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Supplementary Table 1 | Top twenty multiple linear regression models describing the relationship between functional morphology and niche score

Model	*F*	d.f.	*P*	multiple R^2	adjusted R^2	ΔAIC	C_p
iso_PC1 = (-0.117 x short_gill_rakers) + (11.1 x lower_jaw_opening_inlever) + (-4.42 x upper_jaw_protrusion) + 1.85	30.14	3, 545	$P < 2.2 \times 10^{-16}$	0.1423	0.1376	0	4.3907
iso_PC1 = (-0.116 x short_gill_rakers) + (-2.06 x gape) + (11.8 x lower_jaw_opening_inlever) + (-3.71 x upper_jaw_protrusion) + 1.82	23.14	4, 544	$P < 2.2 \times 10^{-16}$	0.1454	0.1391	0.0057	4.4131
iso_PC1 = (-0.114 x short_gill_rakers) + (-2.53 x gape) + (2.22 x epaxial_width) + (11.2 x lower_jaw_opening_inlever) + (-3.54 x upper_jaw_protrusion) + 1.80	18.82	5, 543	$P < 2.2 \times 10^{-16}$	0.1477	0.1398	0.5503	4.9744
iso_PC1 = (-0.115 x short_gill_rakers) + (-2.25 x gape) + (0.842 x epaxial_height) + (11.3 x lower_jaw_opening_inlever) + (-3.67 x upper_jaw_protrusion) + 1.80	18.78	5, 543	$P < 2.2 \times 10^{-16}$	0.1474	0.1396	0.7236	5.1456
iso_PC1 = (-0.116 x short_gill_rakers) + (0.714 x epaxial_height) + (10.6 x lower_jaw_opening_inlever) + (-4.44 x upper_jaw_protrusion) + 1.83	22.83	4, 544	$P < 2.2 \times 10^{-16}$	0.1438	0.1375	1.0700	5.4676
iso_PC1 = (-0.116 x short_gill_rakers) + (1.42 x epaxial_width) + (10.6 x lower_jaw_opening_inlever) + (-4.42 x upper_jaw_protrusion) + 1.83	22.75	4, 544	$P < 2.2 \times 10^{-16}$	0.1433	0.1370	1.3669	5.7621
iso_PC1 = (-0.0206 x long_gill_rakers) + (-0.106 x short_gill_rakers) + (-2.08 x gape) + (11.8 x lower_jaw_opening_inlever) + (-3.66 x upper_jaw_protrusion) + 2.08	18.56	5, 543	$P < 2.2 \times 10^{-16}$	0.1459	0.1381	1.6686	6.0796
iso_PC1 = (-0.0192 x long_gill_rakers) + (-0.108 x short_gill_rakers) + (11.1 x lower_jaw_opening_inlever) + (-4.38 x upper_jaw_protrusion) + 2.09	22.65	4, 544	$P < 2.2 \times 10^{-16}$	0.1428	0.1365	1.7066	6.0993
iso_PC1 = (-0.114 x short_gill_rakers) + (-2.59 x gape) + (1.80 x epaxial_width) + (0.651 x epaxial_height) + (11.0 x lower_jaw_opening_inlever) + (-3.54 x upper_jaw_protrusion) + 1.79	15.79	6, 542	$P < 2.2 \times 10^{-16}$	0.1488	0.1394	1.8345	6.2682
iso_PC1 = (-0.017 x long_gill_rakers) + (-0.106 x short_gill_rakers) + (-2.54 x gape) + (2.14 x epaxial_width) + (11.2 x lower_jaw_opening_inlever) + (-3.50 x upper_jaw_protrusion) + 2.01	15.70	6, 542	$P < 2.2 \times 10^{-16}$	0.1480	0.1386	2.3208	6.7479
iso_PC1 = (-0.0216 x long_gill_rakers) + (-0.104 x short_gill_rakers) + (-2.27 x gape) + (0.853 x epaxial_height) + (11.3 x lower_jaw_opening_inlever) + (-3.61 x upper_jaw_protrusion) + 2.07	15.69	6, 542	$P < 2.2 \times 10^{-16}$	0.1480	0.1386	2.3523	6.7790
iso_PC1 = (-0.0200 x long_gill_rakers) + (-0.106 x short_gill_rakers) + (0.723 x epaxial_height) + (10.6 x lower_jaw_opening_inlever) + (-4.39 x upper_jaw_protrusion) + 2.08	18.31	5, 543	$P < 2.2 \times 10^{-16}$	0.1443	0.1364	2.7531	7.1534
iso_PC1 = (-0.116 x short_gill_rakers) + (1.01 x epaxial_width) + (0.596 x epaxial_height) + (10.4 x lower_jaw_opening_inlever) + (-4.43 x upper_jaw_protrusion) + 1.83	18.30	5, 543	$P < 2.2 \times 10^{-16}$	0.1442	0.1363	2.7708	7.1710
iso_PC1 = (-0.0168 x long_gill_rakers) + (-0.108 x short_gill_rakers) + (1.34 x epaxial_width) + (10.6 x lower_jaw_opening_inlever) + (-4.38 x upper_jaw_protrusion) + 2.04	18.22	5, 543	$P < 2.2 \times 10^{-16}$	0.1436	0.1358	3.1439	7.5409
iso_PC1 = (-0.0185 x long_gill_rakers) + (-0.104 x short_gill_rakers) + (-2.59 x gape) + (1.70 x epaxial_width) + (0.671 x epaxial_height) + (11.0 x lower_jaw_opening_inlever) + (-3.49 x upper_jaw_protrusion) + 2.02	13.55	7, 541	$P = 3.47 \times 10^{-16}$	0.1492	0.1382	3.5624	8.0000
iso_PC1 = (-0.0182 x long_gill_rakers) + (-0.107 x short_gill_rakers) + (0.919 x epaxial_width) + (0.615 x epaxial_height) + (10.4 x lower_jaw_opening_inlever) + (-4.39 x upper_jaw_protrusion) + 2.05	15.20	6, 542	$P = 3.33 \times 10^{-16}$	0.1446	0.1352	4.5099	8.9124
iso_PC1 = (-0.0499 x long_gill_rakers) + (-2.18 x gape) + (12.0 x lower_jaw_opening_inlever) + (-3.80 x upper_jaw_protrusion) + 0.997	21.84	4, 544	$P < 2.2 \times 10^{-16}$	0.1383	0.1320	4.5311	8.9110
iso_PC1 = (-0.0491 x long_gill_rakers) + (11.3 x lower_jaw_opening_inlever) + (-4.56 x upper_jaw_protrusion) + 0.982	28.32	3, 545	$P < 2.2 \times 10^{-16}$	0.1349	0.1301	4.7490	9.1290
iso_PC1 = (-2.15 x gape) + (12.1 x lower_jaw_opening_inlever) + (-3.99 x upper_jaw_protrusion) - 0.0285	28.29	3, 545	$P < 2.2 \times 10^{-16}$	0.1348	0.1300	4.8166	9.1967
iso_PC1 = (11.4 x lower_jaw_opening_inlever) + (-4.74 x upper_jaw_protrusion) - 0.0271	41.29	2, 546	$P < 2.2 \times 10^{-16}$	0.1314	0.1282	4.9532	9.3421

'Niche score' is the score of F_2 hybrids along the first principal component of stable isotope space (here 'iso_PC1', the variable name used in R). These models were found by performing all-subsets multiple linear regression (using the R package 'leaps'[70]) on the set of functional morphological traits that were identified to be statistically associated with PC1 in univariate linear models at the $P < 0.05$ probability level. Functional morphological traits are illustrated in Fig. 2 of the main paper. Models in the table are ordered in terms of increasing ΔAIC score, with the single 'best' model overall at the top. All functional morphological traits in models for which ΔAIC ≤ 2 were considered when modelling the genetic basis of niche score[73] (genetic modelling results summarised in Fig. 3 of the main paper). 'ΔAIC' is the difference between AIC of the current model and AIC of the single 'best' model overall. Adjusted R^2 follows Theil[79]. 'C_p' is Mallows' C_p statistic[71].

Supplementary Table 2 | Top twenty multiple linear regression models describing the relationship between body shape coordinates and niche score

