Supplementary Information

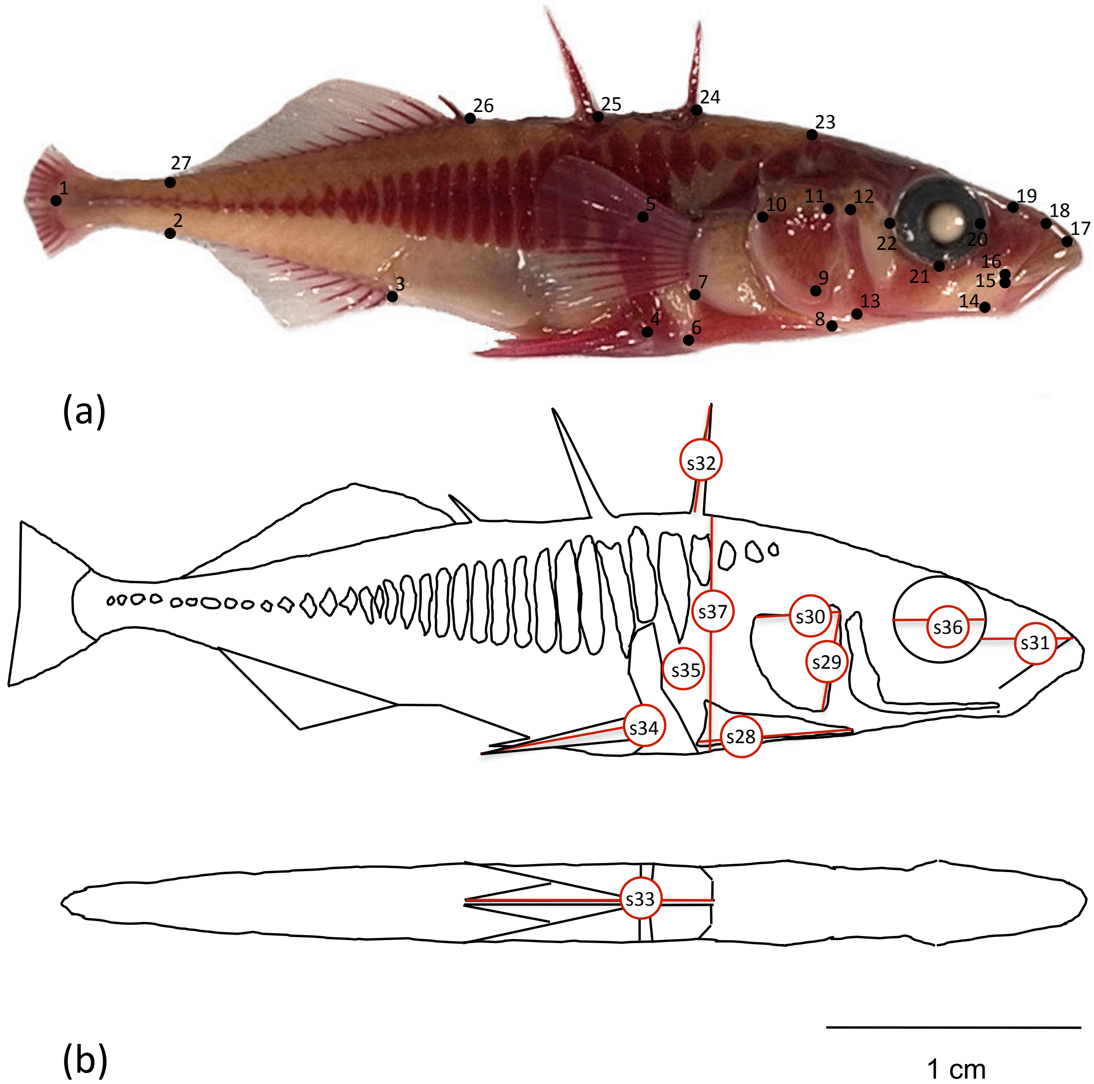


Figure S1. (A) An adult F2 Little Campbell Marine x Paq Lake stickleback stained with alizarin red to highlight bony tissue. Dots indicate the locations of the 27 landmarks. (B) A lateral and ventral tracing of the same individual indicating the 11 metric traits measured for QTL analyses.

