Table S1
Sample sizes from each family in each developmental treatment

	Family 1	Family2	Family 3	Family 4	Family 5	Family 6
12 °C Development	2	0	1	2	1	0
18 °C Development	1	1	0	3	0	1
24 °C Development	2	1	1	2	0	0

Table S2
Genomic mean and median percent methylation

Treatment	Mean genomic percent DNA methylation	Standard deviation	Median genomic percent DNA methylation	Standard deviation
12 °C development, 18 °C acclimation	73.66%	2.21%	82.62%	3.68%
24 °C development, 18 °C acclimation	72.31%	0.89%	80.30%	0.74%
18 °C development, 18 °C acclimation	72.03%	2.20%	80.98%	3.32%
18 °C development, 5 °C acclimation	73.62%	1.24%	83.38%	2.07%
18 °C development, 25 °C acclimation	73.96%	1.48%	84.12%	3.56%

(n=6)

Table S3
Kolmogorov-Smirnov test statistics

Treatment	p-value	D statistic
12 °C development, 18 °C acclimation	2.20E-16	0.02336
24 °C development, 18 °C acclimation	2.20E-16	0.042084
18 °C development, 5 °C acclimation	2.20E-16	0.05126
18 °C development, 25 °C acclimation	2.20E-16	0.081229