Model	*F*	d.f.	*P*	multiple R^2	adjusted R^2	ΔAIC	C_p
iso_PC1 = (2.85 x y2) + (-14.2 x x4) + (-6.39 x x5) + (6.32 x x12) + (-14.4 x x13) + (-25.6 x y13) + (12.2 x y14) + (7.85 x x15) + (7.26 x y17) + 1.13	27.42	9, 601	$P < 2.2 \times 10^{-16}$	0.2911	0.2805	0	0.4329
iso_PC1 = (-13.9 x x4) + (-6.16 x x5) + (7.02 x x12) + (-15.5 x x13) + (-24.6 x y13) + (13.4 x y14) + (7.32 x x15) + (6.58 x y17) + 1.05	30.49	8, 602	$P < 2.2 \times 10^{-16}$	0.2883	0.2789	0.3918	0.7527
iso_PC1 = (3.01 x y2) + (-13.8 x x4) + (-6.06 x x5) + (6.90 x x12) + (-13.6 x x13) + (-25.4 x y13) + (11.3 x y14) + (8.28 x x15) + (2.89 x x16) + (6.65 x y17) -1.41	24.70	10, 600	$P < 2.2 \times 10^{-16}$	0.2916	0.2798	1.5601	2.0073
iso_PC1 = (2.81 x y2) + (-14.7 x x4) + (-6.48 x x5) + (6.16 x x12) + (-14.1 x x13) + (-26.4 x y13) + (11.6 x y14) + (7.67 x x15) + (6.48 x y17) + (1.12 x y19) + 0.795	24.69	10, 600	$P < 2.2 \times 10^{-16}$	0.2915	0.2797	1.6639	2.1077
iso_PC1 = (2.80 x y2) + (-14.1 x x4) + (-6.43 x x5) + (6.30 x x12) + (-14.6 x x13) + (-25.3 x y13) + (12.1 x y14) + (7.60 x x15) + (1.48 x y16) + (6.63 x y17) + 1.39	24.67	10, 600	$P < 2.2 \times 10^{-16}$	0.2914	0.2796	1.7482	2.1892
iso_PC1 = (3.14 x y2) + (-14.1 x x4) + (-6.50 x x5) + (1.78 x y8) + (6.44 x x12) + (-14.7 x x13) + (-25.6 x y13) + (12.6 x y14) + (7.62 x x15) + (6.73 x y17) + 1.24	24.67	10, 600	$P < 2.2 \times 10^{-16}$	0.2914	0.2796	1.7491	2.1901
iso_PC1 = (2.88 x y2) + (-14.0 x x4) + (-6.32 x x5) + (6.54 x x12) + (-14.6 x x13) + (-25.5 x y13) + (12.2 x y14) + (7.92 x x15) + (1.49 x y15) + (6.62 x y17) + 0.954	24.67	10, 600	$P < 2.2 \times 10^{-16}$	0.2914	0.2796	1.7601	2.2007
iso_PC1 = (2.84 x y2) + (-14.4 x x4) + (-6.34 x x5) + (1.47 x y5) + (6.53 x x12) + (-14.3 x x13) + (-25.2 x y13) + (12.0 x y14) + (8.34 x x15) + (8.40 x y17) + 0.924	24.67	10, 600	$P < 2.2 \times 10^{-16}$	0.2914	0.2796	1.7654	2.2059
iso_PC1 = (2.83 x y2) + (0.875 x x3) + (-14.9 x x4) + (-6.32 x x5) + (6.45 x x12) + (-14.5 x x13) + (-25.5 x y13) + (12.3 x y14) + (7.90 x x15) + (7.35 x y17) + 1.18	24.67	10, 600	$P < 2.2 \times 10^{-16}$	0.2914	0.2795	1.7883	2.2280
iso_PC1 = (2.77 x y2) + (-14.4 x x4) + (-6.53 x x5) + (6.11 x x12) + (-14.5 x x13) + (-25.7 x y13) + (12.4 x y14) + (8.02 x x15) + (-1.41 x x17) + (7.28 x y17) + 2.09	24.66	10, 600	$P < 2.2 \times 10^{-16}$	0.2913	0.2795	1.8195	2.2582
iso_PC1 = (2.89 x y2) + (-14.3 x x4) + (-6.53 x x5) + (-1.23 x x9) + (6.18 x x12) + (-14.8 x x13) + (-25.8 x y13) + (12.3 x y14) + (7.63 x x15) + (7.11 x y17) + 2.18	24.65	10, 600	$P < 2.2 \times 10^{-16}$	0.2912	0.2794	1.9150	2.3506
iso_PC1 = (2.78 x y2) + (-14.0 x x4) + (-6.29 x x5) + (0.841 x x10) + (6.29 x x12) + (-14.1 x x13) + (-25.5 x y13) + (12.2 x y14) + (7.98 x x15) + (7.38 x y17) + 0.519	24.65	10, 600	$P < 2.2 \times 10^{-16}$	0.2912	0.2794	1.9349	2.3699
iso_PC1 = (-0.412 x x1) + (2.96 x y2) + (-14.4 x x4) + (-6.40 x x5) + (6.38 x x12) + (-14.2 x x13) + (-25.8 x y13) + (12.1 x y14) + (8.02 x x15) + (7.32 x y17) - 0.305	24.65	10, 600	$P < 2.2 \times 10^{-16}$	0.2912	0.2794	1.9358	2.3708
iso_PC1 = (2.95 x y2) + (-14.3 x x4) + (-6.46 x x5) + (-0.630 x x6) + (6.20 x x12) + (-14.5 x x13) + (-25.8 x y13) + (12.2 x y14) + (7.57 x x15) + (7.15 x y17) + 1.72	24.65	10, 600	$P < 2.2 \times 10^{-16}$	0.2912	0.2794	1.9474	2.3820
iso_PC1 = (-14.4 x x4) + (-6.27 x x5) + (6.84 x x12) + (-15.2 x x13) + (-25.5 x y13) + (12.8 x y14) + (7.14 x x15) + (5.73 x y17) + (1.22 x y19) + 0.678	27.12	9, 601	$P < 2.2 \times 10^{-16}$	0.2888	0.2782	1.9888	2.3611
iso_PC1 = (2.86 x y2) + (-14.2 x x4) + (-6.40 x x5) + (-0.290 x y10) + (6.33 x x12) + (-14.4 x x13) + (-25.8 x y13) + (12.1 x y14) + (7.86 x x15) + (7.25 x y17) + 1.10	24.64	10, 600	$P < 2.2 \times 10^{-16}$	0.2911	0.2793	1.9941	2.4272
iso_PC1 = (-16.2 x x4) + (-6.71 x x5) + (6.02 x x12) + (-14.6 x x13) + (-27.0 x y13) + (12.4 x y14) + (4.87 x x15) + 2.46	34.19	7, 603	$P < 2.2 \times 10^{-16}$	0.2841	0.2758	2.0007	2.2701
iso_PC1 = (-13.8 x x4) + (-6.21 x x5) + (6.98 x x12) + (-15.8 x x13) + (-24.3 x y13) + (13.3 x y14) + (7.04 x x15) + (1.71 x y16) + (5.86 x y17) + 1.34	27.11	9, 601	$P < 2.2 \times 10^{-16}$	0.2887	0.2781	2.0548	2.4253
iso_PC1 = (-14.1 x x4) + (-6.35 x x5) + (6.72 x x12) + (-15.7 x x13) + (-24.7 x y13) + (13.6 x y14) + (7.57 x x15) + (-1.90 x x17) + (6.63 x y17) + 2.34	27.11	9, 601	$P < 2.2 \times 10^{-16}$	0.2887	0.2781	2.0617	2.4320
iso_PC1 = (-14.1 x x4) + (-6.10 x x5) + (1.56 x y5) + (7.24 x x12) + (-15.5 x x13) + (-24.2 x y13) + (13.2 x y14) + (7.84 x x15) + (7.79 x y17) + 0.828	27.10	9, 601	$P < 2.2 \times 10^{-16}$	0.2886	0.2780	2.1312	2.4994

'Niche score' is the score of F_2 hybrids along the first principal component of stable isotope space (here 'iso_PC1', the variable name used in R). These models were found by performing all-subsets multiple linear regression (using the R package 'leaps'[70]) on the set of morphometric landmark coordinates that were identified to be statistically associated with PC1 in univariate linear models at the $P < 0.05$ probability level. The key at the bottom-right of Extended Data Fig. 3 illustrates positions of the 19 morphometric landmarks, for which x- and y- coordinates were treated as separate variables (e.g., 'y12' in the table above indicates the y-, or vertical-, coordinate of morphometric landmark 12). Models in the table are ordered in terms of increasing ΔAIC score, with the single 'best' model overall at the top. All shape coordinates in models for which ΔAIC ≤ 2 were considered when modelling the genetic basis of niche score[73] (genetic modelling results summarised in Fig. 3 of the main paper). 'ΔAIC' is the difference between AIC of the current model and AIC of the single 'best' model overall. Adjusted R^2 follows Theil[79]. 'C_p' is Mallows' C_p statistic[71].

Supplementary Table 3 | Locations and morphological effects of all significant QTLs detected in the F_2 hybrid mapping population

Trait	LOD	LG	Position (cM)	Nearest SNP	PVE	Morphological Trait Mean ± s.e.m. (by Genotype)		
						Homozygous LL	Heterozygous LB	Homozygous BB
first dorsal spine development	4.08	4	28.8	chrIV:10997988	2.55	1.44 ± 0.10	1.12 ± 0.06	0.93 ± 0.08
pelvic girdle development	83.50	7	58.0	chrVII:27918897	47.81	1.98 ± 0.05	1.75 ± 0.04	0.46 ± 0.16
number of lateral plates	5.96	7	40.0	chrVII:25986275	4.06	3.00 ± 0.05	2.79 ± 0.03	2.74 ± 0.05
number of lateral plates	4.04	11	13.5	chrXI:9039275	2.66	2.96 ± 0.05	2.84 ± 0.03	2.68 ± 0.06
number of long gill rakers	18.23	7	27.0	chrVII:19857837	11.7	21.28 ± 0.14	20.58 ± 0.07	19.69 ± 0.14
number of long gill rakers	4.89	8	16.0	chrVIII:13412707	2.57	20.07 ± 0.14	20.60 ± 0.07	20.84 ± 0.12
number of long gill rakers	4.49	12	41.1	chrXII:7504339	2.72	20.86 ± 0.12	20.64 ± 0.08	20.16 ± 0.11
number of long gill rakers	5.34	20	12.0	chrXX:9279241	3.38	21.04 ± 0.13	20.57 ± 0.08	20.26 ± 0.11
number of short gill rakers	4.97	1	36.0	chrI:25560380	3.10	16.35 ± 0.10	15.95 ± 0.06	15.69 ± 0.08
number of short gill rakers	7.24	7	30.0	chrVII:24203557	5.15	16.40 ± 0.10	15.91 ± 0.05	15.65 ± 0.11
number of short gill rakers	3.99	8	32.2	chrVIII:17359071	2.35	15.71 ± 0.14	15.92 ± 0.05	16.24 ± 0.11
number of short gill rakers	5.88	20	16.8	chrXX:14462157	3.48	16.28 ± 0.10	16.00 ± 0.06	15.68 ± 0.08
upper jaw protrusion length (size corrected residual)	10.96	9	28.4	chrIX:6126845	8.82	0.02393 ± 0.00541	0.00076 ± 0.00289	-0.02095 ± 0.00543
lower jaw-opening inlever length (size corrected resid.)	8.06	4	28.8	chrIV:10997988	5.95	-0.01051 ± 0.00270	0.00100 ± 0.00148	0.00907 ± 0.00204
lower jaw-opening inlever length (size corrected resid.)	5.54	21	18.0	chrXXI:11060209	4.07	-0.00564 ± 0.00331	0.00037 ± 0.00144	0.01226 ± 0.00399
buccal cavity length (size corrected residual)	4.51	2	14.0	chrII:17312835	2.66	0.00294 ± 0.00152	0.00224 ± 0.00093	-0.00427 ± 0.00129
buccal cavity length (size corrected residual)	4.72	9	28.0	chrIX:6126845	3.05	0.00496 ± 0.00156	0.00012 ± 0.00084	-0.00238 ± 0.00167
buccal cavity length (size corrected residual)	4.44	14	33.7	chrXIV:3598443	2.84	-0.00546 ± 0.00178	0.00108 ± 0.00083	0.00423 ± 0.00193
neurocranium outlever length (size corrected residual)	4.21	2	10.4	chrII:6475468	2.57	0.00521 ± 0.00142	0.00076 ± 0.00087	-0.00267 ± 0.00118
neurocranium outlever length (size corrected residual)	4.52	9	28.0	chrIX:6126845	2.97	0.00410 ± 0.00149	0.00042 ± 0.00077	-0.00194 ± 0.00149
anterior epaxial muscle height (size corrected residual)	4.28	14	30.0	chrXIV:3414352	3.63	-0.01614 ± 0.00777	-0.00058 ± 0.00338	0.01851 ± 0.00693
anterior epaxial muscle width (size corrected residual)	10.27	4	28.8	chrIV:10997988	8.88	-0.01289 ± 0.00264	-0.00119 ± 0.00153	0.00913 ± 0.00207
anterior epaxial muscle width (size corrected residual)	7.31	12	38.0	chrXII:15046849	6.32	-0.00718 ± 0.00242	-0.00202 ± 0.00156	0.00955 ± 0.00229
anterior epaxial muscle width (size corrected residual)	5.19	16	9.1	chrXVI:5562355	4.34	-0.00444 ± 0.00245	-0.00319 ± 0.00159	0.00820 ± 0.00216
anterior epaxial muscle width (size corrected residual)	4.08	21	30.0	chrXXI:11414383	2.79	-0.00438 ± 0.00306	-0.00167 ± 0.00154	0.00921 ± 0.00458
suction feeding index score	3.90	12	18.0	chrXII:548804	3.33	0.00634 ± 0.00029	0.00714 ± 0.00013	0.00774 ± 0.00030
landmark 1 x-coordinate (x1)	3.68	8	18.0	chrVIII:16299555	1.78	-2.7750 ± 0.0029	-2.7825 ± 0.0017	-2.7862 ± 0.0026
landmark 1 x-coordinate (x1)	9.17	14	39.5	chrXIV:4632223	5.10	-2.7929 ± 0.0030	-2.7823 ± 0.0015	-2.7717 ± 0.0029
landmark 2 y-coordinate (y2)	5.57	16	13.5	chrXVI:9981125	4.33	-0.2433 ± 0.0022	-0.2507 ± 0.0014	-0.2584 ± 0.0020
landmark 3 x-coordinate (x3)	6.89	10	4.0	chrX:1275840	4.45	-0.4186 ± 0.0039	-0.4036 ± 0.0016	-0.3975 ± 0.0026
landmark 3 y-coordinate (y3)	4.41	4	28.8	chrIV:10997988	3.62	-0.3110 ± 0.0021	-0.3175 ± 0.0012	-0.3225 ± 0.0016
landmark 3 y-coordinate (y3)	4.30	7	60.3	chrVII:27918897	3.01	-0.3207 ± 0.0021	-0.3185 ± 0.0011	-0.3096 ± 0.0027
landmark 3 y-coordinate (y3)	3.99	14	18.0	chrXIV:1442872	2.42	-0.3122 ± 0.0025	-0.3178 ± 0.0011	-0.3235 ± 0.0024
landmark 4 x-coordinate (x4)	3.69	1	30.7	chrI:15145305	2.61	-0.3459 ± 0.0022	-0.3510 ± 0.0013	-0.3520 ± 0.0019
landmark 4 x-coordinate (x4)	4.56	16	14.0	chrXVI:9981125	3.29	-0.3442 ± 0.0021	-0.3501 ± 0.0013	-0.3552 ± 0.0018
landmark 4 x-coordinate (x4)	4.10	21	10.0	chrXXI:9820534	2.69	-0.3453 ± 0.0026	-0.3492 ± 0.0013	-0.3591 ± 0.0040
landmark 4 y-coordinate (y4)	4.58	4	28.0	chrIV:11367975	3.83	-0.1986 ± 0.0015	-0.2022 ± 0.0008	-0.2070 ± 0.0012
landmark 4 y-coordinate (y4)	3.54	6	0.0	chrVI:487411	3.28	-0.2027 ± 0.0023	-0.2018 ± 0.0011	-0.2074 ± 0.0022
landmark 4 y-coordinate (y4)	3.68	14	38.0	chrXIV:4632223	2.32	-0.2004 ± 0.0018	-0.2025 ± 0.0008	-0.2067 ± 0.0016
landmark 4 y-coordinate (y4)	5.75	16	14.8	chrXVI:12996432	4.58	-0.1996 ± 0.0014	-0.2018 ± 0.0009	-0.2079 ± 0.0012

