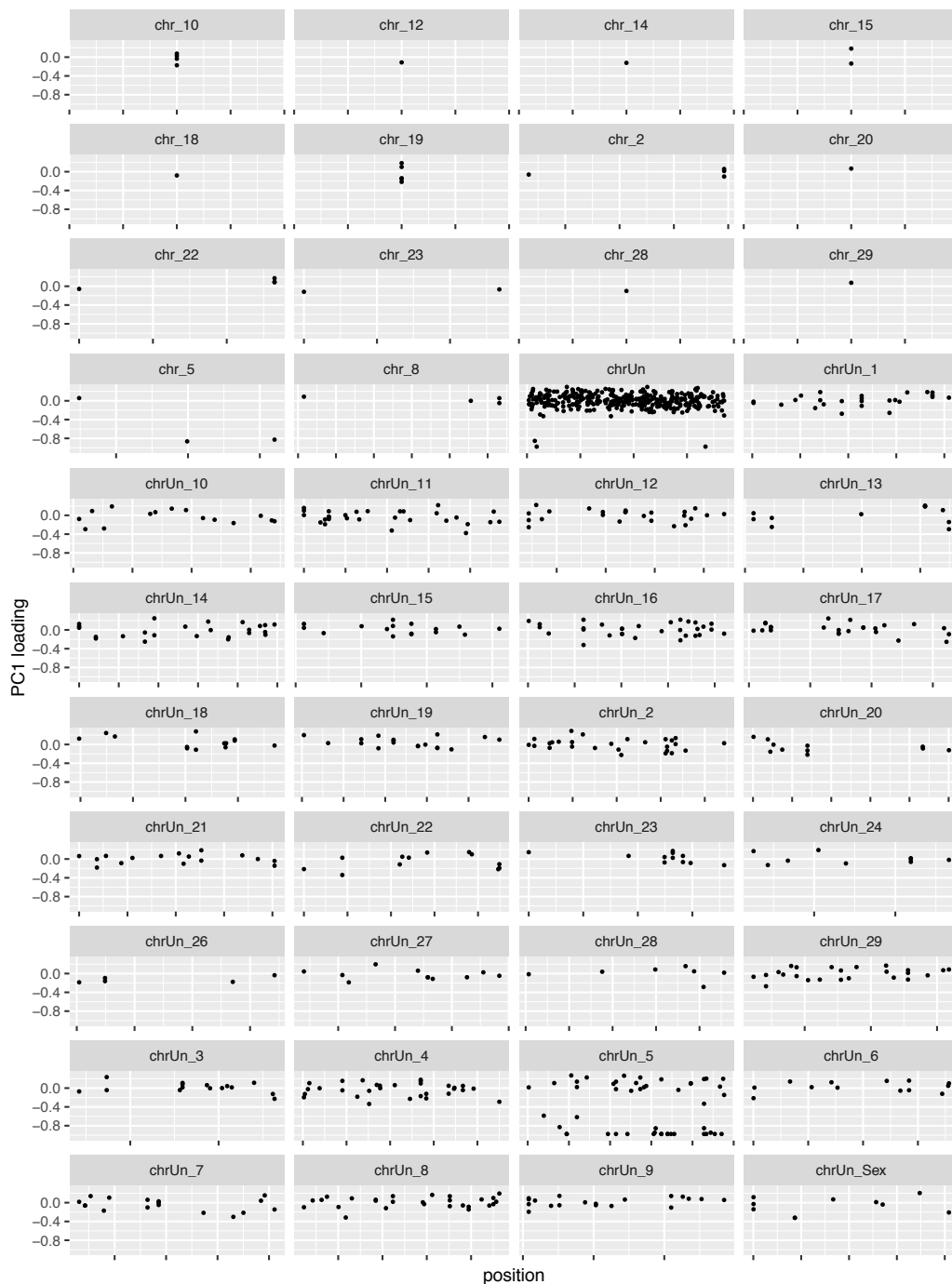


## File S1. Methow RAD - genome alignment & LD analysis

Principal component analysis of RAD data in the hatchery and natural origin (HOR and NOR, respectively) revealed the presence of two clusters separated along the PC1 axis. However, these clusters did not correspond to the hatchery and natural-origin samples. To determine if there was a pattern in the genomic location for loci driving the separation of the clusters, RAD tags were assigned to *O. mykiss* chromosomes based on their alignment to scaffolds. The distribution of PC1 loadings are illustrated in Figure S1. Generally speaking, markers with PC1 loadings in the extreme of the distribution are located on chromosome 5, with a few also on chrUn. Those markers are also likely to belong on chromosome 5, and linkage disequilibrium (LD) between these markers was further explored.