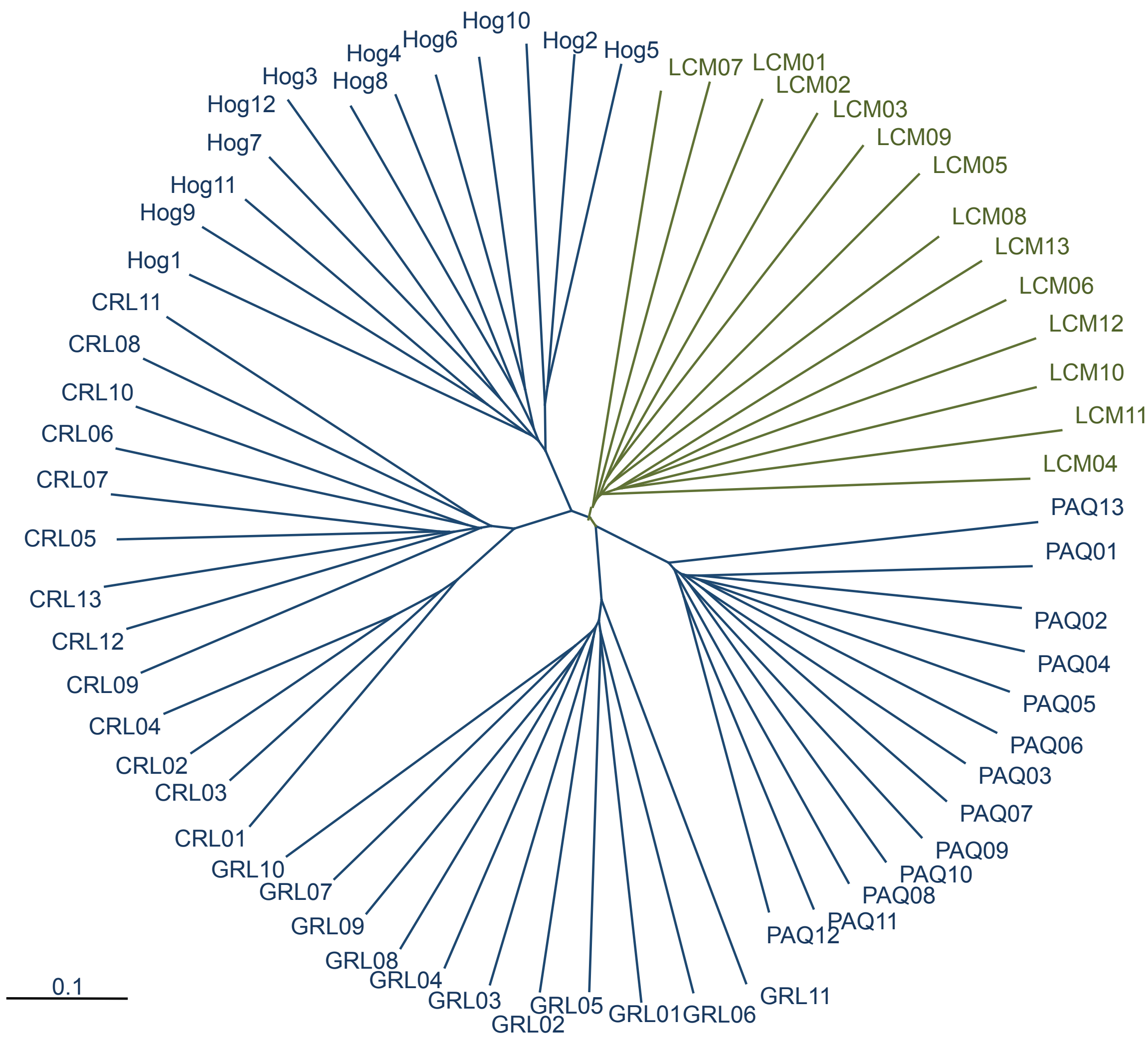


Figure S2. Individual-based Neighbor Joining (NJ) phylogeny created using 104 microsatellite loci and the Chord distance for the marine population (Little Campbell River; LCM), Paq Lake (PAQ), Cranby Lake (CRL), Graham Lake (GRL), and Hoggan Lake (HOG). The unrooted tree suggests all freshwater populations are genetically distinct and arose independently and simultaneously from the marine population.