landmark 6 x-coordinate (x6)	6.76	12	38.0	chrXII:15046849	3.99	0.1715 ± 0.0015	0.1667 ± 0.0010	0.1624 ± 0.0014
landmark 6 x-coordinate (x6)	3.90	13	20.0	chrXIII:17392141	2.12	0.1685 ± 0.0021	0.1674 ± 0.0009	0.1630 ± 0.0021
landmark 6 x-coordinate (x6)	4.02	16	23.8	chrXVI:16058672	1.89	0.1724 ± 0.0018	0.1661 ± 0.0010	0.1636 ± 0.0015
landmark 6 x-coordinate (x6)	8.10	17	12.0	chrXVII:2232080	5.02	0.1587 ± 0.0016	0.1674 ± 0.0009	0.1735 ± 0.0020
landmark 7 x-coordinate (x7)	4.24	2	10.4	chrII:6475468	2.20	-0.0880 ± 0.0021	-0.0813 ± 0.0014	-0.0752 ± 0.0019
landmark 7 x-coordinate (x7)	3.85	4	20.0	chrIV:339710	2.55	-0.0773 ± 0.0031	-0.0805 ± 0.0012	-0.0862 ± 0.0026
landmark 7 x-coordinate (x7)	5.96	8	18.0	chrVIII:16299555	2.91	-0.0897 ± 0.0027	-0.0785 ± 0.0018	-0.0793 ± 0.0019
landmark 7 x-coordinate (x7)	4.21	16	14.8	chrXVI:12111717	2.16	-0.0764 ± 0.0021	-0.0806 ± 0.0013	-0.0858 ± 0.0019
landmark 8 x-coordinate (x8)	6.28	4	18.4	chrIV:339710	4.53	0.1917 ± 0.0022	0.1910 ± 0.0008	0.1841 ± 0.0016
landmark 8 x-coordinate (x8)	5.39	7	27.0	chrVII:19857837	4.22	0.1855 ± 0.0013	0.1899 ± 0.0007	0.1952 ± 0.0014
landmark 8 y-coordinate (y8)	5.43	4	22.8	chrIV:5165268	5.11	0.1604 ± 0.0020	0.1667 ± 0.0008	0.1706 ± 0.0014
landmark 8 y-coordinate (y8)	3.61	12	34.9	chrXII:13045611	3.56	0.1621 ± 0.0014	0.1675 ± 0.0009	0.1690 ± 0.0013
landmark 9 x-coordinate (x9)	4.10	7	24.0	chrVII:16848769	3.02	0.2118 ± 0.0013	0.2156 ± 0.0007	0.2199 ± 0.0014
landmark 9 y-coordinate (y9)	4.48	17	28.0	chrXVII:9881295	2.96	0.0905 ± 0.0016	0.0928 ± 0.0010	0.0990 ± 0.0016
landmark 9 y-coordinate (y9)	4.37	18	9.7	chrXVIII:4836241	2.88	0.0900 ± 0.0014	0.0937 ± 0.0011	0.0980 ± 0.0015
landmark 10 x-coordinate (x10)	7.25	8	18.0	chrVIII:16299555	4.69	0.2872 ± 0.0018	0.2933 ± 0.0008	0.2979 ± 0.0014
landmark 10 x-coordinate (x10)	4.47	20	6.0	chrXX:2312273	2.77	0.2919 ± 0.0016	0.2920 ± 0.0009	0.2975 ± 0.0016
landmark 11 y-coordinate (y11)	10.28	4	20.0	chrIV:339710	6.40	-0.1920 ± 0.0025	-0.2009 ± 0.0007	-0.2066 ± 0.0018
landmark 11 y-coordinate (y11)	4.37	16	13.5	chrXVI:9981125	2.84	-0.1979 ± 0.0013	-0.2000 ± 0.0008	-0.2048 ± 0.0012
landmark 12 y-coordinate (y12)	4.92	4	27.5	chrIV:11367975	2.75	-0.0592 ± 0.0015	-0.0652 ± 0.0009	-0.0680 ± 0.0012
landmark 14 x-coordinate (x14)	3.91	2	28.0	chrII:20796189	2.45	0.8350 ± 0.0026	0.8265 ± 0.0011	0.8308 ± 0.0022
landmark 14 x-coordinate (x14)	4.08	13	18.5	chrXIII:17392141	2.96	0.8252 ± 0.0036	0.8269 ± 0.0011	0.8363 ± 0.0034
landmark 14 x-coordinate (x14)	4.13	21	0.1	chrUn:7381868	2.70	0.8279 ± 0.0017	0.8266 ± 0.0012	0.8338 ± 0.0019
landmark 15 x-coordinate (x15)	3.83	16	16.0	chrXVI:14283264	2.93	0.7831 ± 0.0012	0.7871 ± 0.0007	0.7896 ± 0.0011
landmark 15 y-coordinate (y15)	6.26	4	36.0	chrIV:29034665	4.80	0.1357 ± 0.0018	0.1417 ± 0.0009	0.1467 ± 0.0013
landmark 15 y-coordinate (y15)	4.17	16	0.0	chrXVI:2483136	3.28	0.1376 ± 0.0016	0.1424 ± 0.0010	0.1452 ± 0.0015
landmark 16 x-coordinate (x16)	4.93	2	12.0	chrII:10092618	3.25	0.3890 ± 0.0011	0.3919 ± 0.0007	0.3951 ± 0.0010
landmark 16 x-coordinate (x16)	3.90	9	52.0	chrIX:19745222	2.10	0.3893 ± 0.0015	0.3918 ± 0.0007	0.3975 ± 0.0029
landmark 16 y-coordinate (y16)	4.17	20	18.0	chrXX:14859034	3.28	0.1318 ± 0.0016	0.1362 ± 0.0009	0.1400 ± 0.0013
landmark 17 y-coordinate (y17)	3.96	2	17.1	chrII:17453243	2.42	-0.0439 ± 0.0016	-0.0394 ± 0.0009	-0.0368 ± 0.0014
landmark 17 y-coordinate (y17)	7.43	4	27.5	chrIV:11367975	4.76	-0.0478 ± 0.0015	-0.0388 ± 0.0009	-0.0361 ± 0.0012
landmark 17 y-coordinate (y17)	5.96	20	14.6	chrXX:9279241	3.56	-0.0443 ± 0.0016	-0.0401 ± 0.0009	-0.0354 ± 0.0013
landmark 18 y-coordinate (y18)	4.91	4	26.0	chrIV:11367975	3.56	0.3994 ± 0.0021	0.4038 ± 0.0012	0.4108 ± 0.0016
landmark 19 x-coordinate (x19)	3.91	2	14.0	chrII:17312835	2.52	-1.3403 ± 0.0026	-1.3387 ± 0.0016	-1.3292 ± 0.0023
landmark 19 x-coordinate (x19)	5.21	17	6.0	chrXVII:1264852	2.75	-1.3276 ± 0.0027	-1.3370 ± 0.0015	-1.3450 ± 0.0034
landmark 19 x-coordinate (x19)	3.69	20	38.0	chrXX:17708486	1.89	-1.3402 ± 0.0032	-1.3368 ± 0.0017	-1.3325 ± 0.0030

Abbreviations in the column headers are defined as follows: 'LOD', logarithm$_{10}$ of odds score; 'LG', linkage group; 'cM', centimorgan; 'SNP', single nucleotide polymorphism marker; 'PVE', percentage variance explained; 'L', limnetic allele at the SNP locus located nearest to the quantitative trait locus (QTL); and 'B', benthic allele at the nearest SNP locus. Supplementary Table 4 defines the physical position of each nearest SNP locus in the *Gasterosteus aculeatus* genome and provides the corresponding ID Number in the Single Nucleotide Polymorphism Database (hosted by the U.S. National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/projects/SNP/). Following the scheme in Extended Data Fig. 3, red text indicates QTLs for 'component traits' of niche use, and blue text indicates QTLs for all other traits. The four QTLs that were identified for armour traits (i.e., extent of first dorsal spine development, extent of pelvic girdle development, and number of lateral plates) are coloured blue because we excluded all armour traits *a priori* from the statistical tests that were used to identify component traits. We did this to focus the present investigation on the genetic architecture of feeding niche divergence — the central axis of divergence between benthic and limnetic stickleback — as mediated by the currently best-known class of traits underlying adaptation to alternate feeding niches in this evolutionary model system (i.e., morphological traits). Supplementary Discussion Section 6 provides an expanded rationale for this approach. All other QTLs in the table are coloured according to outcomes of the statistical tests and criteria used to identify component traits (described fully in Methods; also see Supplementary Tables 1 and 2).

Supplementary Table 4 | Identities, map positions, and physical locations of the 408 single-nucleotide polymorphisms (SNPs) used for linkage analysis and QTL mapping

Linkage Group (LG)	Map Position (cM)	SNP Marker Name (Chromosome : Position in b.p.)	NCBI SNP ID Number (ss#)	SNP Identifier # in Extended Data Fig. 3
1	0	chrUN:37631434	244223001	SNP 001
1	0.73	chrI:913033	120258411	SNP 002
1	6.00	chrI:1550	418641979	SNP 003
1	7.85	chrI:1245655	418641981	SNP 004
1	8.47	chrI:1320011	418641982	SNP 005
1	19.65	chrI:3310077	244222768	SNP 006
1	25.69	chrI:4219350	244222770	SNP 007
1	27.71	chrI:7545826	418641993	SNP 008
1	30.28	chrI:14261764	418641998	SNP 009
1	30.72	chrI:15145305	418642000	SNP 010
1	32.79	chrI:26879230	244222777	SNP 011
1	33.49	chrI:25560380	418642013	SNP 012
1	40.12	chrI:22361077	120258417	SNP 013
1	41.37	chrI:22899825	418642011	SNP 014
1	42.64	chrI:20584613	418642006	SNP 015
1	43.82	chrI:20740719	418642007	SNP 016
1	45.73	chrI:19946499	418642005	SNP 017
2	0	chrII:377479	244222779	SNP 018
2	2.01	chrII:3516452	120258422	SNP 019
2	2.79	chrII:640670	418642019	SNP 020
2	4.26	chrII:3931852	418642025	SNP 021
2	5.41	chrII:4530808	120258423	SNP 022
2	7.55	chrII:919438	244222781	SNP 023
2	7.96	chrII:5935944	252841148	SNP 024
2	8.08	chrII:5914538	418642030	SNP 025
2	8.30	chrII:5590307	252841076	SNP 026
2	10.43	chrII:6475468	244222782	SNP 027
2	11.80	chrII:10092618	418642034	SNP 028
2	15.34	chrII:17312835	418642041	SNP 029
2	17.06	chrII:17453243	418642042	SNP 030
2	20.98	chrII:19985741	244222785	SNP 031
2	23.70	chrII:22644752	418642054	SNP 032
2	25.26	chrII:22443700	244222787	SNP 033
2	27.33	chrII:20796189	120258427	SNP 034
2	32.36	chrII:21013052	418642052	SNP 035
2	35.01	chrII:21231538	244222786	SNP 036
3	0	chrIII:186390	418642056	SNP 037
3	0.45	chrIII:269753	418642057	SNP 038
3	5.95	chrIII:706165	244222789	SNP 039
3	7.24	chrIII:1651721	252841079	SNP 040
3	14.95	chrIII:11302839	418642071	SNP 041
3	15.77	chrIII:10376395	244222790	SNP 042
3	15.97	chrIII:11836494	418642072	SNP 043
3	17.01	chrIII:12433574	418642074	SNP 044
3	18.49	chrIII:12930427	418642076	SNP 045
3	19.65	chrIII:13397314	418642078	SNP 046
3	22.61	chrIII:13520975	252841102	SNP 047
3	23.45	chrIII:13929118	244222792	SNP 048
3	24.09	chrIII:14135608	418642081	SNP 049
3	24.48	chrIII:13911180	418642080	SNP 050
3	24.93	chrIII:14048561	252841058	SNP 051
3	25.46	chrIII:14393183	418642084	SNP 052
3	26.44	chrIII:14456990	252841063	SNP 053
3	29.38	chrIII:14892994	244222794	SNP 054
3	31.50	chrIII:15185662	418642088	SNP 055
3	31.72	chrIII:15157782	418642087	SNP 056
3	37.02	chrIII:15793968	418642089	SNP 057
3	37.56	chrIII:15933767	244222795	SNP 058

