This is an electronic appendix to the paper by Hendry *et al*. 2004 Adaptive variation in senescence: reproductive life span within a wild salmon population. *Proc. R. Soc. Lond.* B **271**, 259–266. (DOI 10.1098/rspb.2003.2600.)

Electronic Appendix A

1. Microsatellite analyses

Total genomic DNA was isolated from fin tissue using the Qiagen 96-well Dneasy procedure and quantified using a Hoefer DyNA Quant™ 200 Fluorometer. Nine microsatellite loci were used: *Ots107*, *Ots3*, *One102*, *One103*, *One105*, *One108*, *One109*, *One111*, and *One114* (Appendix Table 2; Banks *et al.* 1999; Nelson & Beacham 1999; Olsen *et al.* 2000, In press). PCR amplification was carried out on an MJResearch™ DNA Engine™ PCT-200 or a DNA Engine Tetrad™ PCT-220 with 10 µl reaction volumes consisting of approximately 30 ng DNA, 1.5-2.5 mM $MgCl_2$, 8 mM dNTPs, 0.4 µM unlabeled-labeled forward primer mix, and 0.4 µM unlabeled reverse primer. Cycling conditions were 2 min at 92 C; 30 cycles of 15 seconds at 92 C, 15 seconds at 51-56 C (Appendix Table 2), and 30 seconds at 72 C; and a final extension for 10 min at 72 C. Amplicons were separated and visualized on 64-well denaturing polyacrylamide gels using a Li-Cor IR$^{2®}$scanner and scored with Li-Cor Saga™ GT version 2.0 (Lincoln, NE). Li-Cor 50-350 bp or 50-500 bp size standards were loaded in the first and last lanes and at intervals of 14 lanes across each gel. To ensure consistency of allele scores, positive controls (2-4 alleles of predetermined size) were loaded in three lanes spread evenly across the gels. Two researchers independently scored all alleles. Samples with score discrepancies were re-amplified and the double-scoring process repeated until all scores matched.

Statistical analyses were performed using FSTAT version 2.9.3 (Goudet 1995). Estimates of allele frequencies, observed heterozygosities (H_O), and expected heterozygosities (H_E) were calculated for each combination of locus and sample (i.e., early vs. late). Randomization tests (N=360) of the statistic *f* were used to test for conformity to Hardy Weinberg equilibrium (HWE) within each locus/sample combination. Randomization tests (N=720) of the log-likelihood *G*-statistic were used to test for genotypic disequilibrium among locus pairs (the two samples pooled). *G*-tests of genotypic frequency homogeneity were then used to test for genetic differentiation between early and late samples at each locus. Statistical significance was evaluated at an uncorrected nominal level (α = 0.05), and after sequential Bonferroni correction (Rice 1989).

Allele frequencies are given in Appendix Table 3 and H_O and H_E are given in Appendix Table 4. The only significant deviation from HWE was *One103* in the late sample, and this was no longer significant after correction for multiple comparisons. Significant deviations from genotypic equilibrium were found for 12 of 36 locus pairs but were not consistently associated with a particular pair. Only two of the deviations remained significant after correction for multiple comparisons, and these probably reflect

the mixing of two distinct samples (early and late). We conclude that the loci were assorting independently. Overall genetic differentiation between the early and late samples was very low (Appendix Tables 3 and 4), which was not surprising given they were collected only 29 d apart from the same location in a small stream. The effective number of migrants (N_em) between early and late breeders was 7.83, estimated with the rare alleles method (Slatkin 1985) as implemented in GENEPOP (Raymond & Rousset 1995). The *rate* of gene flow between early and late breeders (i.e., the *proportion* of genes exchanged per generation, m) was estimated using the maximum-likelihood coalescent program MIGRATE (Beerli & Felsenstein 1999). The accuracy of our estimate was maximized by using the microsatellite ladder model with a threshold of 30 (Beerli & Felsenstein 1999).

2. Selection analyses

We used standard regression methods (Lande & Arnold 1983; Janzen & Stern 1998) to determine the relationship between each trait (breeding date and RLS) and relative fitness. For all analyses, each trait was standardized to a mean of zero and a standard deviation of unity. Absolute fitness was set zero for each female whose nest was superimposed and at unity for each female whose nest was not superimposed. Relative fitness was then calculated in the usual way (Lande & Arnold 1983; Janzen & Stern 1998). Actual fitness values were unknown but were probably not zero and unity: some eggs survive with superimposition and some eggs die without superimposition. However, the choice of alternative values would not influence the sign of the selection coefficient (positive or negative) or its significance level.

