MOLECULAR ECOLOGY

Supplemental Information for:

Sperm of more colorful males are better adapted to ovarian fluids in lake char (Salmonidae)

David Nusbaumer, Laura Garaud, Christian de Guttry, Laurie Ançay & Claus Wedekind

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Table S1. Experiment 1: Determining sperm characteristics of males #1700 to #1709 in various activation media, i.e., diluted ovarian fluid of one of 4 females (#GAA to #GAD) or water only. The entries give the number of independent sets of measurements.

	Male									
Activation medium	#1700	#1701	#1702	#1703	#1704	#1705	#1706	#1707	#1708	#1709
Ovarian fluid of #GAA	2	2	2	2	2	2	2	2	2	2
Ovarian fluid of #GAB	2	2	2	2	2	2	2	2	2	2
Ovarian fluid of #GAC	2	2	2	2	2	2	2	2	2	2
Ovarian fluid of #GAD	2	2	2	2	2	2	2	2	2	2
Water only	2	2	2	2	2	2	2	2	2	2

Table S2. Experiment 2: The sperm competition trials, with 2 times 24 eggs per experimental cell. Asterisks indicate experimental cells that were accidentally lost.

Female	Activation	Dyad				
		#1700 vs #1701	#1702 vs #1704	#1703 vs #1705	#1706 vs #1707	#1708 vs #1709
#GAA	ovarian fluid	2 x 24	2 x 24	2 x 24	2 x 24	2 x 24
#GAA	water	2 x 24	2 x 24	2 x 24	2 x 24	2 x 24
#GAB	ovarian fluid	2 x 24	2 x 24	2 x 24	2 x 24	2 x 24
#GAB	water	2 x 24*	2 x 24*	2 x 24*	2 x 24*	2 x 24*
#GAC	ovarian fluid	2 x 24	2 x 24	2 x 24	2 x 24	2 x 24
#GAC	water	2 x 24	2 x 24	2 x 24	2 x 24	2 x 24
#GAD	ovarian fluid	2 x 24	2 x 24	2 x 24	2 x 24	2 x 24
#GAD	water	2 x 24	2 x 24	2 x 24	2 x 24	2 x 24

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Table S3. The 6 microsatellite markers used for paternity assignment, and the sex marker that was added to the first multiplex.

Marker	Multiplex	Sequence forward primer	Sequence reverse primer	Dye	$N_{alleles}$	Range [bp]
Cocl3-N	1	5'-TTCAGGTTTGGTAAGCAAG-3'	5'-AGTGTAATAAATCACCCGAG-3'	atto550	8	226-270
OtsG253b	1	5'-GAGCAGGCCGAGCAGGTGTCT-3'	5'-AATTGGGTCATTAAGGCTCTGTGG-3'	fam	16	88-118
Ssa456	1	5'-CTTCCCAGGAGTCATCATAAATCT-3'	5'-TAAACCCCACTGCTTGTTGAGTGT-3'	hex	4	209-215
Sfo8	2	5'-CAACGAGCACAGAACAGG-3'	5'-CTTCCCCTGGAGAGGAAA-3'	hex	12	248-275
Sfo23	2	5'-GTGTTCTTTTCTCAGCCC-3'	5'-AATGAGCGTTACGAGAGG-3'	atto550	11	136-217
Ssa85	2	5'-AGGTGGGTCCTCCAAGCTAC-3'	5'-ACCCGCTCCTCACTTAATC-3'	fam	11	156-189
Sdy[1]	1	5'-CCCAGCACTGTTTTCTTGTCTCA-3'	5'-CTTAAAACCACTCCACCCTCCAT-3'	atto620	1	226-226

[1]Sex marker, used for a parallel study on sex-specific embryo development (Nusbaumer *et al.* 2021).

Table S4. Identity of focal males (ID = #1700 to #1709) if it is the more yellow than its competitor, less inbred, or least kin to a given female (ID = from #GAA to #GAD).