3	40.41	chrIII:16123944	418642090	SNP 059
3	41.27	chrIII:16224572	120258430	SNP 060
3	43.84	chrIII:16463929	244222796	SNP 061
3	50.10	chrUN:30323959	418642642	SNP 062
3	50.28	chrUN:27040022	418642631	SNP 063
3	50.77	chrUN:30223426	418642641	SNP 064
4	0	chrIV:2045971	418642099	SNP 065
4	3.86	chrIV:2274657	418642100	SNP 066
4	4.40	chrIV:1942641	244222798	SNP 067
4	15.09	chrIV:3334208	418642103	SNP 068
4	15.73	chrIV:4065598	244222799	SNP 069
4	16.24	chrIV:5313693	120258433	SNP 070
4	16.77	chrIV:219384	418642093	SNP 071
4	17.43	chrIV:4034002	120258432	SNP 072
4	18.37	chrIV:339710	418642094	SNP 073
4	22.83	chrIV:5165268	418642111	SNP 074
4	27.52	chrIV:11367975	120258435	SNP 075
4	28.76	chrIV:10997988	244222801	SNP 076
4	31.60	chrIV:15052901	244222804	SNP 077
4	32.17	chrIV:15721538	244222806	SNP 078
4	32.17	chrIV:15737291	244222807	SNP 079
4	35.03	chrIV:29763654	120258443	SNP 080
4	36.07	chrIV:29034665	418642133	SNP 081
4	38.15	chrIV:31350187	418642140	SNP 082
4	40.86	chrIV:30568387	252841083	SNP 083
4	42.41	chrIV:31740478	244222809	SNP 084
4	44.07	chrIV:31583885	252841078	SNP 085
4	44.92	chrIV:31486885	418642142	SNP 086
4	46.13	chrIV:32236655	418642145	SNP 087
4	46.65	chrIV:32005807	120258445	SNP 088
4	48.27	chrIV:32148857	418642144	SNP 089
4	48.40	chrIV:32092919	252841132	SNP 090
4	49.57	chrIV:32277841	418642146	SNP 091
4	50.19	chrIV:32350814	418642148	SNP 092
4	50.29	chrIV:32387818	120258447	SNP 093
4	53.56	chrIV:32592491	418642150	SNP 094
4	53.66	chrIV:32487875	244222812	SNP 095
4	58.84	chrUN:27478064	244222993	SNP 096
4	59.88	chrUN:27402745	252841068	SNP 097
4	60.21	chrUN:32765800	244222999	SNP 098
5	0	chrUN:12390868	120258569	SNP 099
5	7.89	chrV:7791830	252841093	SNP 100
5	9.16	chrV:4819972	418642158	SNP 101
5	12.08	chrV:2528528	244222814	SNP 102
5	12.14	chrV:1727383	418642153	SNP 103
5	14.20	chrV:1238066	120258448	SNP 104
5	16.34	chrV:8562218	244222817	SNP 105
5	17.24	chrV:8327818	244222816	SNP 106
5	21.78	chrV:9521704	418642162	SNP 107
5	23.74	chrV:9884672	418642164	SNP 108
5	30.51	chrV:10355139	418642170	SNP 109
5	32.79	chrV:10781761	418642175	SNP 110
5	33.86	chrV:10674055	418642173	SNP 111
5	39.77	chrV:11509827	418642178	SNP 112
5	43.50	chrV:11316476	252841077	SNP 113
5	44.48	chrUN:25946639	244222990	SNP 114
5	45.63	chrUN:25691760	252841096	SNP 115
5	47.51	chrV:12015986	418642182	SNP 116
6	0	chrVI:487411	418642183	SNP 117
6	4.26	chrVI:1440771	244222823	SNP 118
6	6.88	chrVI:11873663	120258454	SNP 119
6	10.62	chrVI:7249692	418642188	SNP 120
6	12.64	chrVI:11954719	418642192	SNP 121
6	14.23	chrVI:13220597	252841044	SNP 122

6	17.30	chrVI:14131973	252841117	SNP 123
6	17.41	chrVI:13775642	418642196	SNP 124
6	18.43	chrVI:13911632	418642197	SNP 125
6	20.22	chrVI:14571427	418642200	SNP 126
6	24.89	chrVI:14976508	418642201	SNP 127
6	25.93	chrVI:15041940	244222829	SNP 128
6	26.57	chrVI:15274689	244222830	SNP 129
6	28.54	chrVI:15413799	418642203	SNP 130
6	31.62	chrVI:15654034	418642204	SNP 131
6	44.88	chrVI:16870159	244222834	SNP 132
7	0	chrVII:835236	252841091	SNP 133
7	3.59	chrUN:28671327	244222995	SNP 134
7	5.28	chrVII:537136	252841113	SNP 135
7	6.24	chrVII:330141	418642212	SNP 136
7	6.37	chrUN:29087782	244222996	SNP 137
7	9.71	chrVII:1569236	418642218	SNP 138
7	11.99	chrVII:1481322	418642217	SNP 139
7	16.68	chrVII:2559099	418642220	SNP 140
7	20.16	chrVII:5936068	120258457	SNP 141
7	25.94	chrVII:16848769	418642232	SNP 142
7	26.99	chrVII:19857837	418642237	SNP 143
7	27.24	chrVII:21302029	418642238	SNP 144
7	27.63	chrVII:20883742	252841067	SNP 145
7	30.80	chrVII:24203557	120258459	SNP 146
7	31.53	chrVII:24217606	418642245	SNP 147
7	34.16	chrVII:25193081	418642246	SNP 148
7	38.11	chrVII:25910223	418642247	SNP 149
7	38.66	chrVII:25986275	418642248	SNP 150
7	41.51	chrVII:26227403	120258461	SNP 151
7	43.85	chrVII:26448674	252841125	SNP 152
7	45.19	chrVII:26538823	244222842	SNP 153
7	53.37	chrVII:26769148	418642251	SNP 154
7	60.25	chrVII:27918897	418642257	SNP 155
7	60.28	chrUN:29400087	418642638	SNP 156
8	0	chrVIII:1929053	244222843	SNP 157
8	2.96	chrVIII:868226	418642258	SNP 158
8	6.90	chrVIII:2505620	418642263	SNP 159
8	8.22	chrVIII:3281178	120258463	SNP 160
8	8.90	chrVIII:3627706	244222844	SNP 161
8	9.30	chrVIII:3987295	120258464	SNP 162
8	10.56	chrVIII:6680213	418642268	SNP 163
8	11.12	chrVIII:4503012	244222845	SNP 164
8	15.16	chrVIII:13412707	244222846	SNP 165
8	19.26	chrVIII:16299555	418642283	SNP 166
8	22.34	chrVIII:16148618	244222850	SNP 167
8	25.88	chrVIII:16649103	418642284	SNP 168
8	28.62	chrVIII:16843576	418642285	SNP 169
8	31.80	chrVIII:16954373	244222853	SNP 170
8	32.20	chrVIII:17359071	252841141	SNP 171
8	34.16	chrVIII:17576018	120258466	SNP 172
8	40.02	chrVIII:18047605	244222854	SNP 173
8	42.09	chrVIII:19282658	418642286	SNP 174
8	42.13	chrVIII:18760705	244222855	SNP 175
8	45.62	chrVIII:18432598	120258467	SNP 176
9	0	chrIX:803523	252841065	SNP 177
9	8.26	chrIX:1571056	418642294	SNP 178
9	9.50	chrIX:430462	418642289	SNP 179
9	10.31	chrIX:2089567	244222858	SNP 180
9	12.65	chrIX:1273244	244222857	SNP 181
9	15.06	chrIX:2310926	418642299	SNP 182
9	17.13	chrIX:16779825	244222869	SNP 183
9	17.44	chrIX:15670033	244222868	SNP 184
9	17.96	chrIX:15475540	418642312	SNP 185
9	18.72	chrIX:13852312	418642311	SNP 186

9	19.56	chrIX:13553866	252841127	SNP 187
9	22.81	chrIX:10468143	244222864	SNP 188
9	22.92	chrIX:7893416	418642306	SNP 189
9	26.56	chrIX:5403530	120258474	SNP 190
9	26.64	chrIX:5441237	244222862	SNP 191
9	27.00	chrIX:7146708	418642304	SNP 192
9	27.15	chrIX:5568375	244222863	SNP 193
9	27.17	chrIX:20090929	244222871	SNP 194
9	28.38	chrIX:6126845	252841056	SNP 195
9	37.30	chrIX:18826248	418642319	SNP 196
9	38.37	chrIX:18494397	418642317	SNP 197
9	38.38	chrIX:18942598	120258478	SNP 198
9	46.53	chrIX:19322448	418642320	SNP 199
9	53.67	chrIX:19745222	418642321	SNP 200
10	0	chrUN:14043112	418642618	SNP 201
10	0.63	chrUN:14127611	418642619	SNP 202
10	0.76	chrUN:29017220	418642637	SNP 203
10	0.87	chrUN:24511995	418642628	SNP 204
10	2.41	chrX:1245433	120258479	SNP 205
10	2.88	chrX:1275840	418642326	SNP 206
10	8.30	chrX:4696470	418642330	SNP 207
10	10.42	chrX:8703061	120258485	SNP 208
10	10.71	chrX:7113953	120258483	SNP 209
10	11.01	chrX:8647016	244222873	SNP 210
10	14.03	chrX:11139448	252841128	SNP 211
10	20.08	chrX:12507632	244222877	SNP 212
10	22.08	chrX:13452742	418642354	SNP 213
10	23.40	chrX:13132917	418642352	SNP 214
10	23.92	chrX:12844036	418642350	SNP 215
10	33.42	chrX:14265366	120258486	SNP 216
10	36.44	chrX:14456479	252841100	SNP 217
10	36.44	chrX:14549101	252841122	SNP 218
10	37.27	chrX:14831394	418642358	SNP 219
11	0	chrXI:1449684	120258489	SNP 220
11	0.50	chrXI:1017481	120258488	SNP 221
11	1.48	chrXI:234849	120258487	SNP 222
11	2.13	chrXI:457909	244222878	SNP 223
11	11.48	chrXI:7355052	418642370	SNP 224
11	13.51	chrXI:9039275	252841094	SNP 225
11	16.34	chrXI:10976029	244222883	SNP 226
11	18.26	chrXI:12097498	418642375	SNP 227
11	18.43	chrXI:12746496	244222884	SNP 228
11	19.18	chrXI:12550151	418642376	SNP 229
11	27.69	chrXI:14616764	120258494	SNP 230
11	28.48	chrXI:14691162	418642380	SNP 231
11	28.64	chrXI:14830913	244222885	SNP 232
11	29.35	chrXI:14631875	418642379	SNP 233
11	31.12	chrXI:14286902	120258493	SNP 234
11	33.04	chrXI:14426451	418642377	SNP 235
11	34.65	chrXI:15005173	244222886	SNP 236
11	39.96	chrUN:32523521	418642646	SNP 237
11	40.34	chrXI:15600885	244222887	SNP 238
11	52.96	chrXI:16494449	418642385	SNP 239
11	55.12	chrXI:16655205	120258495	SNP 240
11	55.95	chrXI:16701186	244222888	SNP 241
12	0	chrUN:30606854	244222997	SNP 242
12	5.24	chrUN:36788692	418642650	SNP 243
12	7.52	chrUN:38378170	120258576	SNP 244
12	10.45	chrXII:70478	418642386	SNP 245
12	20.77	chrXII:548804	252841119	SNP 246
12	29.88	chrXII:16454328	418642411	SNP 247
12	30.15	chrXII:1589655	120258497	SNP 248
12	30.43	chrUN:17470353	418642620	SNP 249
12	32.82	chrXII:2181073	252841129	SNP 250

