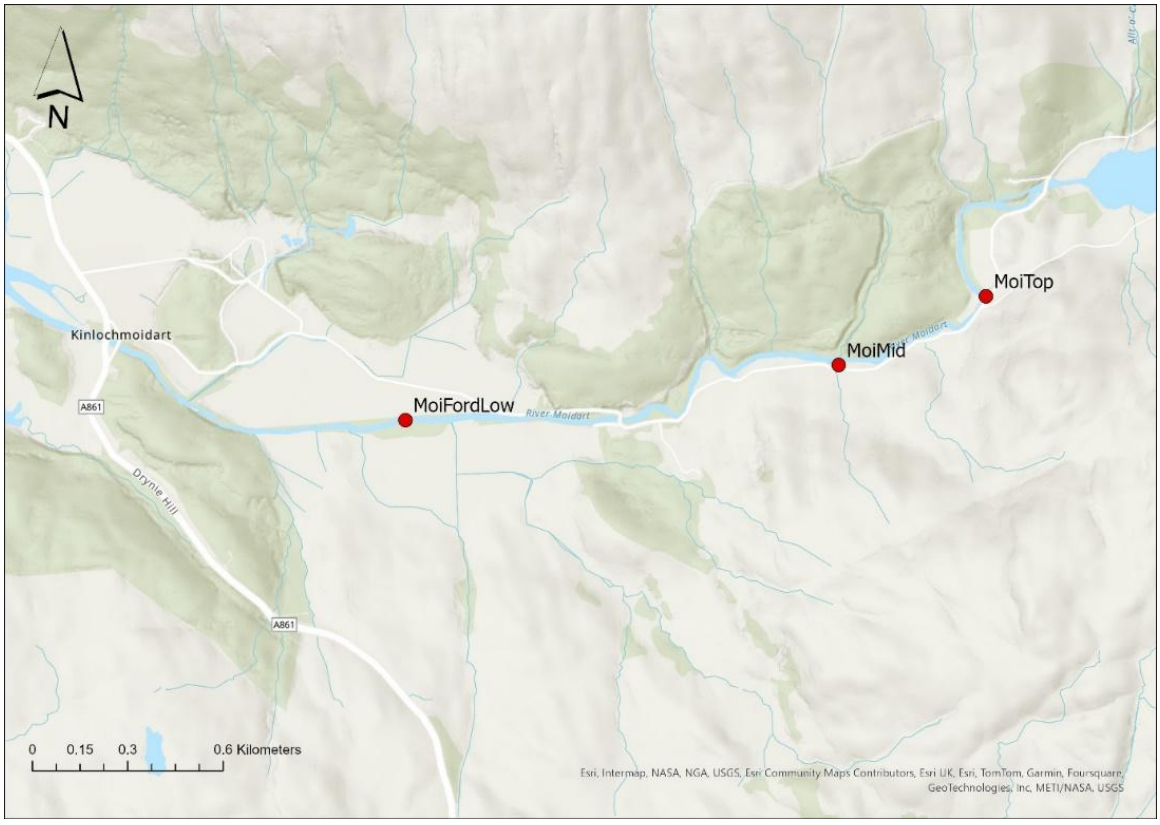

Figure 5. Sites on the Moidart, marked by red dots.

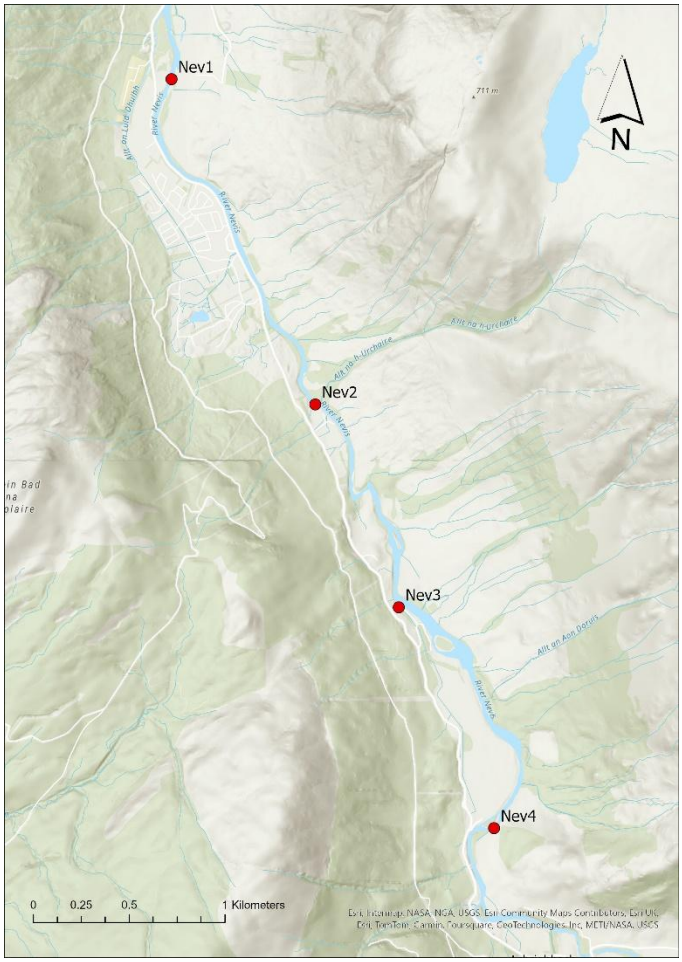

Figure 6. Sites on the Nevis, marked by red dots.