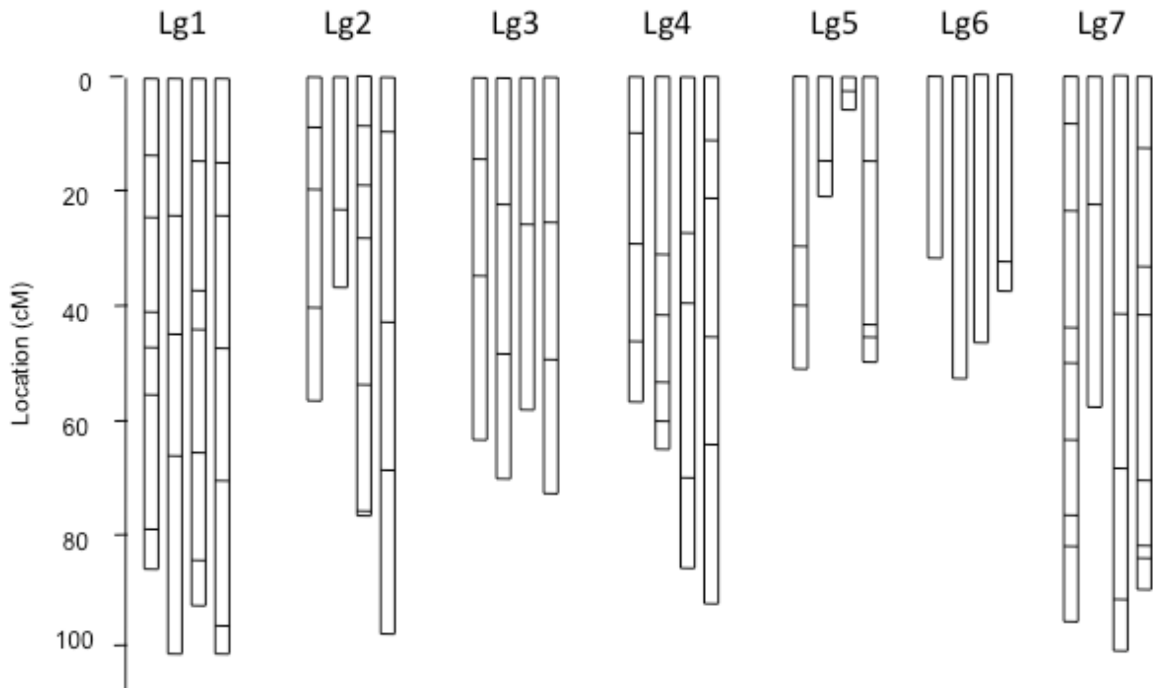


Figure S3. Genetic linkage maps assembled for F2 Marine x lake populations. The number of linkage groups corresponds to the known haploid number of chromosomes in stickleback. For each linkage group (Lg), the maps appear in the following order of lakes; Cranby, Hoggan, Graham, and Paq. Horizontal lines along each linkage group represent locations of mapped microsatellite loci (for identification see Table S2).

Figure S3 (continued)