			Least kin to female			
Dyad	More yellow	Less inbred	#GAA	#GAB	#GAC	#GAD
1	#1701	#1701	#1701	#1701	#1701	#1700
2	#1702	#1704	#1704	#1702	#1704	#1702
3	#1703	#1705	#1703	#1705	#1705	#1705
4	#1706	#1707	#1707	#1707	#1707	#1707
5	#1709	#1708	#1709	#1708	#1709	#1709

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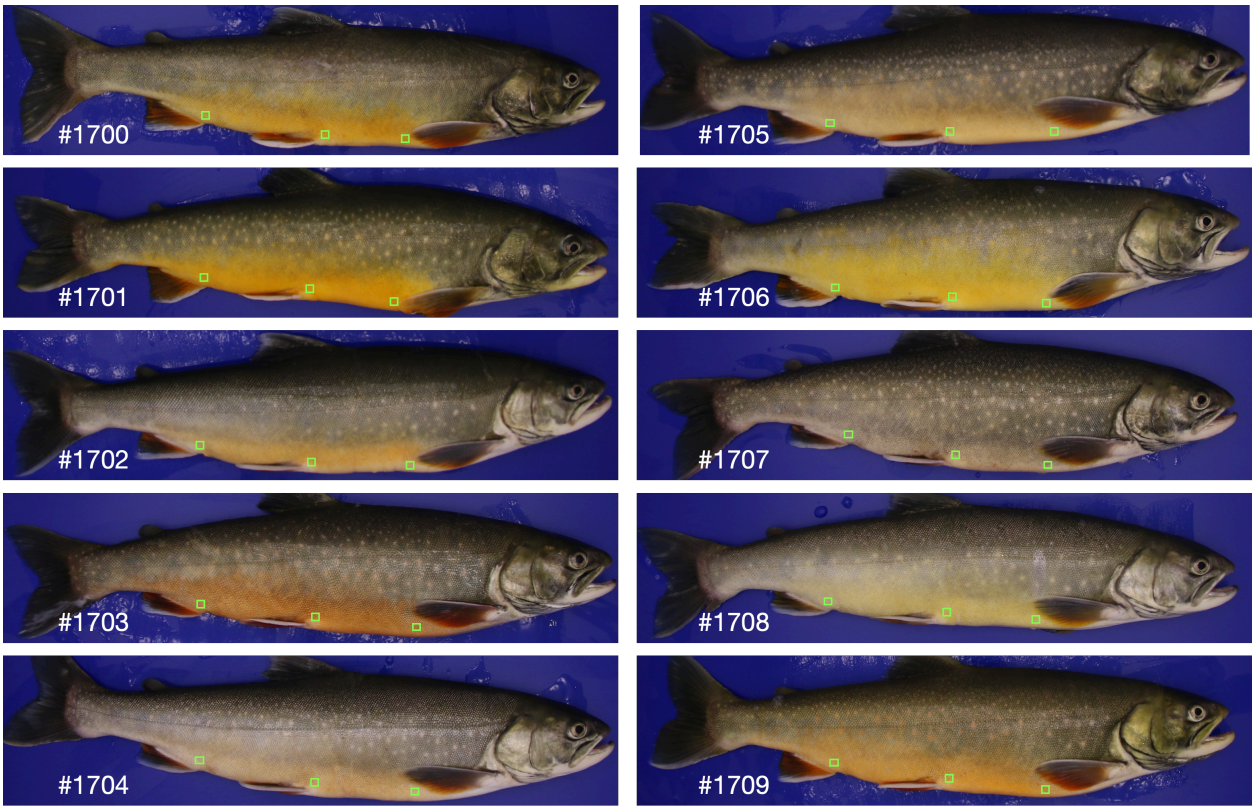

Figure S1. The 10 males sampled in 1017 and used for experiments 1 and 2, and the locations of the 3 squares (in green) from which color measurements of the ventral area (yellow and red) were taken. When including the 24 males sampled in 2018, mean gray value was positively correlated with yellowness, i.e., the yellower the lighter ($r = -0.47$, $p < 0.005$) and negatively with redness, i.e., the redder the darker ($r = -0.66$, $p < 0.001$).

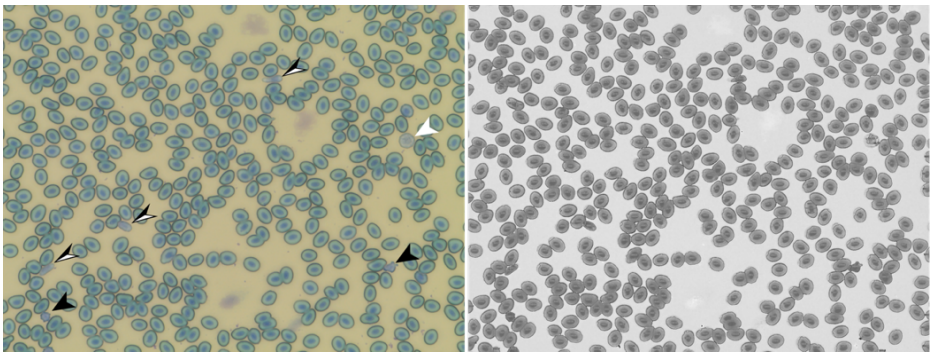

Figure S2. Example of a blood smear image as taken under the microscope (left) and its output of automated cell count (right). On the left image, black arrow heads point towards lymphocytes, the white arrow towards a granulocyte, and the black and white arrow towards thrombocytes. The rest are erythrocytes.