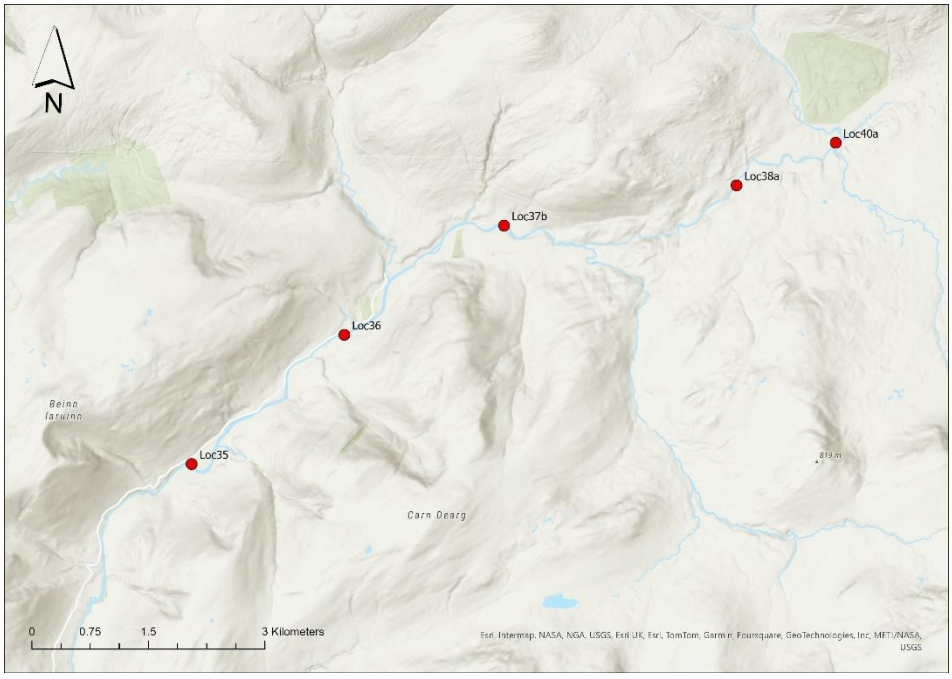

Figure 7. Sites on the Roy, marked by red dots.

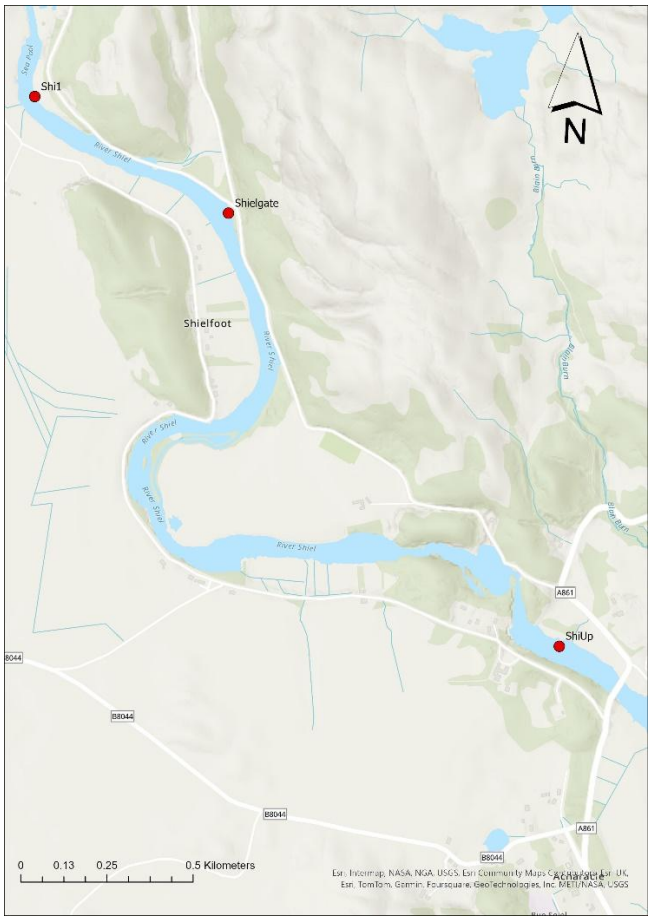

Figure 8. Sites on the Shiel, marked by red dots.

Results

Genetic analyses of fin samples were carried out by UHI. The full UHI report is included in Appendix A and contains a discussion of findings along with numbers of breeding adults.

Recommendations

Repeating this survey in future would allow for any changes in the numbers of breeding adults to be observed and thus give an indication of the health of populations.

Acknowledgements

This work was only possible thanks to the funding provided by Salmon Scotland's Wild Fisheries Fund (formerly Wild Salmonid Fund).

We would also like to thank the University of the Highlands and Islands (UHI) for carrying out and writing up the genetic analyses of the samples.

