ONLINE SUPPLEMENTARY MATERIAL

PREDATION'S ROLE IN REPEATED PHENOTYPIC AND GENETIC DIVERGENCE OF ARMOR IN THREESPINE STICKLEBACK

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Supplementary Methods

Crossing, fertilization and fish husbandry

The crossing of wild caught individuals from parental populations to generate F_1 hybrid lines was accomplished using standard laboratory protocol. Fertilization was performed by first stripping a female's eggs into a Petri dish containing a small amount of de-chlorinated tap water. Next, a male was anaesthetized in clove oil, both testes were removed and placed into the Petri dish with the eggs, and then crushed to release sperm. The macerated testes were then removed from the Petri dish after 20 minutes, the eggs transferred to plastic egg-cups (pint cup with the bottom replaced with fine fiberglass mesh) and submerged in an egg-tank (20 L) containing methylene blue to reduce fungal growth. Eggs remained in aerated egg-tanks for eight days, after which they were transferred to a 102 L tank. F_1 hybrid lines were raised from hatchlings to adults in 102 L tanks. Hatchlings were fed newly hatched live brine shrimp twice a day for the first six weeks, after which diets were supplemented with chopped frozen blood worms. At about three months post-hatching, F_1 lines were split into two to four 102 L tanks to maintain roughly 20 individuals per tank throughout the following winter, during which time individuals were fed frozen bloodworms once daily.

The following spring, when F_1 males began to show nuptial coloration and females were gravid, brother-sister mating pairs were chosen from each F_1 hybrid line. Using the protocol described above, eggs from an F_1 female were stripped and fertilized with sperm from an F_1 male of the same family to establish an F_2 family to be used in the predation trials. One mating pair from each of the six F_1 hybrid lines was created from

the PBxOLM cross, resulting in six F_2 families. A total of ten brother-sister mating pairs were chosen from the single MLxLCM F_1 hybrid line, resulting in ten F_2 families. F_2 hatchlings were fed newly hatched live brine shrimp twice a day and raised for about three weeks in 102 L tanks until they reached about 11 mm standard length. At this time, hatchlings were transported from the laboratory to the UBC experimental ponds to begin the predation experiment.

Table S1. Means, standard deviations and selection differentials for standard length and all size-adjusted armor traits from each F_2 family in the Paxton and McKay lines.

Table S1.A. Paxton lines		No-predation		Predation		selection
Trait	family	Mean (X_B)	S.D.	Mean (X_A)	S.D.	differential (i)
standard length	1	14.708	1.390	15.051	1.003	0.247
	2	13.909	1.192	14.217	0.895	0.258
	3	13.556	1.229	14.845	1.061	1.049
	4	12.451	1.130	12.847	0.995	0.351
	5	15.107	0.805	16.323	1.068	1.511
	6	13.967	1.616	14.702	0.944	0.455
anterior dorsal spine length	1	0.023	0.114	-0.043	0.137	-0.577
	2	0.008	0.118	-0.015	0.116	-0.194
	3	0.015	0.116	-0.044	0.128	-0.511
	4	0.006	0.136	-0.017	0.104	-0.164
	5	0.012	0.131	-0.022	0.154	-0.256
	6	0.011	0.116	-0.017	0.140	-0.236
second dorsal spine length	1	0.032	0.124	-0.060	0.137	-0.741
	2	0.006	0.129	-0.011	0.125	-0.132
	3	0.000	0.101	0.000	0.121	0.002
	4	0.007	0.095	-0.019	0.098	-0.271
	5	-0.005	0.093	0.009	0.097	0.145
	6	-0.008	0.157	0.012	0.120	0.128

Table S1A. Paxton lines continued

pelvic spine length	1	0.045	0.249	-0.072	0.205	-0.470
	2	0.021	0.186	-0.037	0.203	-0.313
	3	0.036	0.159	-0.107	0.227	-0.896
	4	-0.025	0.174	0.072	0.141	0.557
	5	-0.054	0.057	0.080	0.195	2.337
	6	-0.020	0.148	0.030	0.181	0.335
pelvic girdle length	1	-0.018	0.776	0.033	0.480	0.065
	2	0.054	0.207	-0.086	0.481	-0.676
	3	0.063	0.479	-0.152	0.698	-0.450
	4	-0.054	0.211	0.153	0.201	0.986
	5	0.013	0.721	-0.018	0.982	-0.043
	6	-0.015	0.120	0.023	0.181	0.314

		No-predation	Predation
	family	before(after)	before(after)
sample size	1	49(45)	49(24)
	2	42(42)	43(21)
	3	36(20)	35(7)
	4	31(20)	31(7)
	5	40(11)	39(6)
	6	41(31)	41(20)