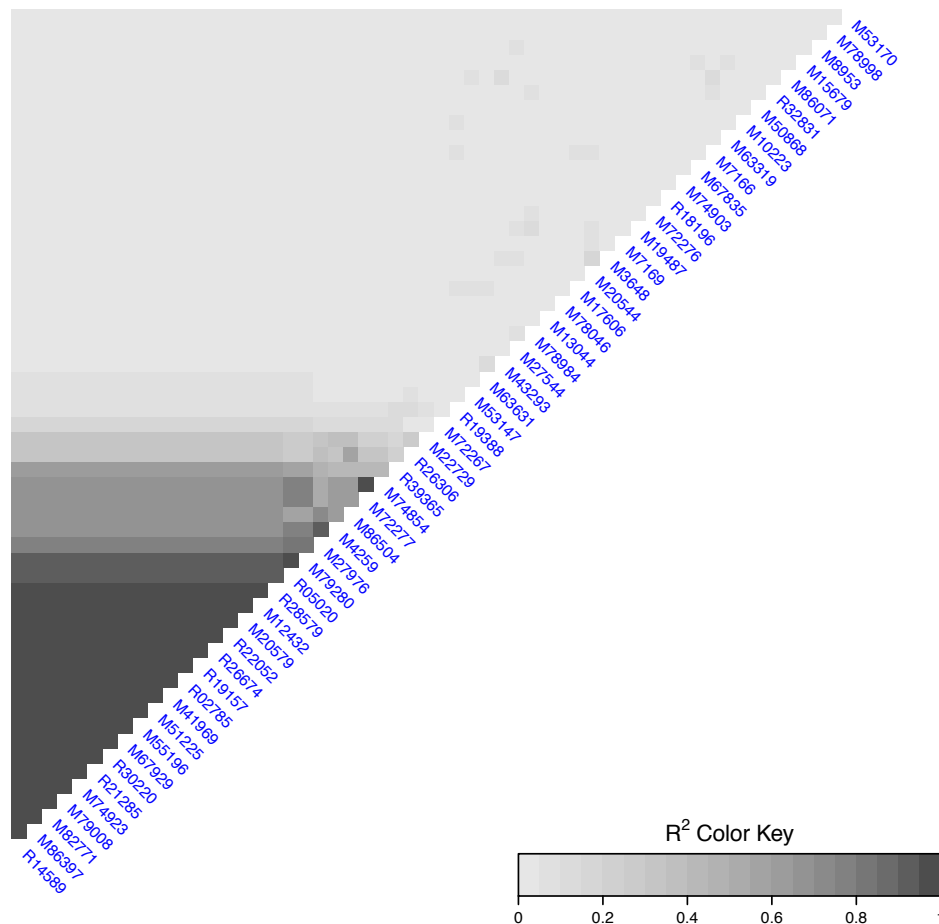
**File S1-Figure 1.** PC1 loadings by *O. mykiss* chromosome