Selection coefficients were estimated with logistic regressions, followed by the conversion of logistic coefficients to their linear equivalents (Janzen & Stern 1998). First, simple logistic regressions were used to estimate total directional selection acting on each trait. Coefficients from these regressions (rows one and two of Table 2 in the MS) represent the combined strength of direct and indirect selection acting on the trait. (Indirect selection acts on a trait solely through its correlation with other traits under selection). Second, a multiple logistic regression including both traits was used to estimate direct directional selection acting on each trait. Partial coefficients from this regression (rows three and four of Table 2 in the MS) represent the strength of direct selection acting on each trait (i.e., after removing indirect selection acting through the other trait). Third, a multiple logistic regression including both traits, squared terms for both traits (univariate quadratic coefficients), and the cross-product term (bivariate quadratic coefficient) was used to estimate quadratic selection acting on each trait, as well as the combination of the two traits. Partial coefficients for the squared terms (rows five and six of Table 2 in the MS) represent the strength of stabilizing selection (when negative) or disruptive selection (when positive). The partial coefficient for the cross-product term (row seven of Table 2 in the MS) represents the strength of selection favoring similar trait combinations (when positive: i.e., late breeding with long RLS and/or early breeding with short RLS) or different trait combinations (when negative: i.e., late breeding with short RLS and/or early breeding with long RLS). Fourth, simple logistic regressions were performed separately for early and late females to estimate directional selection on RLS *within* each group.

3. Age-specific survival and hazard.

Using LIFEREG with breeding day as a covariate, a constant hazard model (exponential model) was rejected in favor of a Weibull model in all three years: 1995, 1996, and 2000 (each $P < 0.0001$). A Weibull model was not rejected for a generalized gamma model in 1995 ($P = 0.150$) but was marginally rejected in 1996 ($P = 0.010$) and 2000 ($P = 0.049$). We continue to focus on the Weibull parameter estimates (Appendix Table 5) rather than the gamma estimates because (1) the former are easy to interpret ($\ln T = \beta_0 + \beta_1 x + \sigma\varepsilon$, with time until death as T and x as breeding day), (2) the fit of the two model forms was so similar, and (3) the coefficients for breeding date were nearly identical even when the gamma model was superior: 1996 (gamma: $\beta_1 = -0.031 \pm 0.003$) and 2000 (gamma: $\beta_1 = -0.024 \pm 0.003$). The percent increase in time until death for each additional day of breeding was calculated using the conversion $100(e^{-\beta 1/\sigma} - 1)$.

4. ESS model.

We assumed female fitness was influenced by the number of eggs deposited, the probability of superimposition, and the loss of eggs in the event of superimposition. In the following, we explain how each of these effects was parameterized for the Pick Creek population.

The number of eggs deposited was assumed to be a function of a female's RLS. In Pick Creek, the seasonal decline in RLS and corresponding increase in gonad mass represent a 1.5% decrease in gonad mass for each additional day of life. Egg size varies little with respect to breeding date in Pick Creek (Hendry *et al.* 1999), and so a 1.5% decrease in gonad mass corresponds to a 1.5% decrease in egg number. The breeding season in Pick Creek is approximately 35 d long but our model used a 15 d season to speed computer processing time. We therefore set the cost of an additional day of life as a 3.6% decrease in egg number.

We considered three factors that influence the probability of superimposition: the distribution of female breeding dates, daily predation rate (i.e., extrinsic mortality), and nest site availability. The distribution of breeding dates was estimated as a beta distribution ($\alpha = \beta = 1.5$; for more details see Morbey & Ydenberg 2003). The daily predation rate was estimated at 2.7%, based on our extensive data for tagged females in Pick Creek (A. Hendry & S. Gende, unpublished). For the 15 d season in our model, daily predation rate was therefore set at 6.3%. Nest site availability was unknown for Pick Creek but the absence of waiting behavior (females that enter the creek but do not breed immediately, see Morbey & Ydenberg 2003), suggests that enough sites are available for all (or most) females. Nest site availability was therefore set at 10,000 for a population of 10,000 females, roughly similar to the size of the actual breeding population (T. Quinn, Univ. of Washington, unpublished data).