Table S5

Difference in percent DNA methylation level for DMRs common to all treatments

DMR position			12 °C Development		24 °C Devlopment		5 °C Acclimation		25 °C Acclimation	
chr	start	end	qvalue	DiffMeth	qvalue	DiffMeth	qvalue	DiffMeth	qvalue	DiffMeth
groupI	9551801	9551900	3.52E-02	21	1.29E-02	23	9.78E-04	26	1.03E-02	24
groupI	24585101	24585200	1.23E-05	-16	6.34E-05	-14	3.24E-05	-15	9.61E-04	-13
groupI	27994101	27994200	7.90E-05	20	4.49E-03	17	2.48E-08	28	6.72E-03	17
groupII	18989201	18989300	8.13E-06	15	4.91E-08	18	3.00E-16	24	3.31E-04	16
groupII	21905101	21905200	1.44E-02	10	5.17E-03	11	4.30E-06	15	5.37E-03	11
groupIII	879601	879700	5.51E-09	23	3.10E-06	19	4.03E-03	12	1.15E-15	31
groupIII	9626701	9626800	2.23E-67	32	3.89E-07	12	1.18E-18	19	2.31E-30	25
groupIV	132901	133000	1.57E-03	14	3.36E-05	16	7.14E-03	11	9.56E-03	13
groupIV	2314301	2314400	2.43E-15	-10	8.36E-17	-10	9.82E-30	12	3.58E-34	-15
groupIV	2758901	2759000	3.15E-02	14	3.08E-02	15	3.91E-03	18	2.05E-03	19
groupIV	11319801	11319900	4.00E-04	-14	3.95E-03	-11	4.07E-04	-13	4.39E-06	-17
groupIV	12200201	12200300	1.07E-03	17	4.32E-03	16	6.87E-06	22	8.46E-04	19
groupIV	29800001	29800100	1.03E-06	15	5.09E-08	18	7.18E-19	26	6.79E-13	28
groupIX	353601	353700	9.20E-06	-13	4.96E-04	-11	4.24E-14	-22	4.86E-11	-18
groupIX	8238301	8238400	7.43E-03	-15	2.42E-04	-19	2.11E-03	-15	6.51E-04	-17
groupIX	17934101	17934200	4.70E-02	13	6.68E-03	16	5.86E-03	15	2.00E-02	16
groupV	9288901	9289000	3.64E-04	-14	1.34E-02	-11	6.73E-10	-25	1.27E-02	-10
groupVI	1530101	1530200	4.31E-08	26	5.66E-03	16	1.97E-11	27	8.73E-03	14
groupVI	4132101	4132200	1.01E-37	13	1.80E-12	-12	1.11E-52	-27	3.86E-37	13
groupVII	5901801	5901900	4.96E-11	-20	3.75E-18	-27	3.17E-51	-55	9.13E-06	-12
groupVII	15623001	15623100	4.60E-06	21	9.36E-03	14	3.56E-04	17	6.62E-04	17
groupVIII	351301	351400	3.04E-02	12	1.23E-03	18	1.08E-08	27	1.33E-05	27
groupX	97101	97200	9.08E-10	36	9.73E-05	22	2.46E-15	37	9.17E-04	23
groupX	2872701	2872800	4.20E-06	-16	2.29E-04	-12	6.29E-05	-13	8.74E-04	-11
groupXI	2274501	2274600	8.61E-05	20	1.22E-02	15	6.30E-07	24	2.93E-06	24
groupXII	7504001	7504100	7.04E-04	14	7.12E-04	14	3.86E-06	18	1.42E-02	11
groupXIII	8471801	8471900	9.75E-06	32	1.38E-04	30	4.85E-09	37	1.28E-03	24
groupXIV	3410601	3410700	2.65E-02	-14	4.41E-02	-13	3.38E-02	-13	2.62E-02	-13
groupXIV	3943001	3943100	3.40E-02	-14	1.56E-02	-14	4.76E-05	-23	8.94E-03	-12
groupXIV	10441601	10441700	2.17E-02	-14	1.60E-04	-20	7.08E-07	-25	7.12E-03	-16
groupXIX	8793401	8793500	8.41E-08	22	2.15E-06	17	1.08E-09	23	1.44E-03	14
groupXIX	10454801	10454900	3.29E-03	-13	1.49E-07	-21	2.58E-10	-23	5.99E-03	-12
groupXIX	15351001	15351100	2.35E-03	15	6.51E-03	13	1.44E-04	18	2.20E-03	15
groupXV	1970801	1970900	1.34E-04	-21	5.07E-06	-25	9.26E-04	-17	1.54E-03	-16
groupXV	4546301	4546400	4.46E-187	36	1.83E-100	27	1.56E-52	19	4.13E-109	29
groupXVI	8645601	8645700	5.14E-03	23	2.09E-02	19	5.51E-03	20	3.47E-02	17
groupXVI	17985101	17985200	2.93E-06	32	4.05E-04	26	6.06E-08	38	1.18E-07	37
groupXVI	17985201	17985300	8.29E-05	23	1.01E-02	16	4.03E-06	26	1.54E-08	31
groupXVII	5773201	5773300	8.78E-03	-14	1.04E-02	-12	9.66E-11	-27	4.55E-02	-12
groupXVII	11445901	11446000	8.57E-04	16	1.13E-03	16	2.00E-05	20	7.49E-03	14
groupXVIII	7284901	7285000	3.63E-02	16	4.98E-02	13	3.09E-04	23	3.65E-05	24
groupXVIII	15625401	15625500	1.20E-12	26	3.85E-06	18	2.37E-04	17	1.06E-04	18
groupXXI	5934501	5934600	5.07E-06	-28	1.44E-05	-30	5.01E-06	-27	1.62E-04	-24
groupXXI	8811701	8811800	7.07E-04	12	2.27E-03	10	1.32E-04	13	1.05E-12	30
scaffold_1331	2901	3000	3.73E-05	-20	9.70E-05	-19	2.79E-07	-22	3.76E-07	-25
scaffold_1498	5201	5300	1.62E-07	11	4.63E-06	10	1.50E-13	16	7.16E-11	15
scaffold_167	70001	70100	4.98E-12	-25	2.32E-03	-15	3.08E-13	-24	3.31E-10	-24
scaffold_235	12101	12200	4.14E-03	17	8.66E-03	17	1.11E-05	23	1.07E-03	19
scaffold_515	5601	5700	5.90E-16	16	2.25E-13	13	5.99E-45	22	6.37E-35	18
scaffold_990	401	500	2.99E-03	22	4.79E-02	13	6.34E-08	32	2.42E-03	19