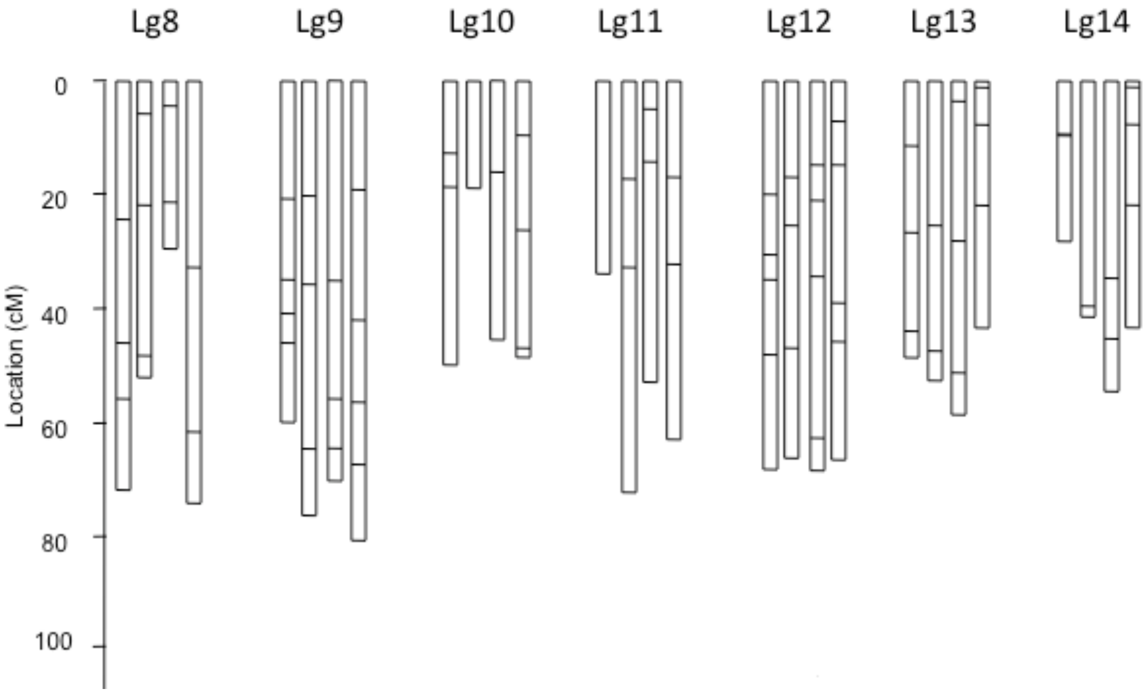
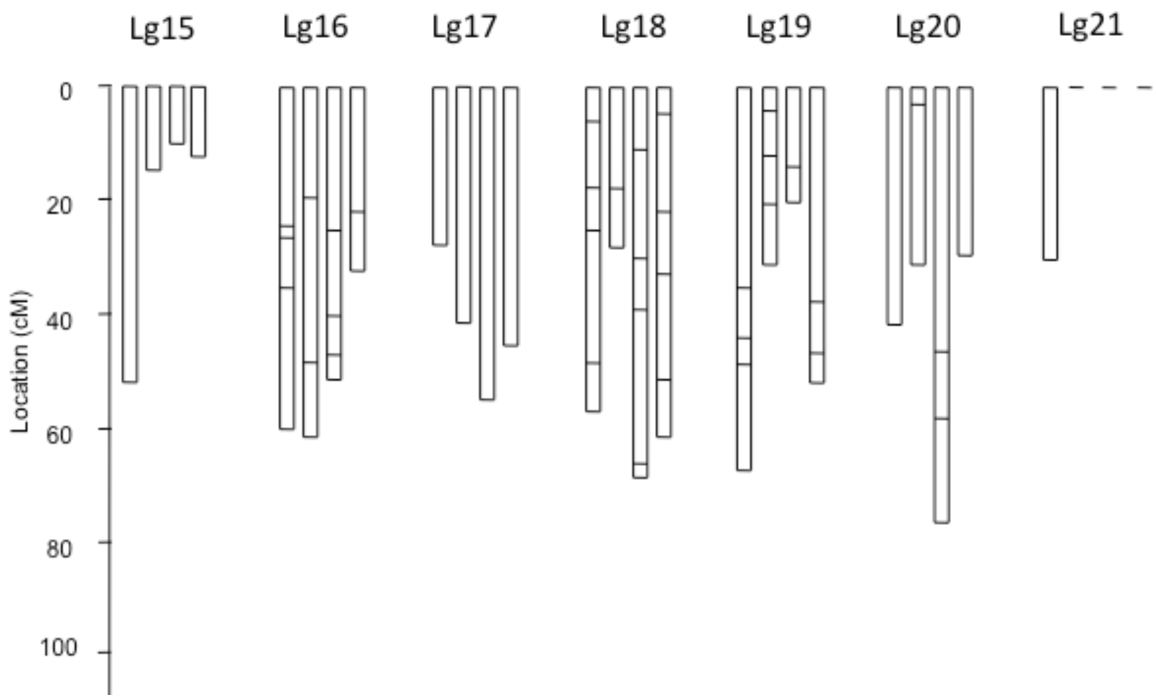


Figure S3 (continued)