Appendix A – University of the Highlands and Islands (UHI) Report

Genetic Evaluation of wild Atlantic Salmon Populations in Lochaber: Project Report, January 2025

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Introduction

The Lochaber region in northwest Scotland contains thirteen major river systems which contain wild Atlantic salmon (*Salmo salar*) populations. Several of these support important recreational fisheries. The wild Atlantic salmon populations in Lochaber have declined in recent years, echoing a similar trend across Scotland and throughout northern Europe. Reasons for these Atlantic salmon population declines are not fully understood, but they are thought to result from a combination of factors that include changing conditions in the marine environment. Atlantic salmon aquaculture – active on the west coast of Scotland and generally using farm strains with Norwegian ancestry – may be one of the contributary factors. Amongst other impacts it presents a potential genetic risk to native wild Atlantic salmon populations due to escaped aquaculture fish hybridizing with wild fish.

Proper management of wild Atlantic salmon populations requires estimations of the number of breeding adults, and identification of escaped aquaculture fish or their offspring within the populations. Genetic tools can provide the means to do both. This project uses a panel of highly variable genetic markers and applies statistical analyses to estimate minimum actual and effective numbers of breeders to and examine aquaculture ancestry within samples of juveniles sampled from wild Lochaber salmon populations.

Sampling

Tissue samples were collected in autumn 2021, 2023 & 2024 by the Lochaber Fisheries Trust. A mixture of fry and parr (length 40-132 mm) were caught be electrofishing at various sampling sites in the Lochy, Moidart, Shiel, Scaddle, Nevis and Roy Rivers (Table 1, Figure 1). These rivers all contain wild-breeding Atlantic salmon populations. At some point in their recent past they are also likely to have been stocked with hatchery-produced juveniles from various Scottish salmon stocks. IBFC was not provided with information about contemporary stocking programmes. Small caudal fin clips were taken from the sampled fish and preserved in pre-labelled tubes containing 70% ethanol. All electrofishing and tissue collection was performed following standard protocols under required licences and permissions. is.

DNA extraction and genotyping-by-sequencing

The fin clip samples were received by the Institute for Biodiversity and Freshwater Conservation in November 2023 and September 2024. All samples were processed in 96-well plates with three 'blank' control wells (containing no salmon tissue) on each plate. DNA was extracted from approximately 2mm^2 of each fin clip using HotSHOT alkaline lysis (Truett *et al*. 2000). DNA concentration was measured by spectrophotometry using the QiaExpert system and diluted with 10mM Tris to a standard concentration of 10ug/µl using a QIAgility liquid handling robot. Each sample was genotyped for a panel of 88 short tandem repeat genetic markers ('microsatellites'). Markers were amplified in two separate multiplex PCR reactions containing the following: 3µl 2x Qiagen Type-IT multiplex master mix, 0.3µl primer multiplex mix (45 or 46 primer pairs at a mean concentration of 1µM per primer),

2.7µl diluted DNA. Thermocycling conditions were: 95°C for 15min, 25x [94°C 30s, 57°C 3min, 72°C 30s], 72°C for 10min. The two sets of PCR products were pooled for each sample and diluted 40x with water. Six to eight 96-well plates were combined for each DNA sequencing run. Sample-specific forward and reverse index combinations and Illumina sequencing tags were added to each sample (including blanks) in 5µl PCR reactions using the following protocol: PCR mix - 2.35µl H_2O, 0.5µl 10x buffer, 0.25U Taq DNA polymerase, 0.1µl dNTPs (10µM each), 1µl forward and reverse index mix (1µM per index); 1µl diluted multiplex PCR product; thermocycler conditions - 98°C for 2 min, 20x [98°C 10s, 62°C 30s, 72°C 15s], 7C for 10 min. Product for all samples was pooled into a single library, and purification and fragment size selection was performed using Agencourt AmPure XP beads. The concentration of the pooled library was measured via fluorometry using a Qubit with a high-sensitivity kit and standardized. Each pooled library was single-end sequenced on an Illumina MiSeq using Illumina V3 sequencing chemistry (150 cycles), with sequence reads demultiplexed to individual samples on the basis of their sample-specific indices and output in fastq format.

Statistical analysis

Microsatellite genotypes were called from DNA sequence reads using MEGASAT (Zhan *et al.* 2017), using an IBFC standard pipeline. Brown trout or first-generation trout-salmon hybrids in the dataset were identified from a known combination of non-amplification of certain microsatellite loci with brown-trout specific alleles at other loci. The package rubias (Moran & Anderson 2018) was used in R 4.0.3 (R Core Team 2020) to check for the presence of genetically identical samples (i.e those taken from the same individual fish). Finally, any remaining fish with > 25% missing data and any genetic marker with >25% missing data was removed from the analysis.

The software COLONY 2.0.6.6 (Jones & Wang 2010) was used to infer family structure among the genotyped juveniles and so infer the number of breeders that produced them. COLONY uses a maximum likelihood approach to infer sibling relationships from shared genetic variation, taking into account possible genotyping error. We made the a-priori assumption of no long-distance movement of juveniles and no widely dispersed spawning locations of individual adults, and therefore performed seven separate analyses with for the following geographical regions – Moidart (MoiFoLow, MoiMid, MoiTop), lower Shiel (ShiGate, Shi01, ShiUp); upper Shiel (Shi12, Shi13); Scaddle (Con01, Con02, Con03, Sca02), Lochy (HapVal, LoyB, Loy01, Loy02, Loy03, Loy04, Loy06, Lun01, Lun03, Lun04, Lunfall), Nevis (Nev01, Nev02, Nev03, Nev04) and Roy (Loc35, Loc36, Loc37b, Loc38a, Loc40). The following parameters were applied: probability of allele drop out 0.001 and other errors 0.001 for all loci; allele frequency not updated; diecious parents; polygamy for both sexes; full sibship scaled; weak sibship prior with an average maternal and paternal sibship size of 2; unknown population allele frequency; combined pairwise likelihood and full likelihood (FLPS) algorithm with medium run length and medium precision. To confirm model convergence, three independent replicate runs were performed with different random seeds. Where results varied, the solution with the largest number of parents was selected. COLONY also generates an estimate of effective number of breeders (Ne_{sib}), based on the inferred sibship relationships.