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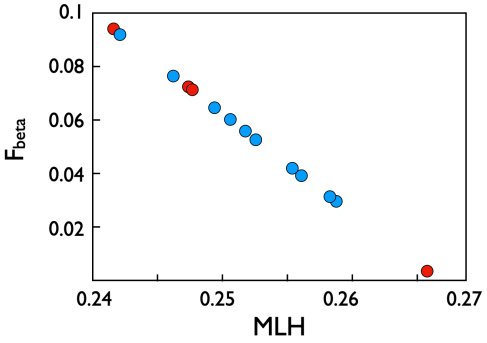

Figure S3. The link between multi-locus heterozygosity (MLH) and individual inbreeding coefficients (F_{beta}) for the males (blue) and females (red) sampled in 2017 and used in experiments 1 and 2.

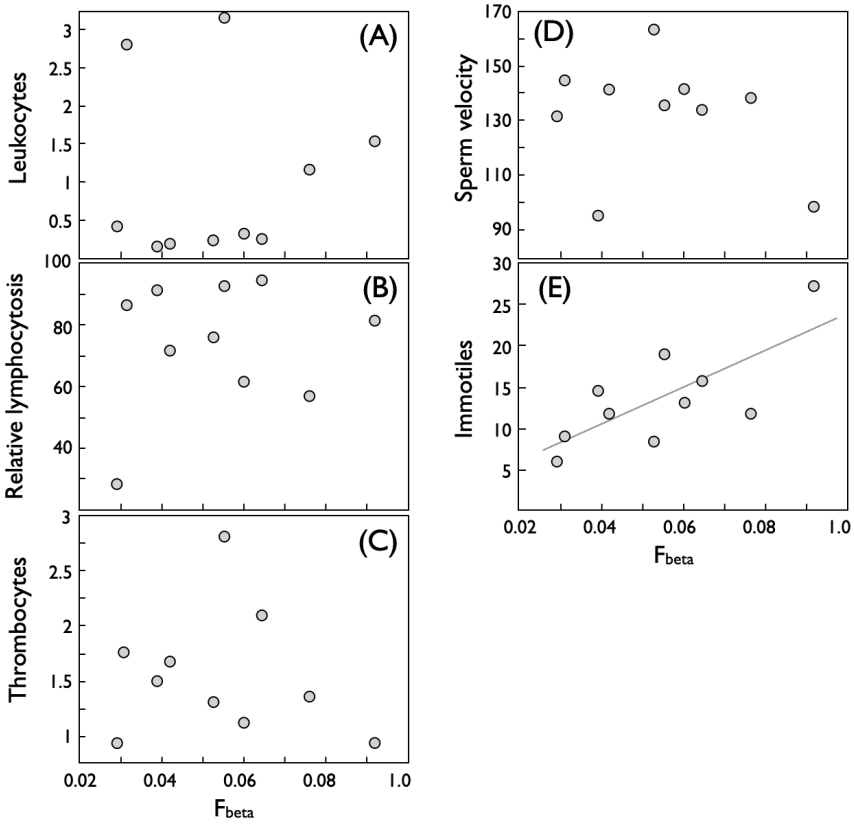

Figure S4. Immunological and sperm characteristics of males relative to their inbreeding coefficient (F_{beta}): (A) leukocytes (% of all blood cells, (B) relative lymphocytosis (% of lymphocytes among leukocytes), (C) thrombocytes per 100 blood cells, (D) sperm velocity (µm/sec), (E) percentage of immotile sperm. Sperm longevity was not measured in 2017. The regression line is given for the significant relationship. See text for statistics.