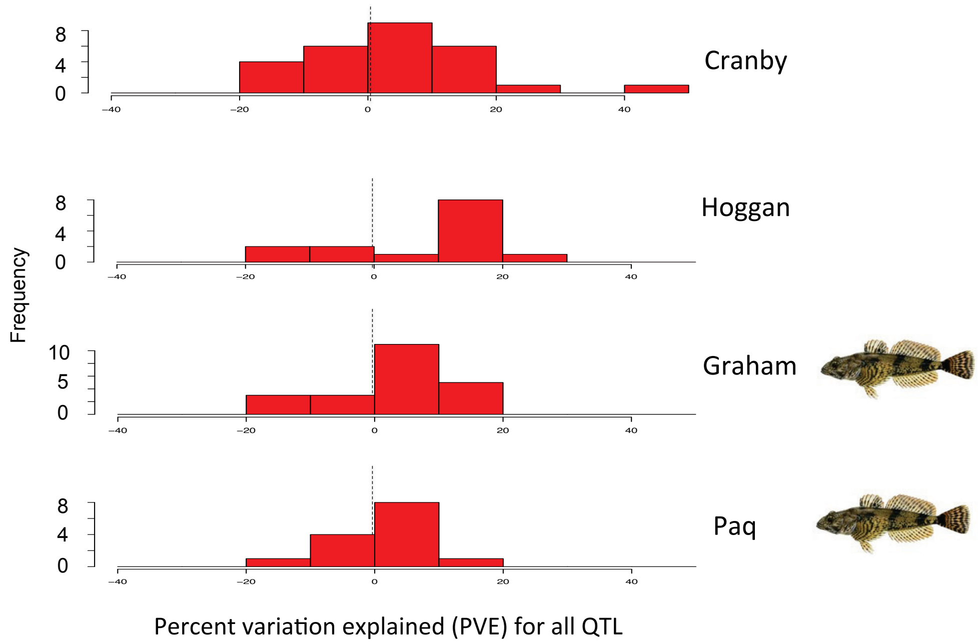


Figure S4. The distribution of all QTL effect sizes as the percentage of variance explained (PVE) in sculpin-absent (Cranby and Hoggan) and sculpin-present (Graham and Paq) Lakes

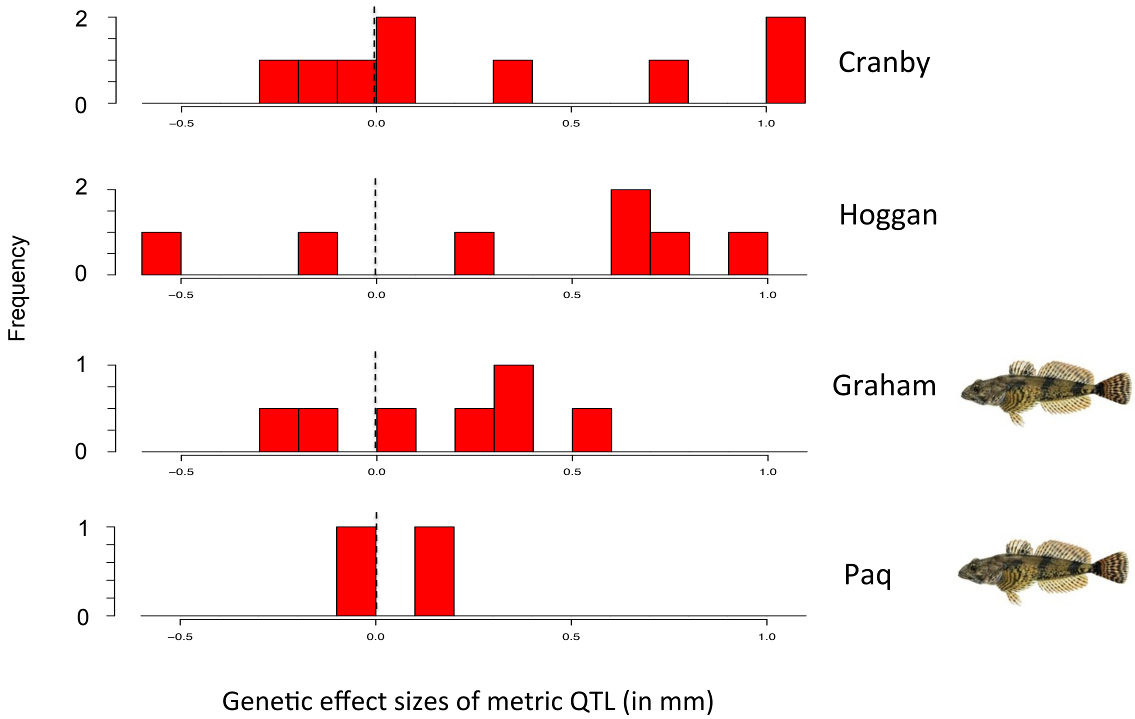


Figure S5. The distribution of QTL effect sizes for size-adjusted metric traits in sculpin-absent (Cranby and Hoggan) and sculpin-present (Graham and Paq) Lakes