Table S1. continued

Table S1.B. McKay lines		No-predation		Predation		selection
Trait	family	Mean (X_B)	S.D.	Mean (X_A)	S.D.	differential (*i*)
standard length	1	15.359	1.696	15.092	1.191	-0.157
	2	14.853	2.144	15.520	0.811	0.311
	3	17.443	1.039	17.558	1.206	0.110
	4	15.131	1.452	15.702	1.154	0.393
	5	17.945	0.897	17.544	1.091	-0.447
	6	16.067	1.579	15.553	1.145	-0.325
	7	14.447	1.159	14.903	1.024	0.394
	8	19.369	2.147	20.513	1.462	0.533
	9	17.944	1.934	18.737	1.990	0.410
	10	14.898	1.089	16.021	1.487	1.032

Table S1B. McKay lines continued

anterior dorsal spine length	1	-0.007	0.161	0.009	0.131	0.099
	2	-0.041	0.221	0.010	0.101	0.230
	3	0.006	0.157	-0.009	0.127	-0.096
	4	0.009	0.104	-0.021	0.087	-0.288
	5	0.023	0.112	-0.038	0.094	-0.542
	6	0.006	0.138	-0.009	0.111	-0.110
	7	0.009	0.111	-0.018	0.137	-0.244
	8	0.018	0.119	-0.037	0.096	-0.460
	9	0.004	0.157	-0.007	0.192	-0.067
	10	-0.009	0.110	0.018	0.119	0.244
second dorsal spine length	1	-0.010	0.163	0.012	0.130	0.132
	2	-0.001	0.115	0.002	0.127	0.028
	3	0.023	0.144	-0.033	0.152	-0.393
	4	0.004	0.106	-0.010	0.107	-0.139
	5	0.034	0.107	-0.057	0.158	-0.852
	6	-0.008	0.137	0.013	0.122	0.155
	7	0.006	0.119	-0.012	0.127	-0.156
	8	-0.006	0.164	0.013	0.140	0.116
	9	0.017	0.147	-0.028	0.179	-0.307
	10	0.004	0.130	-0.009	0.159	-0.103

Table S1B. McKay lines continued

pelvic spine length	1	-0.016	0.241	0.019	0.262	0.144
	2	-0.010	0.238	0.022	0.215	0.132
	3	-0.010	0.269	0.014	0.284	0.086
	4	0.054	0.212	-0.129	0.228	-0.862
	5	0.034	0.202	-0.056	0.269	-0.446
	6	0.040	0.312	-0.065	0.263	-0.338
	7	0.015	0.231	-0.029	0.202	-0.193
	8	-0.027	0.256	0.058	0.250	0.333
	9	0.053	0.314	-0.087	0.195	-0.445
	10	0.001	0.264	-0.002	0.271	-0.012
pelvic girdle length	1	0.001	0.166	-0.001	0.158	-0.010
	2	-0.005	0.180	0.012	0.175	0.094
	3	0.036	0.169	-0.052	0.119	-0.520
	4	0.015	0.105	-0.036	0.148	-0.488
	5	0.031	0.162	-0.051	0.184	-0.508
	6	-0.024	0.190	0.040	0.188	0.338
	7	0.015	0.161	-0.030	0.175	-0.278
	8	0.063	0.162	-0.133	0.119	-1.211
	9	0.035	0.213	-0.058	0.188	-0.439
	10	0.013	0.185	-0.026	0.181	-0.214