The cost of superimposition was estimated as the proportion of eggs lost when a nest was superimposed. Based on our two full nest excavations, the mortality of eggs owing to superimposition was 0.67 (1 - [the proportion of eggs recovered from the superimposed nest / the proportion of eggs recovered not superimposed nest] = 1 - [0.17 / 0.52]). This cost was roughly similar to that assumed, although not measured, by other investigators (e.g., Fleming & Gross 1994).

References

Banks, M. A., Blouin, M. S., Baldwin, B. A., Rashbrook, V. K., Fitzgerald, H. A., Blankenship, S. M. & Hedgecock, D. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). *J. Hered.* 90, 281–288.

Beerli, P. & Felsenstein, J. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics* 152, 763–773.

Fleming, I. A. & Gross, M. R. 1994. Breeding competition in a Pacific salmon (Coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* 48, 637–657.

Hendry, A. P., Berg, O. K. & Quinn, T. P. 1999. Condition dependence and adaptation-by-time: breeding date, life history, and energy allocation within a population of salmon. *Oikos* 85, 499–514.

Goudet, J. 2001. FSTAT. A program to estimate and test gene diversities and fixation indices. Version 2.9.3. (available from http://www.unil.ch/izea/softwares/fstat.html).

Janzen, F. J. & Stern, H. S. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* 52, 1564–1571.

Lande, R. & Arnold, S. J. 1983. The measurement of selection on correlated characters. Evolution 37, 1210–1226.

Morbey, Y. E. & Ydenberg, R. C. 2003. Timing games in the reproductive phenology of female Pacific salmon (*Oncorhynchus* spp.). *Am. Nat*. 161, 284–298.

Nelson, R. J. & Beacham, T. D. 1999. Isolation and cross species amplification of microsatellite loci useful for the study of Pacific salmon. *Anim. Genet.* 30, 228–119.

Olsen, J. B., Habicht, C. & Seeb, J. E. In press. Moderate and highly polymorphic microsatellites provide discordant estimates of population divergence in sockeye salmon (*Oncorhynchus nerka*). *Environ. Biol. Fishes*.

Olsen, J. B., Wilson, S. E., Kretschmer, E. J., Jones, K. C. & Seeb, J. E. 2000. Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. *Mol. Ecol.* 9, 2185–2187.

Raymond, M. & Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86, 248–249.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43, 223–225.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39, 53–65.

Table 2. Information for the microsatellite loci used to estimate gene flow between early and late breeders in Pick Creek.

Locus	Primer sequence (5'-3')	Repeat motif	Allele size range (bp)	Annealing temp. (^{0}C)	Dye label	Multiplex panel	No. of alleles
One105	F: TCT TTA AgA ATA TgA gCC CTg g R: gCT CAA ATA AAC TTA AAC CTg TCC	Tetra	111-163	51	700	A	3
One111	F: ATg ACC Aag gAg CTT CTg R: TAT CCA ggT ACT CCA CTg gC	Tetra	190-330	51	700	A	22
One114	F: TCA TTA ATC Tag gCT TgT CAg C R: TgC Agg TAA gAC Aag gTA TCC	Tetra	207-299	51	800	A	12
Ots3	F: CAC ACT CTT TCA ggA g R: AgA ATC ACA Atg gAA g	Di	67-111	51	800	A	17
One102	F: CAT ggA gAA Aag ACC AAT CA R: TCA CTg CCC TAC AAC AgA Ag	Tetra	194-270	56	700	B	13
One108	F: TgC AgA gCC ATA CTA AAC CA R: Aag AAT TgA gAg Atg Cag gg	Tetra	163-263	56	800	B	30
One109	F: Agg gAg AgA AgA gAg ggA gA R: CCT Cag Aag Tag CAT Cag CTC	Tetra	114-186	56	700	B	7
Ots107	F: ACA gAC Cag ACC TCA ACA R: ATA gAg ACC TgA ATC ggT	Di	84-140	51	800	C	4
One103	F: AAT gTT gAg AgC TAT TTC AAT R: gAT TgA TgA Atg ggT ggg	Tetra	147-363	51	700	C	17

Table 3. Allele frequencies (number of alleles out of 100) in early and late breeders in Pick Creek. "Allele" columns give allele sizes in base pairs.