## **Testing the pattern of LD among markers with the greatest loading on PC1**

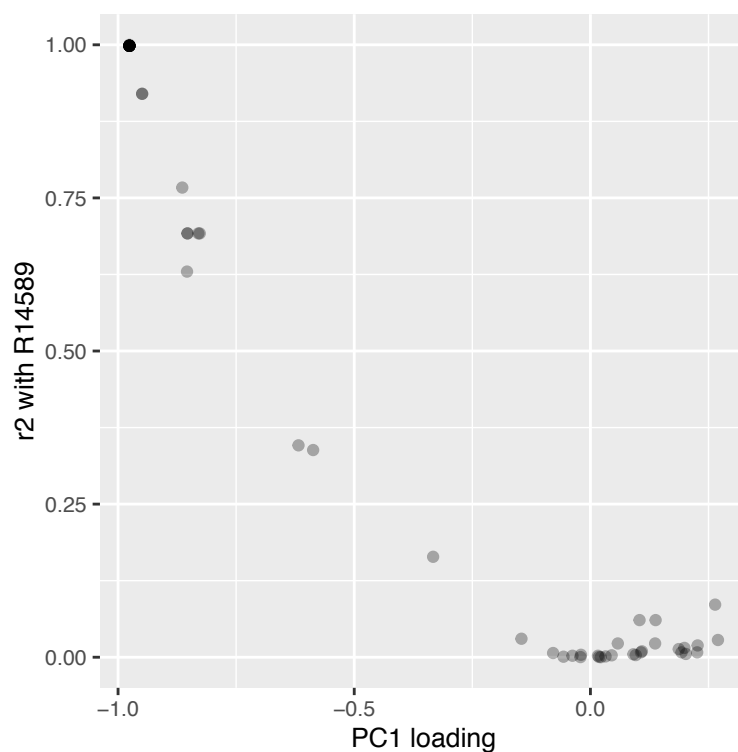
RAD markers on chromosome 5 have previously been shown to be associated with divergence between resident and migrant *O. mykiss* (Pearse et al. 2014), and also associated with QTL for growth (Nichols et al. 2008), age at sexual maturation (O'Malley et al. 2003, Leder et al. 2006), and development rate (Robison et al. 2001, Nichols et al. 2007). To compare markers in the present study, with that of Pearse et al. (2014), RAD markers from this study were reciprocally aligned to RAD markers from Miller et al. (2012) using bowtie2 with default parameters; only markers with reciprocal best hits were called a match. RAD loci in this study matching to those of Miller et al. (2012) were renamed according to that nomenclature, and all other loci from this study were prepended with 'M' (for Methow River). Only four markers used in Pearse et al. (2014) were identified as the same loci in this study.

Any marker from this study that mapped to chromosome 5 in the *O. mykiss* reference genome, to the linkage group that represents chromosome 5 in Miller et al. (2012) (called WS03 in that reference), or with a PC1 loading less than -0.4 was included in LD calculations for loci on that chromosome. The R package LDheatmap was used to calculate and plot LD between these markers. Very high LD ( $r^2 > 0.9$ ) was observed for a group of 20 markers, including R14589 which was identified in a block of strong LD in Pearse et al. (2014). For each of those markers, only two genotypic classes were represented, and the heterozygotes at these markers were found in the smaller cluster of individuals.



**File S1-Figure 2.** Linkage disequilibrium heat plot for markers on chromosome 5, or showing extreme loadings on PC1.

Markers were rank-ordered by LD with marker R14589 to select markers to remove from an additional PCA to determine how this tightly linked block of markers influenced the differentiation between the two groups of points in the PCA with all markers. As a result, marker R14589 and markers with LD greater than 0.2 with this marker (29 total) were removed from the PCA to perform another PCA.



**File S1-Figure 3.** Linkage disequilibrium with marker R14589 plotted with PC1 loading. Darker circles indicate regions of overlapping points.

**File S1-Table 1.** Summary data for chromosome Omy5 loci evaluated for LD. Markers from this study were aligned to the *O. mykiss* reference genome scaffolds (alignments given by CHR and POS) and chromosomes (alignments are given by chrom, chrom\_pos\_start and chrom\_pos\_end), and to the Miller et al. (2012) linkage map (alignments are given to linkage groups (LG) and the cm position of the matched marker).