The presence of genetic material from Norwegian-ancestry aquaculture fish (the predominant type used throughout Scotland) was assessed using the program STRUCTURE (Pritchard *et al.* 2000), which infers the ancestral contribution of different genetically distinct groups to a

focal individual. To do this, we combined the Lochaber genetic dataset with a reference genetic dataset of aquaculture salmon (n = 600). The reference dataset included individuals from three domestic strains commonly stocked on Scotland's west coast, genotyped at IBFC using the same markers, laboratory and bioinformatic pipeline. We ran STRUCTURE specifying two ancestral groups, corresponding to aquaculture fish vs. wild Scottish fish, and applied the following parameters: admixture model with correlated allele frequencies, no prior population information, 100,000 burn-in followed by 150,000 MCMC reps; all other parameters default. Accurate quantification of ancestral proportions is statistically difficult task, particularly when using small numbers of non-diagnostic genetic markers such as microsatellites (Pritchard *et al.* 2007) and when the distinct groups hybridized >2 generations in the past (Pritchard *et al.* 2016). While results of this study can indicate of levels of aquaculture ancestry at different sites, therefore, they should not be considered diagnostic of recent aquaculture ancestry for any individual fish.

Results

Of the 483 samples that were put through the genotyping process, six (all collected from Con03) came from brown trout and 12 failed genotyping quality control due to >25% missing data. Five of the 88 microsatellites were also removed due to missing data, leaving 465 samples and 83 markers for analysis (Table 1)

Sibship reconstruction by COLONY, performed separately for different collection areas, inferred the following number of breeding adults: Moidart 51; Lower Shiel 46; Upper Shiel 31; Scaddle 36; Lochy 115; Nevis 56; Roy 49. Full sib family size ranged from one to 24 fish. Estimated Nb (sib) were: Moidart 56 (37-83); Lower Shiel 56 (38-86); Upper Shiel 10 (5-24); Scaddle 25 (14-46); Lochy 91 (68-123); Nevis 69 (46-103); Roy 60 (40-90) Inferred pedigrees for sampled juveniles are shown in Figure 2.

Estimation of wild vs aquaculture ancestry using Structure inferred varying amounts of aquaculture influence among the sampled geographic regions, with relatively less in Moidart and Lower Shiel compared to Upper Shiel, Scaddle and Lochy. We observed only one individual, in Upper Shiel, that could be a first generation hybrid between aquaculture and wild fish.

Discussion

Genetic reconstruction of sibships among the 464 genotyped juveniles inferred a total of 384 distinct parents, ranging from 31 in the Upper Shiel to 115 in the Lochy. The estimated effective number of breeders per year (Nb_{sib}), a proxy for 'effective population size' was not substantially smaller for any region except upper Shiel, where a very large full sibling family was sampled. Encouragingly, despite high aquaculture activity on the west coast of Scotland, our analysis did not infer large amounts of aquaculture ancestry in most sampled juveniles.

As a 'rule of thumb' a closed population with an effective size >50 but <500 is considered at low risk from problems associated with inbreeding (Frankham *et al.* 2014) but without migrants from other areas it is expected to lose genetic variation and therefore adaptive potential over time as a result of 'genetic drift'. Note that, for species such as salmon where there is likely to be some gene flow among populations, and juveniles spawned in one year return to breed in many different years, the relationship of Nb_{sib} to the true effective population size is not straightforward (Waples, 2024).

We expect the true number of breeders and Nb_{sib} of the investigated population to be higher than these estimates, as the number of distinct parents identified is limited by the number of juveniles sampled. In practice, in species such as salmon where most adults reproduce only once, it is impossible to discover the true adult population size from reconstruction of juvenile sibships (Waples & Feutry 2021). Our ability to obtain a reliable approximation is also limited by the practicality of sampling and genotyping large numbers of juveniles and the non-random distribution of families along the river. A more accurate picture of the number of breeding adults might be obtained through 'parent-offspring close-kin mark-recapture' (Waples & Feutry 2021), whereby genetic samples (e.g. scales, small fin clips or mucus swabs) are taken from captured adults in one year and their possible 0+ offspring in the following year. The breeding adult population size is then estimated by comparing the number of adults that are 'recaptured' – identified as parents of the juveniles to the total number of parents inferred from the juvenile analysis.

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Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML. (2000) Preparation of PCR-quality genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* **29:**52-54.