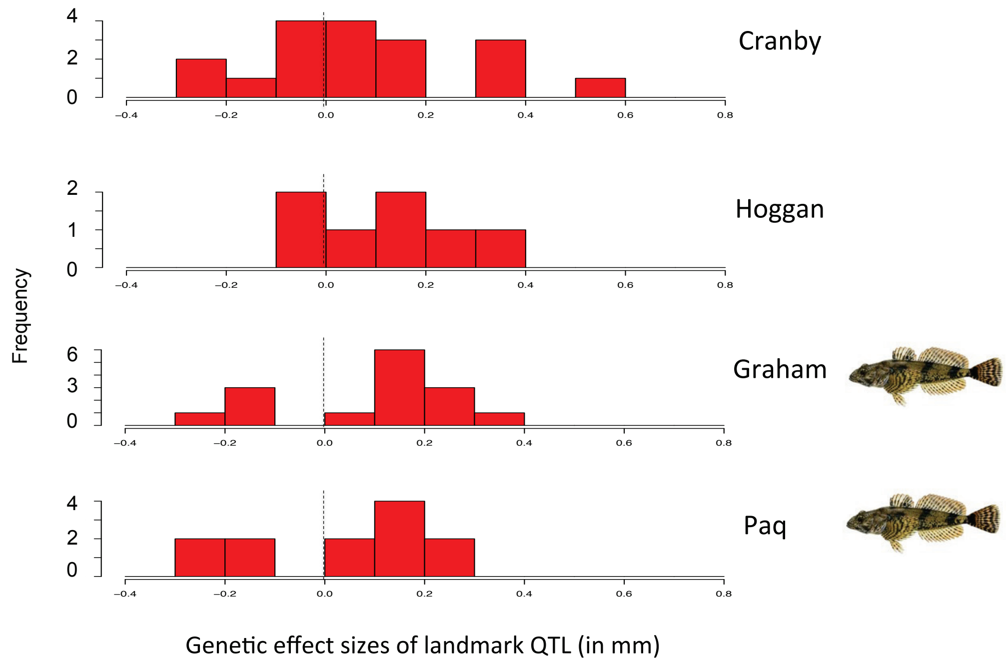


Figure S6. The distribution of QTL effect sizes for landmark traits in sculpin-absent (Cranby and Hoggan) and sculpin-present (Graham and Paq) Lakes

Table S1. The difference between the mean phenotypes of the marine and lake populations, for landmark (x, y), and size-adjusted metric traits (s28-s37). The phenotypic difference between the marine ancestor and each freshwater population (in mm) was used to calculate the direction of QTL effect.

Trait	Cranby	Hoggan	Graham	Paq
x1	-0.240	0.051	-0.173	-0.392
y1	0.105	-0.025	0.278	0.011
x2	0.544	0.322	-0.110	0.064
y2	0.224	0.233	0.431	0.266
x3	0.052	-0.442	1.178	0.782
y3	0.592	0.531	-0.051	-0.022
x4	0.371	0.182	0.173	0.099
y4	0.843	0.733	0.652	0.223
x5	0.648	0.814	0.120	0.308
y5	-0.737	-1.127	-0.533	-0.120
x6	-0.158	0.038	0.026	0.018
y6	0.376	0.575	0.335	0.195
x7	-0.052	0.198	0.429	0.554
y7	0.098	0.108	-0.030	0.074
x8	-0.916	-1.489	-0.054	-0.595
y8	-0.247	0.134	-0.024	-0.019
x9	-0.631	-0.614	-0.657	-0.284
y9	-0.011	0.173	0.257	0.029
x10	-0.629	-0.282	-0.442	-0.153
y10	0.333	0.202	0.391	0.158
x11	-0.416	-0.011	-0.293	-0.277
y11	0.301	0.072	0.070	-0.031
x12	-0.158	-0.043	-0.338	-0.079
y12	0.255	0.083	0.222	-0.006
x13	-0.102	-0.204	-0.222	-0.061
y13	0.022	0.348	0.139	0.073
x14	0.365	0.075	0.177	-0.013
y14	-0.311	-0.063	-0.107	-0.148
x15	0.633	0.137	0.111	0.181
y15	-0.455	-0.339	-0.301	-0.261
x16	0.375	-0.129	-0.374	0.193
y16	-0.484	-0.991	-0.764	-0.151
x17	0.797	0.649	0.654	0.450
y17	-0.026	0.164	0.203	0.046
x18	0.747	0.737	0.661	0.339
y18	-0.034	0.097	0.107	0.023
x19	0.614	0.622	0.492	0.222
y19	0.015	-0.285	-0.180	-0.050
x20	0.209	0.283	0.277	-0.281