12	34.10	chrXII:14223760	244222895	SNP 251
12	34.88	chrXII:13045611	244222894	SNP 252
12	35.32	chrXII:15046849	418642410	SNP 253
12	41.14	chrXII:7504339	418642406	SNP 254
12	42.28	chrXII:6924609	418642405	SNP 255
12	42.38	chrXII:6913126	120258500	SNP 256
12	42.99	chrXII:6745006	244222892	SNP 257
12	44.11	chrXII:5521301	418642402	SNP 258
12	44.58	chrXII:5828898	418642403	SNP 259
12	45.19	chrXII:6012527	418642404	SNP 260
12	47.22	chrXII:4042442	244222891	SNP 261
12	48.20	chrXII:16628544	418642412	SNP 262
12	49.70	chrXII:4123972	418642400	SNP 263
12	51.20	chrXII:3026329	418642398	SNP 264
12	51.47	chrXII:2242677	418642394	SNP 265
12	52.71	chrXII:2713984	418642397	SNP 266
13.1	0	chrXIII:1388673	120258504	SNP 267
13.1	0.58	chrXIII:3109522	120258506	SNP 268
13.1	1.44	chrXIII:2105469	418642423	SNP 269
13.1	1.68	chrXIII:1698554	418642421	SNP 270
13.1	2.08	chrXIII:2523163	120258505	SNP 271
13.1	2.12	chrXIII:2632698	244222901	SNP 272
13.1	3.82	chrXIII:2822352	244222902	SNP 273
13.1	7.90	chrXIII:4401535	418642425	SNP 274
13.1	8.47	chrXIII:4621027	418642426	SNP 275
13.1	13.55	chrXIII:7266499	120258508	SNP 276
13.1	18.46	chrXIII:17392141	120258510	SNP 277
13.1	25.66	chrXIII:19311265	252841123	SNP 278
13.2	0	chrXIII:19693259	252841080	SNP 279
13.2	2.51	chrUN:29831084	418642640	SNP 280
13.2	4.08	chrUN:32210273	418642644	SNP 281
14	0	chrUN:36334731	244223000	SNP 282
14	3.71	chrUN:35285565	418642649	SNP 283
14	5.20	chrUN:38561237	252841055	SNP 284
14	5.83	chrXIV:348659	418642435	SNP 285
14	9.45	chrXIV:721170	244222907	SNP 286
14	12.42	chrXIV:1087388	418642439	SNP 287
14	14.05	chrXIV:1383447	244222908	SNP 288
14	17.18	chrXIV:1442872	120258512	SNP 289
14	22.91	chrXIV:2165223	418642447	SNP 290
14	24.40	chrXIV:1713227	120258513	SNP 291
14	33.35	chrXIV:3414352	120258514	SNP 292
14	33.69	chrXIV:3598443	418642452	SNP 293
14	34.73	chrXIV:3534175	120258515	SNP 294
14	35.66	chrXIV:2908977	418642451	SNP 295
14	39.55	chrXIV:4632223	418642454	SNP 296
14	42.10	chrXIV:7313827	418642457	SNP 297
14	45.41	chrXIV:14049917	252841090	SNP 298
14	45.98	chrXIV:15137805	418642462	SNP 299
14	46.16	chrUN:21213332	120258571	SNP 300
15	0	chrXV:11818	418642463	SNP 301
15	9.35	chrXV:505537	418642465	SNP 302
15	10.21	chrXV:414608	120258519	SNP 303
15	14.23	chrXV:1800560	418642468	SNP 304
15	16.03	chrXV:1987082	418642469	SNP 305
15	16.94	chrXV:1902350	244222911	SNP 306
15	19.63	chrXV:2169610	244222912	SNP 307
15	22.48	chrXV:2507809	244222914	SNP 308
15	33.92	chrXV:6446874	418642477	SNP 309
15	38.80	chrXV:16003465	244222919	SNP 310
15	40.80	chrXV:12281774	418642480	SNP 311
15	41.19	chrXV:13047331	418642481	SNP 312
16	0	chrXVI:2483136	252841051	SNP 313
16	0.79	chrXVI:2392758	244222921	SNP 314

16	4.32	chrXVI:2764206	120258523	SNP 315
16	6.78	chrXVI:3206769	244222923	SNP 316
16	9.10	chrXVI:5562355	244222924	SNP 317
16	9.87	chrXVI:6415385	418642487	SNP 318
16	12.04	chrXVI:8218481	418642488	SNP 319
16	12.86	chrXVI:9428786	244222926	SNP 320
16	13.51	chrXVI:9981125	244222928	SNP 321
16	14.76	chrXVI:12111717	120258526	SNP 322
16	14.80	chrXVI:12996432	244222929	SNP 323
16	16.74	chrXVI:14283264	244222932	SNP 324
16	16.93	chrXVI:14093156	244222931	SNP 325
16	21.13	chrXVI:14963879	244222933	SNP 326
16	22.06	chrXVI:15826700	244222935	SNP 327
16	23.80	chrXVI:16058672	252841101	SNP 328
16	27.34	chrXVI:17895677	244222938	SNP 329
16	28.82	chrXVI:17773420	244222937	SNP 330
16	31.26	chrXVI:17471373	418642502	SNP 331
16	31.82	chrXVI:17405918	418642501	SNP 332
16	34.60	chrXVI:17236926	244222936	SNP 333
16	39.52	chrXVI:16673569	120258528	SNP 334
16	42.34	chrUN:26695645	418642630	SNP 335
16	43.60	chrUN:17922401	120258570	SNP 336
16	45.89	chrUN:17560757	418642621	SNP 337
16	46.08	chrUN:37016121	418642651	SNP 338
17	0	chrXVII:645029	418642506	SNP 339
17	1.01	chrXVII:769372	244222939	SNP 340
17	4.44	chrXVII:1264852	418642508	SNP 341
17	11.96	chrXVII:2232080	120258532	SNP 342
17	14.74	chrXVII:2626658	418642511	SNP 343
17	15.89	chrXVII:2664810	244222940	SNP 344
17	16.23	chrXVII:2872553	418642512	SNP 345
17	21.13	chrXVII:3706521	418642516	SNP 346
17	21.59	chrXVII:3906379	244222942	SNP 347
17	22.28	chrXVII:3843835	120258534	SNP 348
17	24.94	chrUN:2632376	252841074	SNP 349
17	24.50	chrUN:2776586	120258568	SNP 350
17	24.84	chrUN:2474754	418642603	SNP 351
17	25.39	chrXVII:9024413	244222946	SNP 352
17	25.41	chrXVII:9881295	418642523	SNP 353
17	33.04	chrXVII:11037958	252841072	SNP 354
17	34.54	chrXVII:11855617	120258535	SNP 355
17	37.86	chrXVII:12022612	120258536	SNP 356
17	41.88	chrXVII:13795831	252841087	SNP 357
17	43.04	chrXVII:12666712	418642526	SNP 358
17	44.40	chrXVII:12599208	252841154	SNP 359
18	0	chrXVIII:1211531	418642530	SNP 360
18	6.42	chrXVIII:3037920	418642534	SNP 361
18	9.69	chrXVIII:4836241	120258539	SNP 362
18	11.07	chrXVIII:5765162	120258540	SNP 363
18	12.47	chrXVIII:12146976	244222955	SNP 364
18	13.59	chrXVIII:10943853	418642539	SNP 365
18	14.58	chrXVIII:11765327	120258543	SNP 366
18	14.82	chrXVIII:11896010	244222954	SNP 367
18	15.30	chrXVIII:11504306	418642542	SNP 368
18	15.94	chrXVIII:11086837	120258542	SNP 369
18	16.83	chrXVIII:12273872	252841150	SNP 370
18	17.13	chrXVIII:13193140	244222957	SNP 371
18	20.19	chrXVIII:13352631	120258546	SNP 372
18	24.41	chrXVIII:13753579	244222958	SNP 373
19	0	chrXIX:646137	252841146	SNP 374
19	2.93	chrXIX:728155	120258550	SNP 375
19	3.31	chrXIX:707167	418641955	SNP 376
19	5.42	chrXIX:897343	418641956	SNP 377
19	9.03	chrXIX:987461	418641957	SNP 378

19	12.50	chrXIX:1472847	120258551	SNP 379
19	15.25	chrXIX:1546489	418641958	SNP 380
19	27.26	chrXIX:18043409	252841059	SNP 381
19	28.21	chrXIX:18045399	120258558	SNP 382
19	28.22	CH213-119K16:14070	418641977	SNP 383
19	28.22	CH213-119K16:207645	418641978	SNP 384
19	28.22	CH213-21C23:188808	418641953	SNP 385
19	28.22	chrXIX:14650559	418641975	SNP 386
19	28.22	chrXIX:8190806	120258554	SNP 387
20	0	chrXX:1758783	244222962	SNP 388
20	0.84	chrXX:1808773	418642552	SNP 389
20	6.12	chrXX:2312273	418642554	SNP 390
20	8.05	chrXX:2885078	418642555	SNP 391
20	14.62	chrXX:9279241	244222965	SNP 392
20	15.80	chrXX:13893619	252841139	SNP 393
20	16.83	chrXX:14562943	418642569	SNP 394
20	16.84	chrXX:14462157	244222968	SNP 395
20	17.14	chrXX:14859034	418642571	SNP 396
20	19.90	chrXX:15996390	418642573	SNP 397
20	20.51	chrXX:16111163	418642574	SNP 398
20	20.56	chrXX:16253512	252841060	SNP 399
20	39.00	chrXX:17708486	244222970	SNP 400
21	0	chrUN:6720054	244222987	SNP 401
21	0.07	chrUN:7381868	252841045	SNP 402
21	0.85	chrXXI:3082227	418642585	SNP 403
21	8.75	chrXXI:10156751	252841143	SNP 404
21	10.64	chrXXI:9820534	418642589	SNP 405
21	17.35	chrXXI:11060209	120258566	SNP 406
21	31.09	chrXXI:11414383	120258567	SNP 407
21	33.71	chrUN:28158103	418642634	SNP 408

The table provides the linkage group (LG) and map position in centimorgans (cM) of each SNP marker used in the present study. Each marker name combines chromosome location of the SNP (Roman numerals before colon) and physical position of the SNP in base pairs (b.p.; Hindu-Arabic numerals after colon), according to the initial threespine stickleback genome assembly (Broad S1, Feb. 2006). Markers in unassembled genome regions are indicated with 'chrUN'. In these cases, physical position is based on the composite chrUN in the UCSC genome browser. Marker information can be obtained from the Single Nucleotide Polymorphism Database (dbSNP), which is hosted by the National Center for Biotechnology Information (NCBI) of the U.S. National Institutes of Health (available at http://www.ncbi.nlm.nih.gov/projects/SNP/). Data for a specific marker may be queried by a dbSNP search using the submitted NCBI SNP ID Number (i.e., search string 'ss' + nine digit ss# number in the second column from right, all as one eleven-character string with no spaces; e.g., 'ss244223001' retrieves sequence data for the very first SNP locus in the table). For the present study only, a SNP Identifier # was also designated for each marker (column at far right). To simplify visually locating markers and to avoid overly crowded text in Extended Data Fig. 3, these SNP Identifier #s were designated solely for the purpose of cross-referencing the data tabulated here, including the published SNP names, with positions of the same markers (and SNP Identifier #s) in the figure. Note that chromosome XIII was represented by two LGs in the linkage map for this study: LG 13.1 and LG 13.2. These two LGs contained several markers and one marker, respectively, that were physically localized to chromosome XIII. Though LG 13.2 was included in QTL analyses for all traits, no QTLs were mapped to this small LG. Consequently, Extended Data Fig. 3 omits LG 13.2, and uses the simplified label 'LG 13', corresponding to LG 13.1 in this table. Extended Data Fig. 3 also omits any other LG to which no QTLs were mapped.