NAME	locus	CHR	POS	chrom	chrom_pos_start	chrom_pos_end	WR.x.Sw.LG	WR.x.Sw.cM
M10223	10223	scaffold_133	847089	chrUn_5	28757971	30308002	NA	NA
M12432	12432	scaffold_1431	175517	chrUn_5	30578078	30814687	NA	NA
M13044	13044	scaffold_145	73771	chr_5	485803	1988661	NA	NA
M15679	15679	scaffold_1579	117215	chrUn_5	25121519	25326669	NA	NA
M17606	17606	scaffold_1668	139214	chrUn_5	47450542	47642168	NA	NA
M19487	19487	scaffold_1765	66360	chrUn_5	6430656	6605773	NA	NA
M20544	20544	scaffold_181	497994	chrUn_5	32441205	33799332	NA	NA
M20579	20579	scaffold_181	857986	chrUn_5	32441205	33799332	NA	NA
M22729	22729	scaffold_194	38919	chrUn_5	3898256	5200986	NA	NA
M27544	27544	scaffold_223	35481	chrUn_5	10560181	11781655	NA	NA
M27976	27976	scaffold_226	768187	chr_5	1988762	3204057	NA	NA
M3648	3648	scaffold_1110	7552	chrUn_5	26319955	26655648	NA	NA
M41969	41969	scaffold_336	693222	chrUn_5	35562889	36511326	NA	NA
M4259	4259	scaffold_1131	116411	chr_5	3204158	3531843	NA	NA
M43293	43293	scaffold_3495	64030	chrUn_5	47684273	47748869	NA	NA
M50868	50868	scaffold_423	538454	chrUn_5	244261	1077352	NA	NA
M51225	51225	scaffold_4295	30259	chrUn_5	24205506	24258710	NA	NA
M53147	53147	scaffold_44	2152469	chrUn_5	11946418	14162933	NA	NA
M53170	53170	scaffold_44	586509	chrUn_5	11946418	14162933	NA	NA
M55196	55196	scaffold_474	12781	chrUn_5	9471005	10242931	NA	NA
M63319	63319	scaffold_5777	6880	chrUn_5	39564835	39605385	NA	NA
M63631	63631	scaffold_580	585424	chrUn_5	39605486	40265372	NA	NA
M67835	67835	scaffold_636	167843	chrUn_5	20824173	21425557	NA	NA
M67929	67929	scaffold_63871	1878	chrUn	1.004e+09	1.004e+09	NA	NA
M7166	7166	scaffold_1223	194369	chrUn_5	27327322	27621552	NA	NA
M7169	7169	scaffold_1223	43404	chrUn_5	27327322	27621552	NA	NA
M72267	72267	scaffold_700	340182	chrUn_5	42804834	43361543	NA	NA
M72276	72276	scaffold_700	81857	chrUn_5	42804834	43361543	NA	NA
M72277	72277	scaffold_700	81765	chrUn_5	42804834	43361543	NA	NA
M74854	74854	scaffold_74612	556	chrUn	43858657	43860757	NA	NA
M74903	74903	scaffold_746	338531	chrUn_5	43363897	43884976	NA	NA
M74923	74923	scaffold_746	450957	chrUn_5	43363897	43884976	NA	NA
M78046	78046	scaffold_79932	332358	chrUn_5	14329862	14940792	NA	NA
M78984	78984	scaffold_80	370948	chrUn_5	21425658	23241774	NA	NA
M78998	78998	scaffold_80	638491	chrUn_5	21425658	23241774	NA	NA
M79008	79008	scaffold_80	787583	chrUn_5	21425658	23241774	NA	NA
M79280	79280	scaffold_817	62001	chrUn_5	44416933	44896860	NA	NA
M82771	82771	scaffold_902	195155	chrUn_5	45411897	45840962	NA	NA
M86071	86071	scaffold_989	352843	chrUn_5	46669336	47061480	NA	NA

M86397	86397	scaffold_998	311858	chrUn_5	47061581	47447171	NA	NA
M86504	86504	scaffold_99	253800	chrUn_5	7744778	9461712	NA	NA
M8953	8953	scaffold_1298	210095	chrUn_5	28205303	28476615	NA	NA
R02785	36512	scaffold_289	185388	chrUn	54699888	55748466	WS03	63.37
R05020	13295	scaffold_1470	88963	chrUn_5	30827699	31056188	WS03	63.37
R14589	55216	scaffold_474	34869	chrUn_5	9471005	10242931	WS03	63.37
R18196	86651	scaffold_9	3013453	chrUn_11	5987197	9277574	WS03	63.37
R19157	28941	scaffold_23292	5493	chrUn_5	34771175	34777525	WS03	63.37
R19388	56887	scaffold_494	647499	chrUn_5	23391192	24143072	WS03	63.37
R21285	72254	scaffold_700	218953	chrUn_5	42804834	43361543	WS03	63.37
R22052	24027	scaffold_2008	70197	chrUn_5	33946450	34083919	WS03	63.37
R26306	53176	scaffold_44	679589	chrUn_5	11946418	14162933	WS03	63.37
R26674	24028	scaffold_2008	70130	chrUn_5	33946450	34083919	WS03	63.37
R28579	20528	scaffold_181	1344216	chrUn_5	32441205	33799332	WS03	63.37
R30220	71631	scaffold_693	227499	chrUn_5	19482124	20041108	WS03	63.37
R32831	43645	scaffold_3527	28374	chrUn_5	36577154	36640953	WS03	63.37
R39365	14027	scaffold_15031	10579	chrUn_5	31068942	31080929	WS03	63.37

**File S1-Table 2.** Summary statistics for loci aligning to *O. mykiss* chromosome 5. These include the numbers observed in each genotypic class (HOM1, HET, HOM2), the p-value for tests for Hardy Weinberg Equilibrium (HWE), the PC1 loading, observed heterozygosity (Het) and linkage disequilibrium (r2) with marker R14589 identified in Pearse et al. (2014).