y20	0.389	0.319	0.387	0.181
x21	0.091	0.341	-0.099	-0.263
y21	0.389	0.424	0.042	0.357
x22	0.022	0.542	-0.261	-0.028
y22	0.537	0.387	0.389	0.307
x23	-1.032	-0.222	-0.076	-0.165
y23	-0.194	-0.022	-0.377	-0.523
x24	-1.448	-1.755	-0.855	-0.507
y24	-0.685	-0.410	-0.558	-0.348
x25	-0.689	-0.634	-0.300	-0.230
y25	-0.755	-0.636	-0.526	-0.270
x26	0.249	0.243	0.390	0.223
y26	-0.636	-0.611	-0.236	-0.039
x27	0.752	0.591	-0.435	-0.103
y27	0.098	-0.075	-0.216	0.046
s28 (ectocorocoid length)	-0.576	-1.097	-0.085	-0.446
s29 (dorso-ventral opercle length)	0.222	-0.032	-0.126	-0.037
s30 (lateral opercle length)	0.143	0.170	0.063	-0.103
s31, (snout length)	0.454	0.272	0.274	0.551
s32, (dorsal spine length)	-2.072	-1.956	-1.459	-0.991
s33 (pelvic girdle length)	-1.663	-3.365	-0.980	-0.614
s34 (pelvic spine length)	-2.297	-2.252	-2.039	-1.056
s35 (pectoral fin length)	0.047	0.107	0.370	-0.388
s36 (eye diameter)	0.130	0.266	0.318	-0.113
s37 (body depth)	-0.134	0.180	-0.031	-0.266

Table S2. Microsatellite loci mapped in F2 marine x lake populations in this study. Lg refers to linkage group while position refers to the mapped location of microsatellites (in centiMorgans). A blank indicates the locus was not mapped in that population.

Locus	Lg	Cranby	Paq	Graham	Hoggan	Genbank Accession or Reference
stn329	1				0	BV678132
stn248	1				27	BV678074
gac4170	1			0		Largiadér, 1999
stn1	1	0	0			G72126
stn2	1	13	17	12		G72127
stn282	1	24	25			BV678098
stn4	1	42	45	30		G72230
stn5	1	49		35		G72328
stn8	1	57	65	53		G72130
stn9	1				44	G72131
stn13	1	79	91	68		G72234
stn302	1				60	BV678112
stn14	1	88	95	74	79	G72235
stn18	2	0	0	0	0	G72134
stn277	2	9	9	7		BV678094
stn16	2	20		15		G72133
stn21	2				18	BV678098
stn22	2			23		G72239
stn268	2		41		29	BV678088
stn259	2	40	70	43		BV678082
stn265	2			61		BV678086
stn24	2			62		G72138
stn26	2	56	97			G72240
stn262	3	0	0	0	0	BV678084
stn31	3		24			G72242
stn30	3	14		20	17	G72241
stn32	3	34	47	47		G72141
stn270	3				38	BV678090
stn35	3	65	69		55	G72244
stn251	4	0	0	0	0	BV678076
stn38	4	10	11	22		G72145
gac62	4		20			Largiadér, 1999*
stn40	4	29		32	24	G72245
stn42	4				33	G72148
gac4174	4	46			42	AJ010358
stn45	4		43			G72247
stn47	4				47	G72151
stn292	4				52	BV678103
stn46	4			57		G72150
stn309	4		61			BV678116

stn311	4	57	87	69		BV678167
stn52	5	0	0			G72154
stn289	5		15		0	BV678100
stn54	5				11	G72250
stn57	5	30		0		G72155
stn241	5				16	BV678067
stn58	5	40	43	2		G72253
stn60	5		47	5		G72157
stn326	5	51				BV678129
stn62	6		0		0	G72159
stn279	6	0		0		BV678153
stn64	6		31			G72160
stn65	6	34	36	37	41	G72254
stn245	7	0	0			BV678071
stn70	7	9	16			G72164
stn72	7	24	31	0		G72257
stn76	7	45	50			G72259
stn78	7	51	61	34	0	G72261
stn75				55		G72165
stn257	7	64	86		28	BV678148
stn81	7	77		73		G72262
stn80	7		100			G72167
stn82	7	82	122		45	G72168
stn342	7	95	126	81		Peichel, 2001*
stn314	8				0	BV678119
stn84	8			0		G72169
stn83	8			3		G72263
stn89	8			17		G72265
stn88	8	0	0			G72172
stn90	8				5	G72173
stn92	8	21	31			G72175
stn95	8	44	59			G72267
stn250	8				17	BV678075
stn296	8	49				BV678107
stn96	8	64			38	G72176
stn97	8		70		41	G72268
stn118	9	0	0	0	0	G72186
stn99	9				9	G72270
stn106	9	21	17	28		G72274
stn104	9		40			G72272
stn110	9	35	56	45	32	G72182
stn225	9	41	66	52		BV678054
stn114	9	46		56	44	G72277
stn117	9				60	G72185
stn252	9	60	78			BV678077
stn120	10				0	G72187