One103			***One111***			***One114***			***One108***			***One109***		
Allele	Late	Early	**Allele**	Late	Early	**Allele**	Late	Early	**Allele**	Late	Early	**Allele**	Late	Early
167	-	1	**196**	1	2	**222**	-	1	**177**	1	2	**126**	11	21
171	6	10	**200**	30	32	**226**	2	1	**181**	4	4	**130**	10	13
175	2	1	**204**	39	32	**230**	7	5	**185**	6	4	**134**	2	1
179	4	3	**208**	6	10	**234**	7	8	**189**	10	6	**142**	3	3
183	2	-	**212**	4	1	**238**	9	8	**193**	10	12	**146**	2	4
199	1	-	**232**	-	2	**242**	13	10	**197**	13	20	**150**	21	15
219	1	1	**236**	5	3	**246**	14	10	**201**	14	11	**154**	3	7
239	-	1	**240**	2	2	**250**	15	17	**205**	11	7	**158**	3	5
247	3	1	**244**	2	-	**254**	15	7	**209**	4	4	**162**	16	19
251	3	3	**248**	2	1	**258**	6	8	**213**	8	4	**166**	11	6
255	2	-	**252**	1	-	**262**	2	7	**217**	5	5	**170**	11	6
263	-	2	**256**	2	1	**266**	4	9	**221**	5	7	**174**	3	-
267	4	5	**264**	1	-	**274**	4	3	**225**	3	2	**178**	4	-
271	5	3	**268**	1	1	**278**	1	1	**229**	2	5			
275	5	2	**272**	1	3	**282**	1	3	**233**	1	-			
279	4	6	**276**	-	1	**286**	-	1	**245**	3	5	***One102***		
283	6	3	**284**	-	1	**290**	-	1	**249**	-	2	**Allele**	Late	Early
287	9	5	**288**	1	2							**200**	-	1
291	2	7	**292**	1	2							**208**	9	11
295	3	6	**296**	1	1	***Ots107***			***Ots3***			**212**	8	-
299	11	11	**300**	-	1	**Allele**	Late	Early	**Allele**	Late	Early	**220**	2	1
303	5	4	**308**	-	2	**112**	-	1	**75**	1	1	**224**	2	2
307	3	5				**116**	85	76	**83**	1	2	**228**	4	5
311	3	7				**120**	14	23	**87**	13	11	**232**	28	42
315	5	1	***One105***			**124**	1	-	**91**	60	56	**236**	23	19
319	4	2	**Allele**	Late	Early				**95**	10	14	**240**	11	12
323	3	1	**130**	85	84				**97**	14	16	**244**	9	7
327	1	4	**134**	9	10				**99**	1	-	**248**	3	-
331	2	4	**138**	6	6							**264**	1	-
335	1	1												

Table 4. Results of G-tests for genetic differentiation between early and late samples. Also shown are observed (H_O) and expected (H_E) heterozygosities within each sample, and the significance level of tests for deviations from Hardy Weinberg equilibrium (HWE). All *P* values were non-significant after sequential Bonferroni corrections.

Locus	Differentiation (*P* value)	Sample	H_O	H_E	HWE (*P* value)
One105	0.999	Late	0.300	0.268	1.000
		Early	0.320	0.283	1.000
One111	0.761	Late	0.780	0.755	0.742
		Early	0.780	0.788	0.528
One102	0.037	Late	0.820	0.839	0.411
		Early	0.820	0.760	0.872
One108	0.803	Late	0.900	0.920	0.303
		Early	0.880	0.914	0.208
One109	0.092	Late	0.920	0.887	0.858
		Early	0.860	0.872	0.456
One103	0.524	Late	0.880	0.959	0.011
		Early	0.900	0.953	0.086
Ots3	0.929	Late	0.640	0.599	0.839
		Early	0.700	0.634	0.914
Ots107	0.121	Late	0.220	0.261	0.219
		Early	0.360	0.373	0.522
One114	0.712	Late	0.940	0.901	0.892
		Early	0.880	0.919	0.208

Table 5. Parameter estimates ± SE for Weibull models fit to survival data for females dying of senescence in 1995, 1996, and 2000. The intercept (β_0), coefficient for breeding day (β_1), and Weibull scale parameter (σ) are shown in each year. All β_0 and β_1 coefficients are significant ($P < 0.001$).

Year	β_0	β_1	σ
1995	3.35 ± 0.04	-0.037 ± 0.005	0.178 ± 0.017
1996	3.16 ± 0.03	-0.030 ± 0.002	0.182 ± 0.013
2000	2.99 ± 0.03	-0.021 ± 0.002	0.126 ± 0.013