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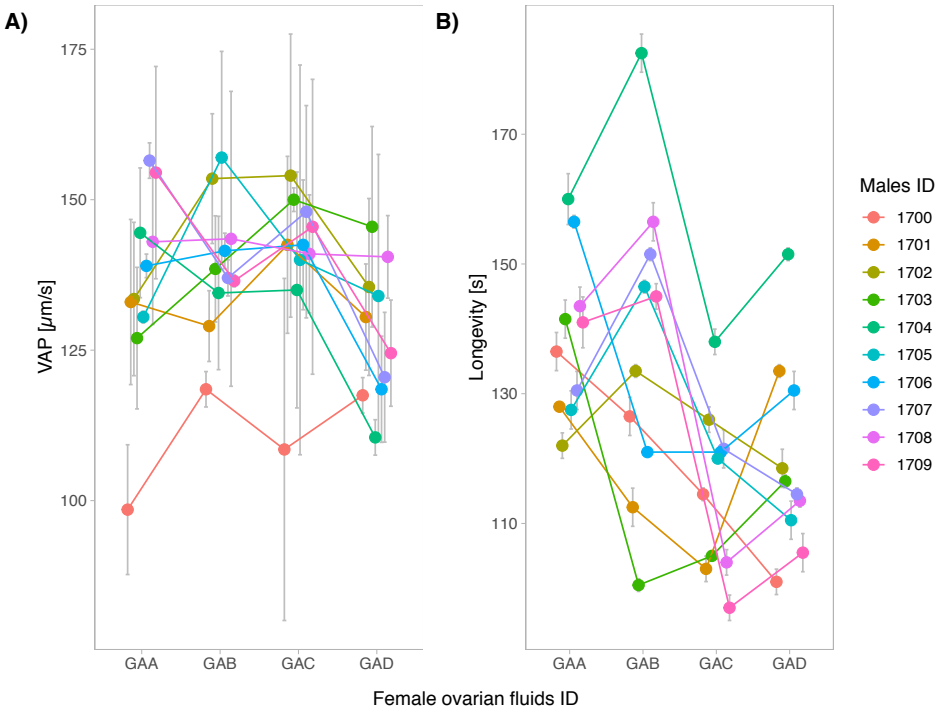

Figure S5. Interactions effects between females' ovarian fluids and males' identities on A) sperm velocity (VAP, in µm/s) and (B) sperm longevity (s). Symbols depicts means of within subject repeated measures with their 95% confidence intervals. See Table 1 for statistics.

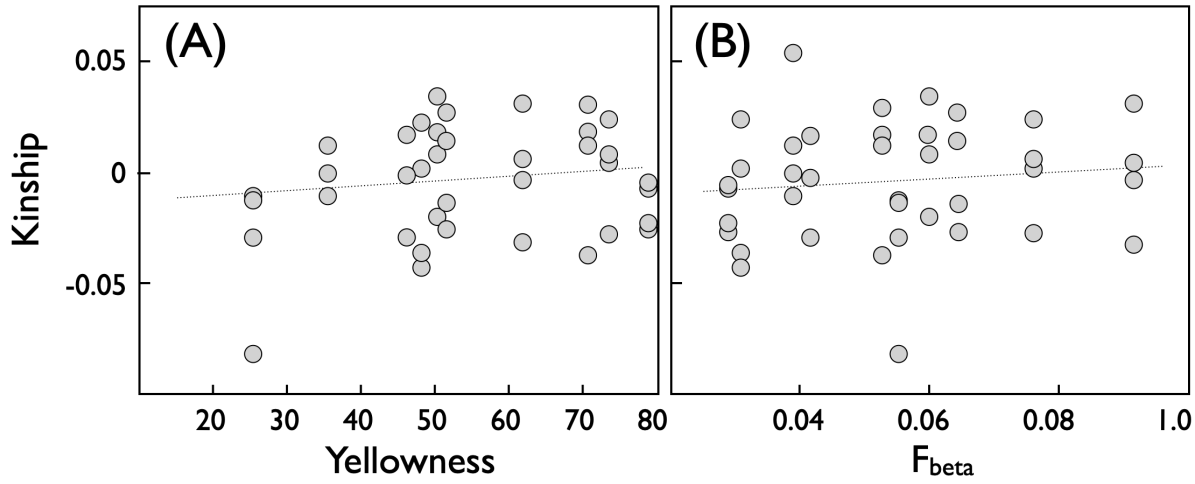

Figure S6. Relatedness between males and females (kinship) that were used in the sperm competition trials (4 females x 10 males = 40 combinations) versus (A) male skin colorations (yellowness) and (B) individual male inbreeding coefficients (F_{beta}). The dotted lines give the non-significant regressions.