stn119	10				15	G72280
stn310	10	0	0			BV678117
stn211	10	8	14	0		BV102489
stn124	10	16	31	13		G72283
stn23	10	47	51	37		G72137
stn125	10		53			G72189
stn228	11			0		BV678056
stn244	11	0	0		0	BV678070
stn224	11			4		BV678144
stn278	11		15		13	BV678095
stn288	11	28	29	11	26	BV678156
stn246	11	68				BV678072
stn133	11	70	58	43		G72194
stn232	12	0	0	0	0	BV678060
stn320	12		7			BV678124
stn319	12	17		12	15	BV678123
stn227	12	27	14	17		BV678145
stn138	12		38		32	G72291
stn143	12	32	44	28		G72196
stn327	12	45	64	50		BV678130
stn134	12	66		55	38	G72287
stn144	12				52	G72197
stn159	13	0	0	0	0	G72206
stn307	13				20	BV678115
stn255	13	11	27	3		BV678080
stn155	13			23		G72299
stn158	13	27	39			G72300
stn153	13	44	57	41	37	G72202
stn149	13				41	G72199
stn150	13	48	66	47		G72200
stn160	14		0	0		G72301
stn161	14				0	G72302
stn163	14	0	20	28		G72304
stn331	14	18	35			BV678134
stn168	14	20	36	36	31	G72209
stn167	14	27	39	44		G72208
stn169	14				33	G72306
stn230	15	0				BV678058
stn170	15	36	0	0	0	G72307
stn230	15			8		BV678058
stn173	15		12		12	G72309
stn176	16		0			G72210
stn177	16	0		0	0	G72211
stn178	16	13				G72312
stn174	16				15	G72310
stn299	16			20		BV678109

stn337	16	27	21	32		BV686435
stn233	16	29				BV678061
stn179	16		30			G72212
stn294	16	38		37	38	BV678105
stn202	17	0	0	0	0	G72322
stn343	17				33	Peichel, 2004
stn323	17	28	45	44		BV678127
stn280	18	0	0	0	0	BV678096
stn261	18	4	5		14	BV678083
stn301	18	16	22	24	22	BV678111
stn317	18	24	32	32		BV678121
stn305	18	47	50	54		BV678114
stn308	18	56	60	55		BV678159
stn290	19	0	0		0	BV678101
stn186	19	30	36	0	8	G72215
stn295	19				15	BV678106
idh	19	39	44	11	21	Peichel, 2004*
stn194	19	43	49	16	24	G72220
stn284	19	62	69			BV678154
stn335	20			0		BV678162
stn214	20	0	0	37		BV102492
stn213	20	34		46	0	BV102491
stn341	20				22	Peichel, 2001*
gac1125	20	81	28	61	24	Largiadér, 1999*
stn180	21				0	G72313
stn218	21	0		0		BV102496
stn222	21	9	0			BV102498

* [1]Largiadér, C.R., Fries, V., Kobler, B., and Bakker, T.C.M. (1999). Isolation and characterization of microsatellite loci from the three-spined stickleback (*Gasterosteus aculeatus* L.). Molecular Ecology *8*, 342-344. [2]Peichel, C.L., Nereng, K.S., Ohgi, K.A., Cole, B.L.E., Colosimo, P.F., Buerkle, C.A., Schluter, D., and Kingsley, D.M. (2001). The genetic architecture of divergence between threespine stickleback species. Nature *414*, 901-905. [3]Peichel, C.L., Ross, J.A., Matson, C.K., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Mori, S., Schluter, D., and Kingsley, D.M. (2004). The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. Current Biology *14*, 1416-1424.