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Table 1: Details of samples collected and genotyped

River	Trib	Site	Lat	Long	Year	Size range (mm)	N	QC Fail	Trout	QC Pass
Lochy	Loy	Loy01	781871	214748	2021	84-100	11	0	0	11
Lochy	Loy	Loy02	782916	213397	2021	99-119	8	0	0	8
Lochy	Loy	Loy03	783043	213222	2021	82-130	12	1	0	11
Lochy	Loy	Loy04	783169	212116	2021	78-108	16	0	0	16
Lochy	Loy	Loy06	784679	209254	2021	59-118	15	0	0	15
Lochy	Loy	LoyBri	783157	212616	2021	46-58	12	0	0	12
Lochy	Lundy	HapVal	777237	214138	2021	77-96	11	0	0	11
Lochy	Lundy	Lun01	776477	212933	2021	90-120	10	0	0	10
Lochy	Lundy	Lun03	776698	213332	2021	54-122	22	1	0	21
Lochy	Lundy	Lun04	777101	214612	2021	79-102	12	0	0	12
Lochy	Lundy	Lunfall	776941	215015	2021	95-126	11	0	0	11
Moidart	Moidart	MoiFoLow	771840	172187	2021	58-128	21	2	0	19
Moidart	Moidart	MoiMid	772013	173551	2021	49-95	22	5	0	17
Moidart	Moidart	MoiTop	772230	174014	2021	50-90	23	0	0	23
Nevis	Nevis	Nev01	772992	212323	2024	72-109	14	0	0	14
Nevis	Nevis	Nev02	771293	213073	2024	64-86	14	0	0	14
Nevis	Nevis	Nev03	770234	213509	2024	62-105	14	0	0	14
Nevis	Nevis	Nev04	769080	214006	2024	77-103	15	1	0	14
Roy	Roy	LOC35	789364	231349	2024	68-124	11	0	0	11
Roy	Roy	LOC36	791025	233313	2024	72-110	12	0	0	12
Roy	Roy	LOC37b	792431	235370	2024	83-118	15	1	0	14
Roy	Roy	LOC38a	792947	238365	2024	80-112	12	0	0	12
Roy	Roy	LOC40a	793494	239641	2024	72-100	5	0	0	5
Scaddle	Cona	Con01	770072	200337	2021	60-102	15	0	0	15
Scaddle	Cona	Con02	771636	197774	2021	111-128	6	0	0	6
Scaddle	Cona	Con03	772430	193695	2021	56-125	7	0	6	1
Scaddle	Scaddle	Sca02	768693	200041	2021	55-103	18	1	0	17
Shiel	Finnan	Shi12a	783767	191114	2023	64-81	14	0	0	14
Shiel	Finnan	Shi12b	783352	191124	2023	69-81	14	0	0	14
Shiel	Finnan	Shi12c	782902	191256	2023	58-104	15	1	0	14
Shiel	Finnan	Shi13	782027	191326	2023	49-87	16	0	0	16
Shiel	Shiel	Shi01	770585	165996	2021	41-60	20	0	0	20
Shiel	Shiel	ShiGate	770247	166558	2021	40-110	20	0	0	20
Shiel	Shiel	ShiUp	768991	167517	2021	46-111	20	0	0	20

Figure 1. Sampling locations.