Supplementary Discussion

1. Establishing a near-natural food web in the experimental pond

Twenty outdoor ponds at an experimental pond facility, located at the University of British Columbia (UBC, Vancouver, BC, Canada), were designed to each contain distinct littoral (i.e., shallow benthic) and open-water (i.e., pelagic) habitats. Extended Data Fig. 1 provides the overhead and profile dimensions of each pond. We used one of these ponds for our genetic mapping experiment (pond no. 4, shown in a photograph in Extended Data Fig. 1). Construction of pond no. 4 was completed in February 2007, when the pond was filled to the level of its two outflow standpipes with City of Vancouver fire hydrant water (approx. 0.75 million litres total volume). Thereafter, rain served as the primary source of water input to the pond. Because ours was the first study ever conducted in pond no. 4, we were certain that no non-experimental fish were present during the investigation described herein.

Paxton Lake is a small (17-hectare), hard water (i.e., high calcium) lake located on Texada Island, BC, Canada (49°42'30" N, 124°31'30" W)[16]. Owing to the lake's high water hardness, marl can be found deposited on parts of the lakebed and encrusted on both *Chara* sp. (stonewort) and fallen trees that become submerged in the lake[83]. We took several steps to condition pond no. 4 for 13 months prior to starting the experiment. This conditioning was done to help simulate the ecology of Paxton Lake, and to promote the development of a near-natural food web of typical prey for the benthic and the limnetic threespine stickleback species (*Gasterosteus aculeatus* spp. complex) that are native to Paxton Lake[14,60,84]. To achieve high water hardness, the littoral zone of pond no. 4 and the littoral zones of the 19 other experimental ponds at UBC were lined with crushed limestone gravel purchased from a quarry adjacent to Paxton Lake (Texada Quarrying Ltd.; see Extended Data Fig. 1). We then supplemented pond no. 4 with the following inputs of inorganic nutrients and organic carbon, as well as fresh sediments, living biota, and propagules derived from the native lake: an initial addition of 1.2 kg of KNO_3 (50% purity) and 47 g of KH_2PO_4 (99% purity), added on 6-March-2007; 8 L of dark sediments (hand dredged from a shallow, inshore region of Paxton Lake on 17-March-2007) plus contents of a 20-min. plankton tow using a 0.5-m diameter plankton net with a mesh size of 80 µm (including the calanoid copepod *Skistodiaptomus oregonensis*, as well as several other native zooplankton taxa, taken from Paxton Lake on 17-March-2007 and stored alive in a separate container filled with 1 L of lake water), both of which were added to pond no. 4 the same day; half of a small, square bale of hay, added on 5-September-2007; a second 8-L sample of fresh, dark sediments from the native lakebed (collected and transported as before) and 10 kg of a living macrophyte bed taken from Paxton Lake, consisting primarily of *Chara* sp. with some *Potamogeton amplifolius* (largeleaf pondweed) and containing large numbers of associated benthic macroinvertebrates (e.g., *Gammarus lacustris*, chironomid larvae, snails, mussels, water mites, etc.), collected from a shallow inshore region of Paxton Lake on 22-February-2008 and added to pond no. 4 later the same day; a second addition of 1.2 kg of KNO_3 (50% purity) and 47 g of KH_2PO_4 (99% purity), added on 29-February-2008; and a final batch of 1.2 kg of KNO_3 (50% purity) and 47 g of KH_2PO_4 (99% purity), added on 10-March-2008. Lastly, reference collections of macroinvertebrates and zooplankton were made from pond no. 4 during the two weeks prior to adding the adult F_1 hybrids (Methods) to the pond (i.e., during the week before and the week after the final addition of inorganic nutrients). Many of the important prey taxa that are commonly consumed by wild Paxton limnetics and/or benthics[14,60,84] were present in each of these baseline collections (M.E. Arnegard, B. Matthews, and D. Schluter, *pers. obs.*).

2. Stable isotopes in natural lakes versus the experimental ponds at UBC

Variation in stable carbon (C) and nitrogen (N) isotope concentrations in animal tissues arises from differential isotopic fractionation during metabolic processes[45] and reflects cumulative dietary patterns over periods of several weeks to months[52-54,85-89]. The standard $\delta^{13}C$ ratio of stable carbon isotopes in a consumer indicates dietary sources of energy, because differences arising from plant metabolic pathways (e.g., in C_3 versus C_4 plants) and food chains in different habitats (e.g., in the plankton-dominated pelagic versus macroinvertebrate-dominated shallow benthic habitats of lakes) are largely conserved during trophic transfers[45]. In contrast, the standard $\delta^{15}N$ ratio of stable nitrogen isotopes typically reflects trophic level within a food web, due to the preferential retention of the heavier nitrogen isotope (^{15}N) with each transfer of energy to a higher trophic level[50]. Together, $\delta^{13}C$ and $\delta^{15}N$ represent a bivariate trophic space in which dietary niche variation among individuals can be quantified and visualised[17,53,57,65,90,91]. In the threespine stickleback species pairs of Paxton and Priest Lakes, both of which are located on Texada Island, limnetic individuals have significantly lower $\delta^{13}C$ and significantly higher $\delta^{15}N$ than the benthic individuals with which they are sympatric[17]. These differences reflect the dominance of carbon sources from pelagic versus benthic prey in the diets of wild limnetics and benthics, respectively, and perhaps, the presence of omnivorous zooplankton species with relatively high trophic positions (e.g., the calanoid copepod *S. oregonensis*) in the food webs of the native lakes[17]. These isotopic differences between wild benthic and limnetic stickleback are also consistent with the finding of an experimental study conducted in aquatic mesocosms, which demonstrated that Paxton limnetics supress the abundance of *S. oregonensis* to a greater degree than either Paxton benthics or individuals from a nearby 'solitary-lake' population (inhabiting Cranby Lake on Texada Island)[55].

Prior to the present experiment, we found that $\delta^{13}C$ was highly significantly different (almost non-overlapping) between pure Paxton limnetic juveniles raised in one experimental pond (#3 at the UBC facility) and pure Paxton benthic juveniles raised in another experimental pond (#2; M.E. Arnegard, B. Matthews, and D. Schluter, *unpublished results*). The pure Paxton limnetic juveniles that were reared in pond no. 3 exhibited lower $\delta^{13}C$ values than the pure Paxton benthic juveniles that were reared in pond no. 2. These pilot measurements of $\delta^{13}C$ are, again, consistent with the observations that limnetic stickleback acquire most of their carbon from pelagic food chains, whereas benthic stickleback acquire most of their carbon from shallow benthic food chains[14,17,60,84]. Moreover, the $\delta^{13}C$ values of the pure limnetics in pond no. 3 and the pure benthics in pond no. 2 (M.E. Arnegard, B. Matthews, and D. Schluter, *unpublished results*) very closely matched those of the F_2 hybrids that, respectively, exhibited the most limnetic-like ('L') and benthic-like ('B') feeding patterns in the experimental population for the present study (see Fig. 1 of the main paper). In addition to threespine stickleback, more pelagic species, ecomorphs, and/or individuals of many other fish taxa generally exhibit lower values of $\delta^{13}C$ than those that feed more from benthic (e.g., littoral) food chains in the same lakes[52,92,93].

In contrast to our preliminary findings for $\delta^{13}C$, the pilot study described above did not show a significant difference in $\delta^{15}N$ between the pure Paxton benthic juveniles and the pure Paxton limnetic juveniles that were raised in separate closed systems (i.e., ponds #2 and #3; M.E. Arnegard, B. Matthews, and D. Schluter, *unpublished results*). However, a significant difference in $\delta^{15}N$ between benthics and limnetics was observed in the native lake, based on individuals that were older than those collected for our pilot study[17]. This lack of a species difference in $\delta^{15}N$ during the pilot study may have been due to the absence of competition from juveniles of the alternate species in each of the pure-species reference ponds at UBC, or it may have resulted largely from our measurement of younger (mid-summer) juveniles in reference ponds #2 and #3 for the pilot study, compared to the autumnal F_2 hybrids sampled from pond

no. 4 (present study). Nevertheless, owing to how general the pattern of $\delta^{13}C$ variation is between pelagic and benthic fishes across many temperate taxa[17,52,56,57,92,93], our pilot $\delta^{13}C$ measurements alone motivated the present effort to map the genetic architecture of feeding niche divergence between Paxton benthic and limnetic threespine stickleback.

3. Justification for delineating regions 'B', 'L', and 'A' in isotope space

We delineated three subsets of F_2 hybrids that represented notable features of the performance-by-isotope landscape shown in Fig. 1a (i.e., landscape regions of highest or lowest mean body size, located near extreme corners of the stable isotope distribution). We took this approach in the main paper because it enabled us to employ several illustrative categorical comparisons, which proved to be the simplest and clearest way to understand and show how F_2 diet, morphology, and feeding performance varied across a roughly triangle-shaped isotope distribution (Fig. 1b–e, Fig. 2a–d, Extended Data Fig. 4, Extended Data Fig. 5). We based the boundaries around regions 'B', 'L', and 'A' on loess-predicted body size contours enclosing 15% of all samples in each region (i.e., the regional boundaries were found by applying a 15% threshold for our inclusion criterion). Selecting this threshold for within-region sample size represented a trade-off between increased statistical power (more individuals in each region) and increased isotopic distinctiveness of the resulting subsets (fewer individuals per region). At the 15% threshold, we achieved reasonably good power for statistical comparisons and fully distinct landscape regions for subsets 'A' and 'B'. However, the 15% threshold resulted in an ill-defined boundary for the third landscape region, 'L'. We nevertheless adopted this threshold, because straightforward application of a reasonable second criterion for region 'L' only (i.e., minimization of PC1 in that subset of F_2 hybrids) yielded an isotopically distinct category of individuals for the intended landscape feature — the second body size peak, located at low $\delta^{13}C$–high $\delta^{15}N$ (Fig. 1a) — while retaining the desired level of power.

Upon testing for variation in diet and morphology among the three resulting subsets of F_2 hybrids, we found that the statistical outcomes of all categorical comparisons were robust and consistent across a range of subset sample sizes (i.e., from that achieved at the 15% threshold, as applied above, down to that achieved at the 10% threshold; at the smaller end of this range, fully distinct regions for 'B', 'A', and 'L' were defined simply on the basis of predicted body size without needing a second criterion; results not shown). Importantly, the outcomes of regression analyses of continuous patterns of dietary or morphological variation using all available samples (Extended Data Fig. 2, Extended Data Fig. 6) support the same conclusions that are more readily illustrated via our categorical approach. Below, we provide detailed results for regression analyses of the diet data on various axes of stable isotope variation among the juvenile F_2 hybrids.