NAME	locus	HOM1	HET	HOM2	P_HWE	PC1_loading	Het	r2_with_R14589
M10223	10223	76	14	1	0.51	0.045	0.1667	0.003086
M12432	12432	72	19	0	0.59	-0.976	0.1755	0.9984
M13044	13044	60	28	3	1	0.058	0.2948	0.02249
M15679	15679	80	11	0	1	-0.057	0.1196	0.0008522
M17606	17606	70	21	0	0.6	0.199	0.2191	0.0152
M19487	19487	80	11	0	1	0.107	0.1308	0.007938
M20544	20544	71	20	0	0.59	0.187	0.201	0.01328
M20579	20579	72	19	0	0.59	-0.976	0.1755	0.9984
M22729	22729	81	10	0	1	-0.587	0.1173	0.3383
M27544	27544	57	28	6	0.36	0.27	0.3299	0.02814
M27976	27976	75	16	0	1	-0.864	0.142	0.7669
M3648	3648	79	11	1	0.37	0.109	0.1562	0.00965
M41969	41969	72	19	0	0.59	-0.976	0.1755	0.9984
M4259	4259	76	15	0	1	-0.827	0.1296	0.692
M43293	43293	67	23	1	1	-0.146	0.2492	0.03016
M50868	50868	72	17	2	0.33	0.016	0.1644	0.002404
M51225	51225	72	19	0	0.59	-0.976	0.1755	0.9984
M53147	53147	37	44	10	0.65	0.138	0.4711	0.06062
M53170	53170	78	12	1	0.42	0.022	0.1412	3.89e-05
M55196	55196	72	19	0	0.59	-0.976	0.1755	0.9984
M63319	63319	52	32	7	0.58	0.096	0.4016	0.003501
M63631	63631	37	40	14	0.65	0.104	0.4614	0.06062
M67835	67835	77	14	0	1	0.091	0.1053	0.00482
M67929	67929	72	19	0	0.59	-0.976	0.1755	0.9984
M7166	7166	46	42	3	0.11	-0.02	0.3947	0.00399
M7169	7169	79	12	0	1	0.226	0.1296	0.007938
M72267	72267	27	50	14	0.29	-0.333	0.49	0.164
M72276	72276	78	12	1	0.42	0.193	0.1292	0.007938
M72277	72277	80	11	0	1	-0.853	0.1296	0.692
M74854	74854	80	11	0	1	-0.853	0.1296	0.692
M74903	74903	80	11	0	1	0.202	0.09336	0.005381
M74923	74923	72	19	0	0.59	-0.976	0.1755	0.9984
M78046	78046	72	15	4	0.03	0.227	0.2589	0.01923
M78984	78984	63	27	1	0.45	0.137	0.2878	0.02249
M78998	78998	77	13	1	0.47	-0.021	0.1412	0.000241
M79008	79008	72	19	0	0.59	-0.976	0.1755	0.9984
M79280	79280	73	18	0	0.59	-0.949	0.1644	0.92
M82771	82771	72	19	0	0.59	-0.976	0.1755	0.9984
M86071	86071	77	14	0	1	0.032	0.1532	0.001291

M86397	86397	72	19	0	0.59	-0.976	0.1755	0.9984
M86504	86504	81	10	0	1	-0.83	0.1292	0.692
M8953	8953	50	36	5	0.79	0.018	0.3819	0.0004274
R02785	36512	72	19	0	0.59	-0.976	0.1755	0.9984
R05020	13295	73	18	0	0.59	-0.949	0.1644	0.92
R14589	55216	72	19	0	0.59	-0.976	0.1755	1
R18196	86651	81	10	0	1	-0.079	0.09259	0.006823
R19157	28941	72	19	0	0.59	-0.976	0.1755	0.9984
R19388	56887	24	53	14	0.14	0.264	0.4942	0.08587
R21285	72254	72	19	0	0.59	-0.976	0.1755	0.9984
R22052	24027	72	19	0	0.59	-0.976	0.1755	0.9984
R26306	53176	74	17	0	1	-0.618	0.2083	0.346
R26674	24028	72	19	0	0.59	-0.976	0.1755	0.9984
R28579	20528	72	19	0	0.59	-0.976	0.1755	0.9984
R30220	71631	72	19	0	0.59	-0.976	0.1755	0.9984
R32831	43645	75	16	0	1	-0.038	0.1651	0.002404
R39365	14027	65	22	4	0.25	-0.854	0.2492	0.6297

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