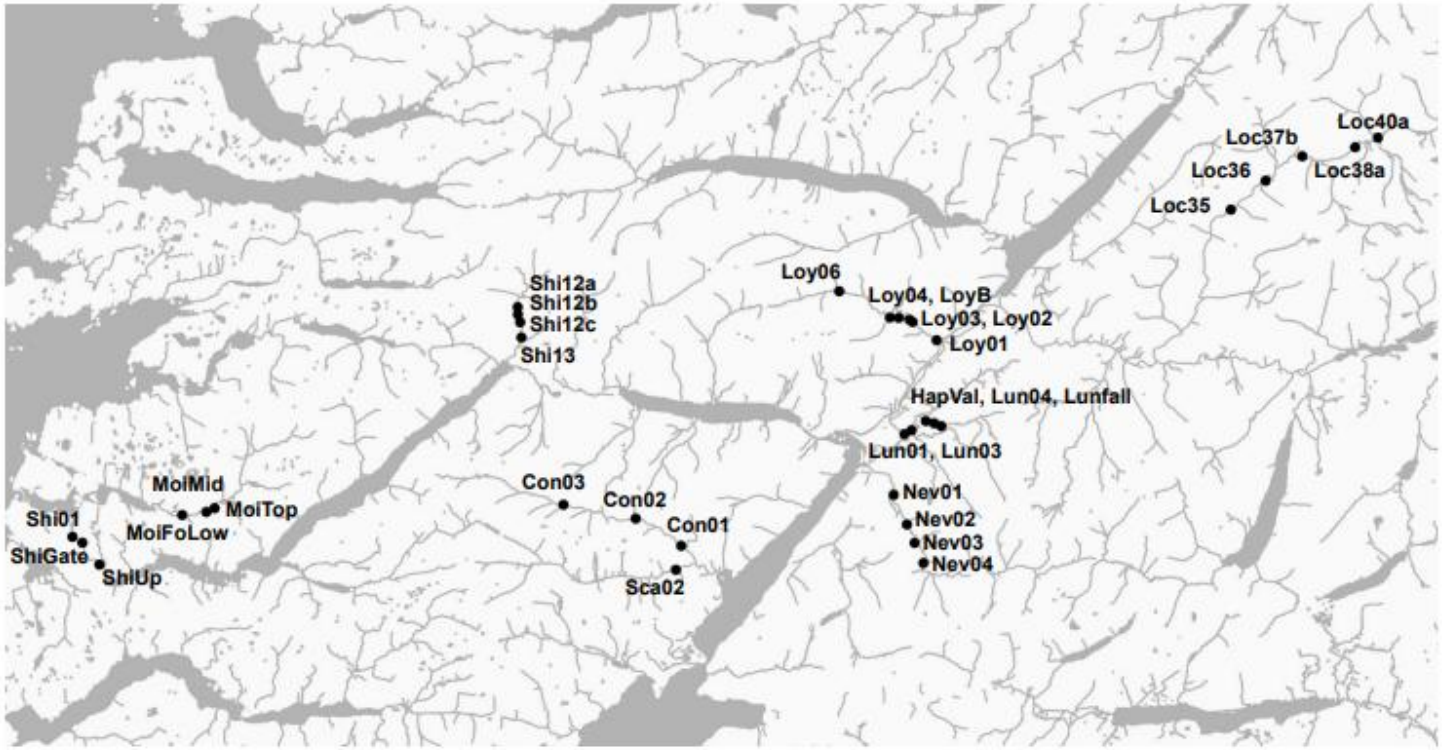

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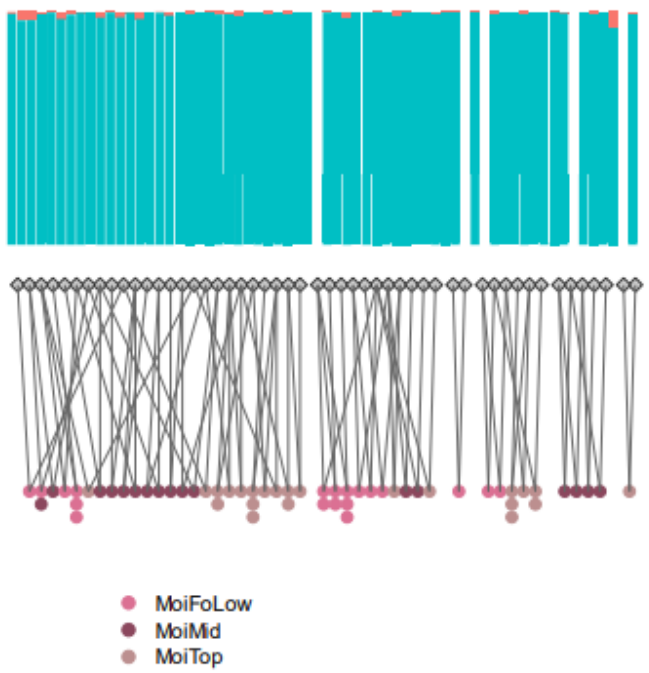
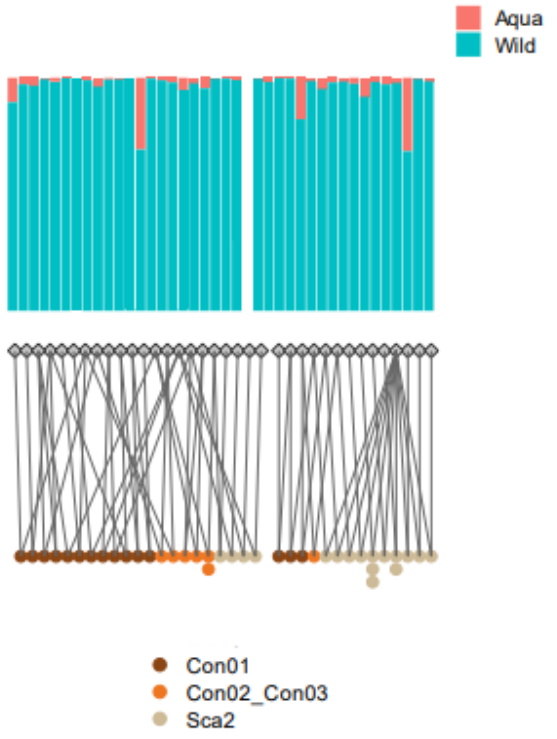
Figure 2 (overleaf). Inferred ancestry of each genotyped fish (top) and inferred pedigree (bottom) with figures in the same order. For ancestry figures, each column represents an individual fish and the colour represents the proportion of their ancestry assigned to wild Scottish or Norwegian-origin aquaculture fish. Note that, using this analysis method, a small amount of aquaculture ancestry (< 1%) is inferred for all wild fish due to statistical noise. For pedigree figures, inferred parents (diamonds, top) are linked by a line to their genotyped offspring (circles, bottom). Offspring arranged in a vertical column are inferred full siblings. Circle colour indicates sampling site. Sampled fish are arranged in the same order in the ancestry and pedigree figures.