First, we performed loess (local 2nd degree polynomial) regression of $\log_e$(number of chironomids consumed + 1), on $\delta^{13}C$ and $\delta^{15}N$, to generate a surface of chironomid feeding by the F_2 hybrids; we did so using the R function 'loess'[51] (span = 0.75). A contour plot of this surface is shown in Extended Data Fig. 2a, in which distinct symbols are used to map how individuals also fed on a second key prey item, the calanoid copepod *S. oregonensis*. We plotted individuals that fed on one or more calanoid copepods with ×, whereas we used ● to indicate individuals that showed no evidence of calanoid copepod feeding. As illustrated, increased chironomid feeding in the F_2 hybrids (a dietary pattern characteristic of wild Paxton benthics[14,19,60,84]) closely tracked an increase in niche score. In contrast, F_2 hybrids that exhibited a capability of foraging on evasive calanoid copepods (a dietary pattern known for wild limnetics[14,19,60,84]) tended to exhibit relatively low values of both niche score (PC1, black arrow) and diet deviation score (PC2, white arrow); stable isotope signatures like these also characterise wild Paxton limnetics[17]. These continuous patterns of dietary variation in the F_2 hybrid mapping population lend support to

interpretations presented in the main paper, which were based on categorical comparisons among delineated subsets of the same F_2 hybrids.

Results of linear and logistic regression analyses using all available samples (Extended Data Fig. 2b–g) further reinforce our biological interpretations about niche score and diet deviation score. These supplementary analyses focussed only on the four prey types that, together, accounted for more than 98% of all food items consumed by the F_2 hybrids. Other consumed foods (data provided with source data for Extended Data Fig. 2) were too rare in the digestive tracts of F_2 hybrids to be informative about the dietary 'meanings' of stable isotope variation under all of the statistical approaches that we used to investigate feeding patterns. Although we only describe our results from linear and logistic regression, below, no outcomes of any other tests (results not shown) were inconsistent with the following findings.

Linear regression applied to the diet data showed that a more benthic F_2 carbon signature[17] (i.e., higher $\delta^{13}C$) was positively associated with the consumption of greater numbers of larval chironomids ($P = 3.70 \times 10^{-6}$; Extended Data Fig. 2b; other statistical details given in the figure legend). In addition, a higher value of niche score, which we also interpret as being more benthic-like, was similarly associated with a higher probability of feeding on larval chironomids ($P = 0.0255$; Extended Data Fig. 2c). By contrast, elevated $\delta^{15}N$, which characterised F_2 hybrids in region 'L' (Fig. 1a) and was also found in wild Paxton limnetics in a prior study[17], was positively associated with the amount of *S. oregonensis* consumed by individuals in the experimental population in pond no. 4 ($P = 0.0139$; Extended Data Fig. 2e).

Based on logistic regression, the probability that F_2 hybrids fed on *S. oregonensis* seemed to rise as niche score decreased ($P = 0.0651$; Extended Data Fig. 2f). Though this trend was not strictly significant at the $P < 0.05$ level, this suggestive result is consistent with an interpretation that F_2 hybrids in region 'L' exhibited a more limnetic-like feeding pattern compared to other individuals. Plankton tows made before and during the experiment (with a 0.3-m diameter, 80-µm mesh plankton net) revealed that *S. oregonensis* was the most abundant zooplankter in the pelagic food web of pond no. 4 (M.E. Arnegard, B.W. Matthews, and D. Schluter, *pers. obs.*). This calanoid copepod is also abundant in Paxton Lake[94]. Compared to other planktonic taxa, calanoid copepods are thought to compose a rather nutritious food resource for fish[95]. *Skistodiaptomus oregonensis* has been found to suffer heavier predation from Paxton limnetics than from Paxton benthics, in both a mesocosm setting[55] and the native lake[14,60]. The trophic position of *S. oregonensis* in Paxton Lake is predicted to be elevated relative to other pelagic prey[17]. If *S. oregonensis* indeed had a similarly elevated trophic position in pond no. 4 as well, its $\delta^{15}N$ composition and the heavier predation pressure it experienced from 'L' individuals during our experiment (Fig. 1c) could have been largely responsible for the elevated $\delta^{15}N$ and reduced niche score characterising this subset of F_2 hybrids (Fig 1a).

Using logistic regression, we also found that the second factor axis through stable isotope space (i.e., PC2 or diet deviation score) was negatively associated both with the probability that calanoid copepods were consumed (Extended Data Fig. 2g) and the probability that larval chironomids were consumed (plot not shown). Similarly, linear regression revealed that PC2 was also negatively associated with the amounts of each of these prey types consumed by F_2 hybrids (also not plotted). Results of these four analyses were the following: (logistic regression, calanoid copepod prey) slope coefficient = -0.958, z = -2.67, $P = 0.00766$; (logistic regression, larval chironomid prey) slope coefficient = -0.960, z = -3.76, $P = 1.68 \times 10^{-4}$; (linear regression, calanoid copepods) slope estimate = -0.270, $R^2 = 0.0586$, $F_{1,97} = 6.03$, $P = 0.0158$; (linear regression, larval chironomids) slope estimate = -0.363, $R^2 = 0.0882$, $F_{1,97} = 9.38$, $P = 0.00284$.

The F_2 hybrids from region 'A' were characterised by the highest diet deviation scores that we observed, overall, in the mapping population. As described in the main paper, we interpret the feeding pattern of these 'A' individuals to be 'alternative' (or 'atypical') in comparison to the diets of native benthics and limnetics from Paxton Lake. This interpretation is bolstered by the negative relationship between diet deviation score and the extent to which F_2 hybrids fed on either larval chironomids or calanoid copepods, which are typical prey of wild benthics and limnetics, respectively. In fact, chironomid larvae and calanoid copepods are considered to be classic markers[56,96] (or surrogates[97,98]) for opposite sides of the benthic–limnetic resource gradient. Collectively, the findings presented above show that niche score was a measure of where each F_2 hybrid fed along the benthic-limnetic resource gradient, which spans feeding niches occupied by the parental species in the natural setting.

Our conclusion that PC2 indicated the extent of dietary 'deviation' away from the classic limnetic–benthic resource axis (as recapitulated in pond no. 4) receives support from the positive relationship between F_2 feeding on symphypleonan collembolans and diet deviation score: (logistic regression) slope coefficient = 1.247, z = 4.262, $P = 2.03 \times 10^{-5}$, Extended Data Fig. 2d; (linear regression) slope estimate = 1.152, $R^2 = 0.328$, $F_{1,97} = 47.3$, $P = 6.04 \times 10^{-10}$, not plotted. Although Hynes[99] demonstrated that some threespine stickleback individuals may feed on collembolans in certain environments, Collembola feeding by either benthic or limnetic sticklebacks has not been observed in Paxton Lake[14,17,84]. Taken together, observed patterns of F_2 hybrid feeding on calanoid copepods, larval chironomids, and symphypleonan collembolans demonstrate that the slowest growing F_2 hybrids in our study, with low $\delta^{13}C$–low $\delta^{15}N$ (in region 'A'), exhibited a divergent pattern of feeding with respect to F_2 hybrids with low (absolute) diet deviation scores and to both of the parental species.

Contrary to data on the consumption of copepods, chironomids, or collembolans by F_2 hybrids, we had no *a priori* grounds for interpreting the pattern of foraging on *Chydorus* sp. with respect to the limnetic–benthic resource spectrum. *Chydorus* sp. (family Chydoridae), a cladoceran microcrustacean taxon, is similar in size to the pelagic zooplankton that are preferred by Paxton limnetics. Yet, this cladoceran is smaller than the average size of prey commonly consumed by sub-adult to adult Paxton benthics[84]. Chydorids tend to be found on aquatic vegetation, leaf litter, and the benthic surfaces of ponds and lakes, rather than among pelagic zooplankton in offshore lentic habitats[84,100]. This distribution pattern applies generally to the Chydoridae, and we also found it to hold true for the *Chydorus* sp. that was common in pond no. 4 during our study (M.E. Arnegard, B. Matthews, and D. Schluter, *pers. obs.*). Paxton benthics and limnetics are both known to feed on cladocerans[14,84], yet the extent to which either pure species specifically consumes littoral chydorids in the native lake remains unclear. Based on linear and logistic regression, we found no hint of a significant relationship between the consumption of *Chydorus* sp. and either the niche score or diet deviation score of F_2 hybrids (results not shown). Thus, our dietary data on *Chydorus* sp., the only other common food resource found in the digestive tracts of F_2 hybrids, are not inconsistent with our data on ingested copepods, chironomids, or collembolans.

Though there are potential limitations to data obtained from the quantification of ingested food items in the digestive tracts of fish[101], this approach was the most direct method practical for measuring the feeding activity of F_2 hybrids in our experimental design. When all such data from all available samples were considered together, we found good support for our interpretations of stable isotope variation among the juvenile hybrid stickleback in our mapping population. The overall consistency of findings on F_2 hybrid diet, both between statistical approaches and among commonly consumed prey, justifies our delineation of regions 'B', 'L', and 'A' in isotope space to help explore and illustrate the biological significance of stable isotope variation in a clear and simple manner.

4. Body size as a measure of feeding performance

We used body size of the F_2 hybrid juveniles at capture as a measure of their feeding performance. For all analyses and figures in the main paper we measured body size as body length (i.e., distance between morphometric landmarks 1 and 13; see Methods and Extended Data Fig. 3). We found that our overall results were essentially unchanged when we quantified body size using centroid size instead of body length (results not shown); centroid size is the square root of the sum of squared distances of all morphometric landmarks from the centre of the body form, or centroid[65,102].

The pre-winter juveniles that we collected for our study had spent their entire lives together in a single closed system (i.e., in pond no. 4), where they had unrestricted access to the same food resource distribution and were able to interact with one another competitively. Given the timeframe and near-natural setting of our experiment, we expected differential foraging success among individuals to be the most important factor responsible for body size (i.e., body length) variation. Although juvenile body size might also reflect differences in fish age, we controlled for this in our analyses by using F_2 family identity (i.e., unique pair of F_1 parents) as a covariate (see Methods). Rather than lifetime reproductive fitness, we were specifically interested in estimating the performance of F_2 hybrids in acquiring food resources during the juvenile growth phase. Moreover, the timing of our sampling was planned to avoid estimating diet and feeding performance after both the onset of the yearly winter crash in zooplankton abundance[103,104] and the increase in northern temperate fish mortality that can occur during severe winters[25,105]. Our rational for pre-winter sampling was that collecting stable isotope samples after such events could have resulted in a compression of the measured trophic range, or it may have otherwise obscured relationships between diet and performance. Our experimental design also required us to measure feeding performance in a non-invasive way, without handling the F_2 hybrids or disturbing the ecology of pond no. 4, including the competitive interactions among the juvenile stickleback. The overall best approach to achieve these aims was to use body size as our measure of F_2 hybrid feeding performance. High feeding performance has a general positive impact on individual fitness across many groups of fish. Given the general strength of the relationship between feeding performance and fitness in fish, body size and/or growth rate have been used as proxies for fitness in numerous evolutionary studies of threespine stickleback and other teleost fishes[13,14,22,105-107].

5. Investigating the body size valley in the stable isotope landscape

We statistically evaluated the dip in body size along the niche score axis by means of polynomial regression applied to the 20 largest F_2 families, using regression models that included only linear and quadratic terms as well as the covariate, F_2 family identity. These models provided a reasonable approximation of the shape of the splines shown in Fig. 1f, while avoiding over-fitting with higher order terms. We excluded all but the 20 largest F_2 families because the remaining families (with fewer F_2 full sibs) were too small to allow meaningful within-family analyses (results presented below). The overall polynomial regression model for all individuals in these 20 largest families, across all values of PC2, was significant (model $R^2 = 33.2\%$; $F_{21,416} = 9.847$; $P < 2.20 \times 10^{-16}$). Though the quadratic term in this model was not significant (coefficient estimate = 0.173 ± 0.101 s.e.; $P = 0.0857$), within-family polynomial regression showed that 16 of the 20 largest families individually exhibited positive quadratic coefficients. The probability of obtaining this outcome by chance is $P = 0.0118$ in a Bernoulli experiment, for which the probability of a positive quadratic term is assumed to be equal to the probability of a non-positive quadratic term (exact binomial test, implemented with R function 'binom.test'[51]; two-sided).