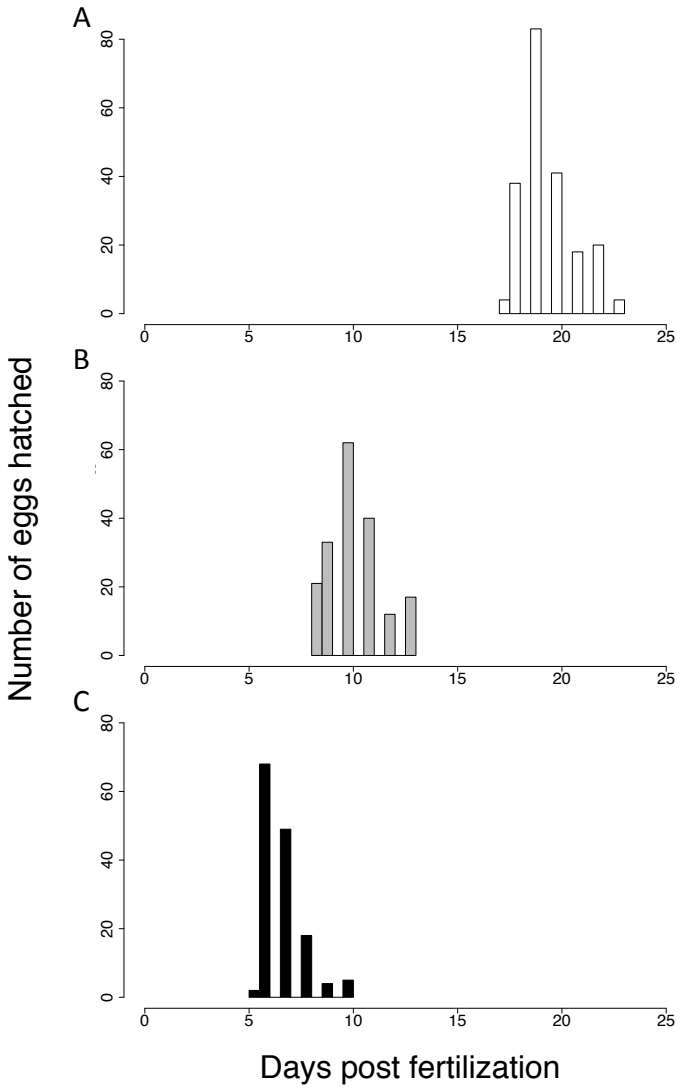


Figure S1: The effects of developmental temperature on time to hatch of threespine stickleback embryos. Histograms represent the number of threespine stickleback embryos that hatched on a particular day post fertilization when reared at 12 °C (A; white), 18 °C (B; grey), or 24 °C (C; black) . Day 0 represents the day of fertilizaiton.

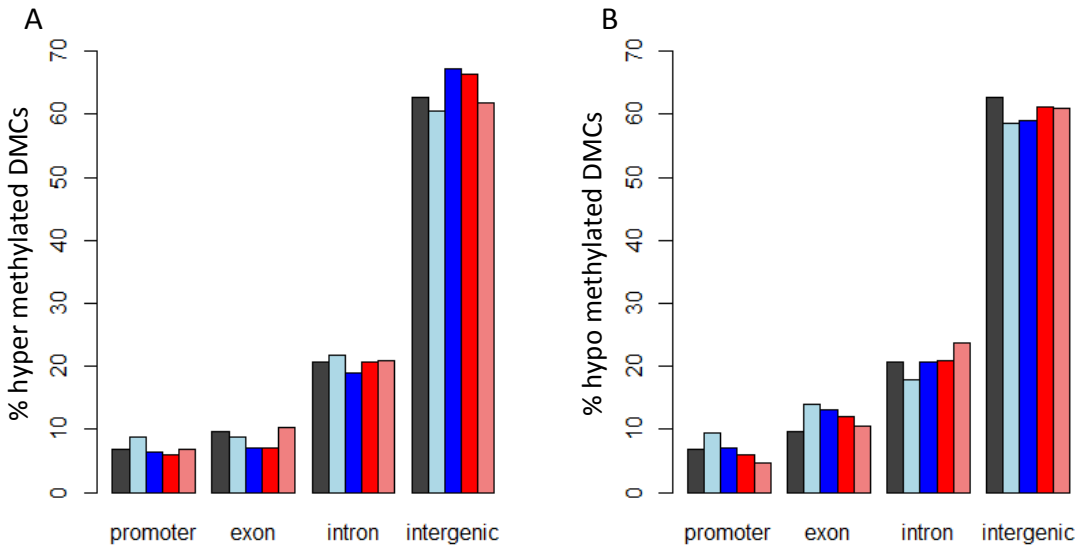


Figure S2
Genomic distributions of (A) significantly hypermethylated and (B) significantly hypomethylated cytosines for each treatment. Different colors correspond to the different treatments with 12 °C development as light blue, 5 °C acclimation as dark blue, 25 °C acclimation as red, 24 °C development as pink. The background distribution of the sequenced CpG loci is in grey and is replicated in panels A and B.