Table S1B. McKay lines continued

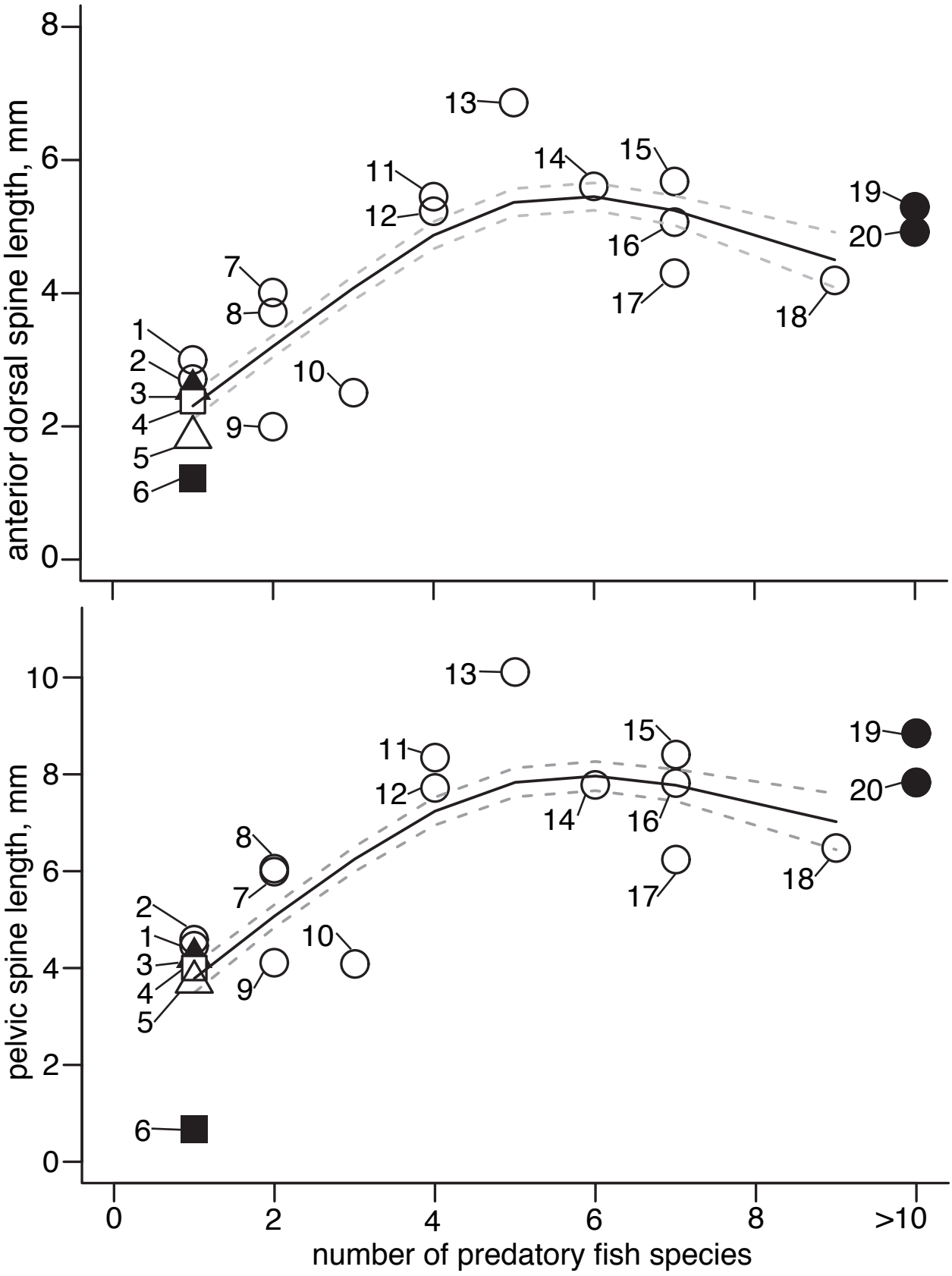
	family	No-predation before(after)	Predation before(after)
sample size	1	37(31)	37(25)
	2	36(31)	36(14)
	3	32(30)	32(21)
	4	29(29)	28(12)
	5	36(25)	36(15)
	6	43(31)	44(19)
	7	70(66)	70(34)
	8	21(19)	20(9)
	9	32(28)	32(17)
	10	48(40)	48(20)

Table S2. Change in frequency (Δp) of the low morph *Ectodysplasin* allele, Eda^L, between no-predator control and predator treatments in F_2 families generated from the Paxton lines.

family	Δp	$t_{0.05(2)5}$	P
1	0.145		
2	0.247		
3	0.025	-2.528	0.053
4	0.050		
5	0.250		
6	-0.002		

Figure S1

Figure S1. The positive association between spine length and the number of predatory fish species present in natural populations of threespine stickleback. Spearman's Rho = 0.762 and 0.781; $P < 0.0002$ for dorsal (top) and pelvic (bottom) spine length respectively. Each symbol represents the size adjusted mean of ten preserved museum specimens from a 20 different population of threespine stickleback from coastal British Columbia, Canada. Spine lengths were adjusted to a standard body length of 51 mm. The curve was estimated using the cubic spline (Schluter 1988); dashed lines represent standard errors generated from 10,000 bootstrap replicates. Open circles represent solitary lake populations, filled circles marine populations, squares represent limnetic (open) and benthic (filled) species from Paxton Lake, and triangles represent limnetic (open) and benthic (filled) species from Priest lake, Texada Island. Numbers connected to symbols indicate the populations used in this study: Klein Lake (1), McKay Lake (2), Priest Lake benthic (3), Paxton Lake limnetic (4), Priest Lake limnetic (5), Paxton Lake benthic (6), Paq Lake (7), North Lake (8), Cranby Lake (9), Dougan Lake (10), Beaver Lake (11), Blackjack Lake (12), Mayer Lake (13), Brannen Lake (14), Erroch Lake (15), Kennedy Lake (16), Fairy Lake (17), Sproat Lake (18), Oyster lagoon marine (19), Little Campbell River marine (20). Data on the number of predatory fish species found in each population were obtained using Fish Wizard (www.fishwizard.com), a database maintained by the provincial government of British Columbia, Canada and the Freshwater Fisheries Society of British Columbia. The two marine populations were excluded from the test of correlation and spline estimation because the number of fish predators is unknown, although likely numerous. The number of predatory fish species in Paq lake shown here differs from the Fish Wizard database based on sightings of Cutthroat trout, *Oncorhynchus clarki*, and prickly sculpin, *Cottus asper* (S.M. Rogers, personal communication).



Supplementary figure S1.