Moidart



Scaddle



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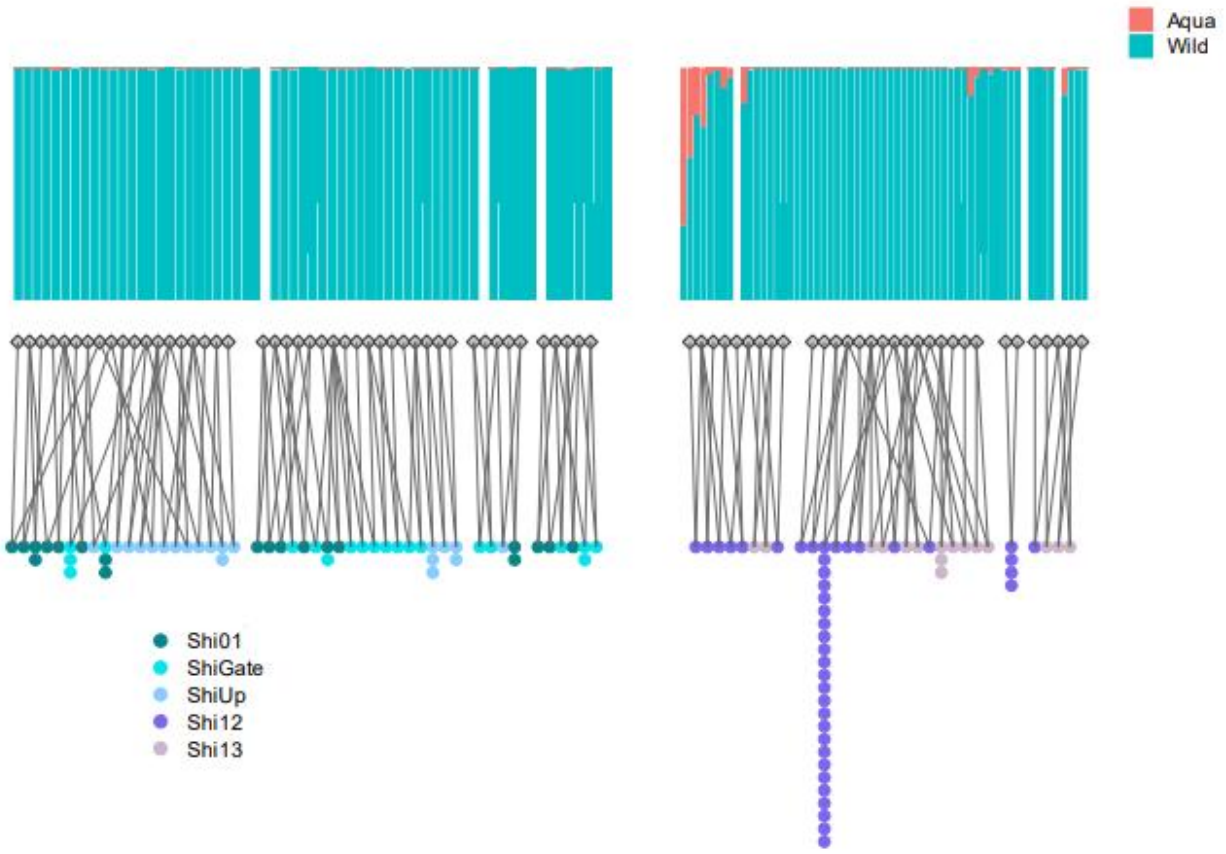
Shiel



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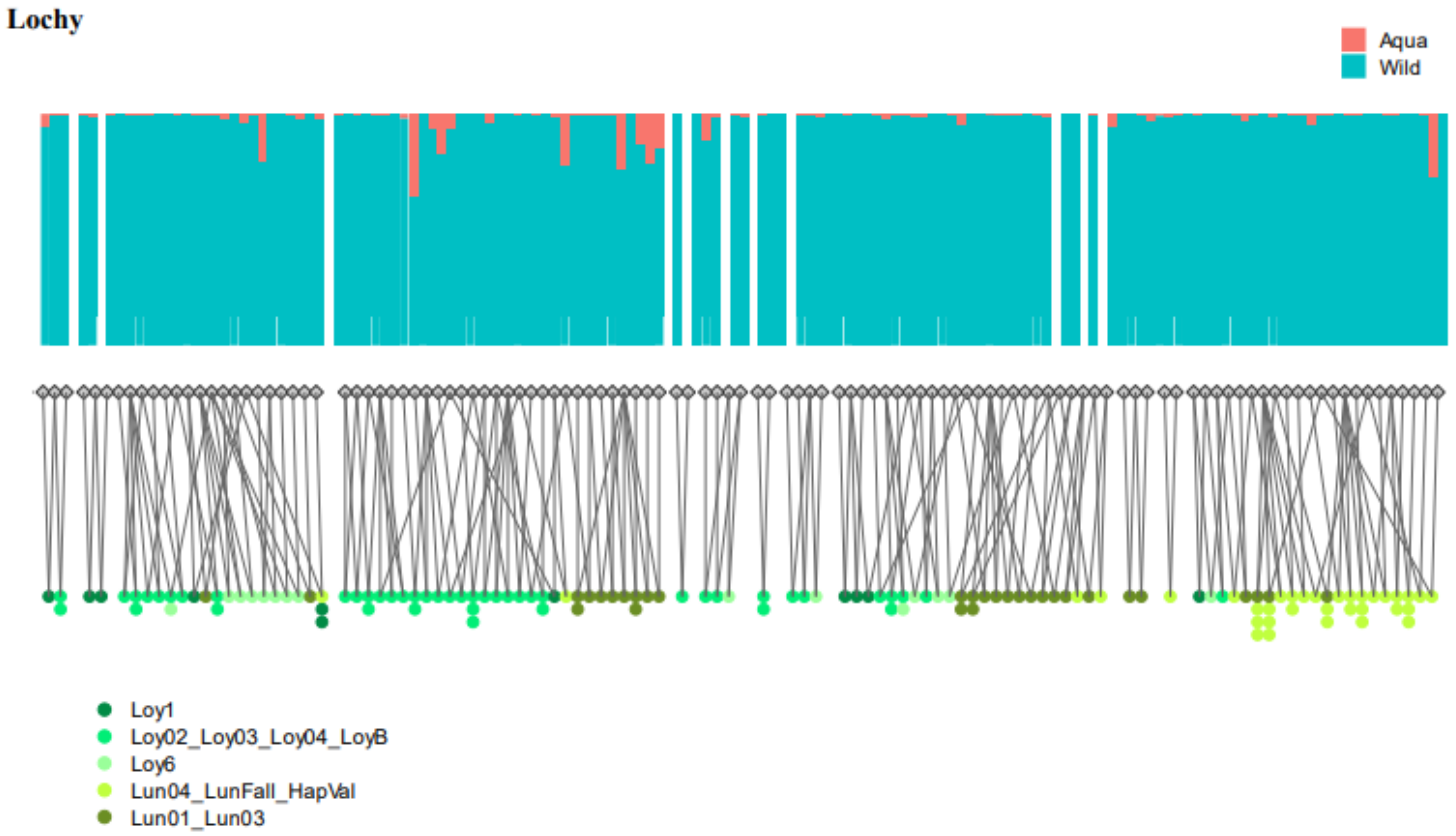

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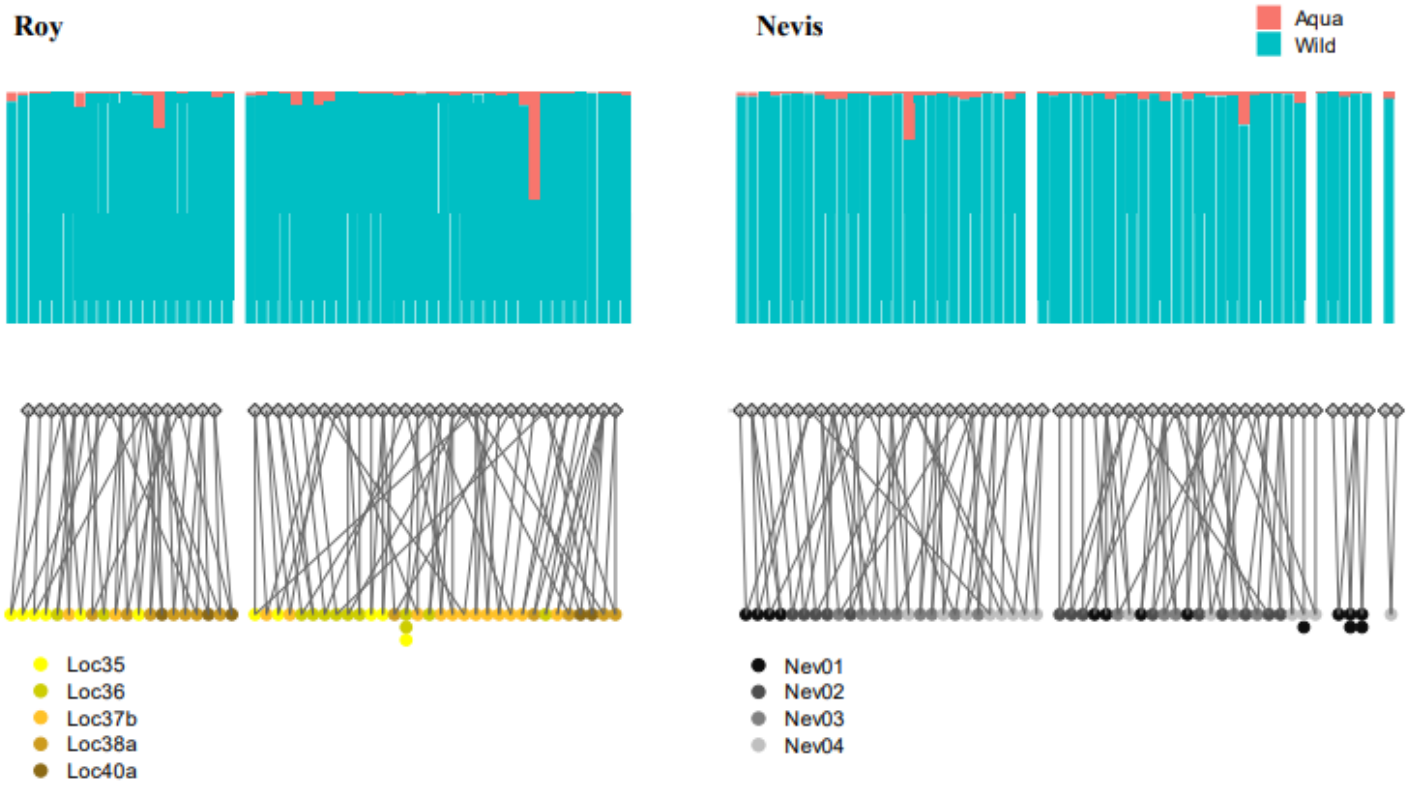

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