Using the same model parameterization and family inclusion criterion, we also found statistical support for a body size valley along the niche use axis when only those individuals with PC2 < 0 were considered (model R^2 = 34.6%; $F_{21,237}$ = 5.973; $P = 4.50 \times 10^{-13}$). Owing to nuances of stable isotope variation among F_2 families (patterns not shown), we found stronger statistical indications of a positive quadratic term in this analysis (quadratic coefficient estimate = 0.244 ± 0.116 s.e.; P = 0.0360) than when all values of PC2 were considered. After restricting the data to PC2 < 0, we found that 17 of the 20 largest F_2 families individually exhibited positive quadratic coefficients (P = 0.00258; exact binomial test; two-sided). Statistical support for a positive quadratic term was also strong when all n = 625 F_2 hybrids with complete isotope and body size data were considered (i.e., all sampled individuals across the full range of PC2) but the F_2 family covariate was excluded from the model (quadratic term: coefficient estimate = 0.542 ± 0.105 s.e.; $P = 3.12 \times 10^{-7}$). Including the F_2 family covariate in this last model was unjustified because many of the 625 F_2 hybrids were from families containing only one sampled individual. No full sibs were detected in the mapping population for a considerable number of these 625 individuals due to the substantially larger total population size of F_2 hybrids (sampled + un-sampled individuals), which were reared together in the experimental pond.

6. Selection of phenotypic traits for inclusion in the study

Here, we explain our rationale for selecting the specific phenotypic traits that we opted to include in our study. Focussing on a pair of species undergoing ecological speciation, our primary aim was to test the genetic architecture of niche divergence by genetically mapping relevant phenotypic traits in F_2 hybrids reared under near-natural conditions. Owing to the reasonably well-established ecological and historical context for niche divergence and speciation in the threespine stickleback species pair of Paxton Lake, and due to the availability of suitable genetic tools and genomic resources for *G. aculeatus*, we based our study on an (intercross) F_2 hybrid mapping population derived from wild Paxton benthic and limnetic F_0 adults. After discussing our reasons for choosing the included traits, we briefly report on the finding that the juvenile F_2 hybrids exhibited slight sexual dimorphism, overall, in the chosen traits.

Adaptive divergence in a whole-organism performance phenotype can involve morphological, behavioural, life history, and/or physiological component traits[108-114]. Any of these trait types can contribute to niche divergence, for example. In animals, one of the most generally important factors connecting component trait variation to how well-adapted individuals are to different niches is their performance in acquiring and consuming different food resources[1,2,5,115-119]. This is certainly true of the stickleback species pair in Paxton Lake, where feeding performance is one of the most important factors affecting the adaptation of benthics and limnetics to inshore and offshore areas of the lake (i.e., to littoral and shallow benthic versus pelagic habitats, respectively)[13,14,16,60,83,84,120]. Among all the different types of traits that have diverged between Paxton benthics and limnetics, morphological traits have been the most intensively studied. Many of the strongest known statistical relationships and/or mechanistic connections between any component traits and feeding performance variation in this species pair have been made for morphological traits. Accordingly, we focussed on morphology alone when deciding which candidate component traits of feeding niche divergence to include in our study.

Numerous morphological traits, including several aspects of body shape, differ significantly between wild Paxton benthics and limnetics[16-20,60,84,121-126]. We attempted to include the largest practical number of such traits, for which significant effects on habitat-specific limnetic-benthic feeding performance have been demonstrated or suggested. Many morphological traits have diverged in parallel in multiple benthic-limnetic species pairs[12,17,18,120,123,127], and the resulting phenotypic differences are

maintained in each species pair in the face of some on-going gene flow[16,20]. The trophic significance of phenotypic variation in some traits is reasonably well understood[16-19].

Reciprocal transplant experiments, carried out in Paxton Lake, have shown that overall divergence of morphological traits between Paxton benthics and limnetics leads to a twofold difference in feeding efficiency between preferred and non-preferred habitats[14,21,22]. Prior work has also established that general body shape and numerous specific morphological characters breed true for these fish in a common laboratory environment[16,122]. Moreover, heritable variation in this species pair has been demonstrated for some of these traits[122,128,129]. Studies of other threespine stickleback populations, including another benthic-limnetic stickleback species pair from a different lake, have also demonstrated heritable variation in several morphological traits thought to underlie trophic variation[66,130,131]. As described in Methods, we selected three classes of morphological traits on which to make phenotypic measurements and perform quantitative trait locus (QTL) mapping: functional morphological traits playing established roles in feeding; morphometric shape traits; and armour traits. We did not measure colour traits known to differ between Paxton benthics and limnetics, because these traits function primarily in sexual or agonistic signalling between breeding adults[132], or in crypsis and predator avoidance[133], rather than in feeding.

Of the three classes of morphological traits that we measured, we only tested the functional morphological traits and the shape traits for significant effects on trophic variation among the juvenile F_2 hybrids. The remaining class of armour traits comprised a small number of defensive characters (pelvic girdle, dorsal spines, and lateral plates), which we were able to rapidly screen alongside our more in-depth measurements of functional morphology and body shape. These armour traits have received a great deal of attention due to their importance in predator avoidance and adaptation of marine stickleback to freshwater environments[24,127,134-136]. Yet, no *a priori* evidence has elucidated specific roles of armour traits in feeding performance variation in the Paxton Lake species pair. Hence, we did not consider armour traits when selecting candidate morphological QTLs for the statistical models that were used to test the genetic basis of feeding niche divergence. We nevertheless performed QTL mapping on these traits (Supplementary Table 3), so that our results could be compared to findings on the genetic architecture of armour trait divergence from other crosses and populations[66,137-140].

Among all traits investigated to date in the Paxton Lake species pair, functional morphological traits generally exhibit the best-understood effects on trophic divergence between limnetics and benthics. In our study, this class of traits included total counts of two series of gill rakers (Fig. 2e)[141], stiff processes that project from the branchial arches and serve to limit the loss or escape of small ingested food particles from the buccal cavities of many different kinds of fish[142,143]. Compared to benthic threespine stickleback, limnetic threespine stickleback have longer and more numerous gill rakers, which are more closely spaced on the gill arches, thereby facilitating the retention of captured zooplankton[14,16,17,60].

Gill rakers were the first functional morphological traits to be identified as having important effects on trophic variation in threespine stickleback[135], and features of this prey-retention system remained the only well-documented functional morphological traits in the *G. aculeatus* species complex for quite some time. However, using a predictive model based on musculoskeletal morphology, McGee and Wainwright[18] recently quantified how Paxton benthics should be capable of generating greater negative suction pressures in their buccal cavities than Paxton limnetics. The model that was used by these authors describes suction feeding, a mode of prey capture that is important to feeding performance in many different fish groups[144]. An enhanced capacity for suction generation in Paxton benthics allows them to dislodge, and feed on, buried or attached macroinvertebrates in littoral and benthic areas of the

lake, their preferred foraging habitats[13,14,60]. Motivated by these recent findings, we included all five functional morphological traits used as predictor variables in the suction feeding index model[18] (Fig. 2f).

In a subsequent study, McGee *et al*.[19] further investigated the functional basis of feeding performance in the Paxton Lake species pair using biomechanical models for three additional prey capture systems: the lower jaw-opening lever (displacement advantage) system, the (upper) jaw protrusion system, and the opercular four-bar linkage (transmission coefficient) system. These authors used kinematic data from high-speed video recordings of feeding trials to test hypothesized effects of craniofacial morphology on feeding performance, which were predicted from the biomechanical models. Significant functional differences were found between benthics and limnetics for these three systems as well as the prey capture system involving suction feeding[19]. Among component morphological traits of these four mechanical systems, upper jaw protrusion length and lower jaw-opening inlever length were found to be two of the most divergent traits between Paxton benthics and limnetics[19]. Given this finding, as well as the general importance of these two oral jaw traits to feeding performance in a wide variety of ray-finned fishes[145-147], we included upper jaw protrusion length and lower jaw-opening inlever length in our functional morphological class of measured traits (Fig. 2g).

As shown by McGee *et al*.[19], upper jaw protrusion is significantly greater in Paxton limnetics than Paxton benthics, resulting in more efficient zooplanktivory by the limnetics. In addition, Paxton limnetics have significantly shorter lower jaw-opening inlevers than benthics, allowing the limnetics to rotate their lower jaws and open their mouths more rapidly than the benthics[19]. The more rapid strike speed of the limnetics, which results from this and other morphological specialisations, facilitates their predation on calanoid copepods (e.g., *S. oregonensis*), a key food resource for limnetics throughout much of the year[14,17,60]. Zooplanktivores like limnetic stickleback require relatively rapid predatory strikes and high jaw protrusion to efficiently capture calanoid copepods, because these microcrustaceans can perform quick escape manoeuvres, called 'jumps', in response to water displacements that are detected via their strain sensitivity antennae[148].

For the shape class of candidate component traits, we employed a geometric morphometric approach[102] to measure several body form features in the juvenile F_2 hybrids. Relative body depth, hydrodynamic streamlining, the sizes and arrangements of fins, and/or mouth position and orientation are thought to generally influence niche occupancy in fish via important habitat-dependent effects on swimming ability, feeding efficiency, and other whole-organism performance traits[149-151]. Parallel evolution of habitat-associated limnetic versus benthic body forms (shallower and more streamlined or deeper and more robust, respectively), in multiple lakes, suggests that these differences in overall body shape have resulted from divergent natural selection for enhanced performance in pelagic versus littoral/benthic habitats[15,84,120,127]. Though some features of stickleback body shape and fin morphology have been related to predator evasion abilities (e.g., fast-start swimming performance) or to swimming abilities in different habitats (e.g., manoeuvrability and sustained swimming performance)[121,152,153], we had very little *a priori* evidence to guide our choice of specific morphological landmarks for measuring 'component shape traits' of trophic divergence in a stickleback species-pair lake. We instead attempted to capture a large number of general body shape features using morphometric landmarks adapted from other threespine stickleback studies[28,61,97,124,125,152,154,155]. We omitted several previously used landmarks and redefined others (Extended Data Fig. 3) to achieve a good balance between: (1) maximal coverage of body regions (especially on the head) that seemed to be most variable among the F_2 hybrids, based on a review of digital images; and (2) use of only those landmarks that could be positioned rather reliably on images of stained juvenile stickleback.

Using the R-package 'leaps'[70], we performed exhaustive searches for the 'best' multiple linear regression models ($0 \leq \Delta AIC \leq 2$) of the relationships between niche score and subsets of morphological characters from each trait class (i.e., subsets from the nine functional morphological traits or the 38 x- and y- coordinates of 19 morphometric landmarks). Results of this model-selection procedure suggested that the 19 landmarks were rather effective in capturing much of the shape variation that underlies niche divergence in the Paxton Lake species pair. For each model considered, 'leaps' returned the standard multiple R^2, as well as Theil's[79] adjusted R^2 (also called McNemar's[156] adjusted R^2). Standard R^2 increases monotonically as more parameters (i.e., explanatory variables) are added to a given multiple regression model. To address this general property of the standard R^2, adjusted R^2 is penalized (i.e., reduced) according to the number of parameters modelled compared to the number of observations used for model fitting[79,156]. Among the best functional morphological trait models predicting niche score, adjusted R^2 ranged from 13.7% to 14.0% (Supplementary Table 1). In contrast, adjusted R^2 ranged from 27.8% to 28.1% among the best shape-trait models predicting niche score (Supplementary Table 2). We cannot conclude from these results that shape variation accounted for approximately twice as much of the total niche score variation as did functional morphology. Despite not being able to quantitatively compare the different trait-class models this way, adjusted R^2 is considered to be a sound goodness-of-fit metric for assessing and contrasting regression equations fitted to different data sets using different numbers of explanatory variables[157]. Thus, whereas the advantage of functional morphological traits was clear from their *a priori* mechanistic connections to niche use, outcomes of the above model comparisons underscore the value of also including body shape traits when investigating the component morphological basis of niche divergence between benthic and limnetic sticklebacks. Though the morphometric landmarks were not as well informed by specific *a priori* hypotheses regarding trophic function, our finding of high adjusted R^2 values for the best shape-trait models (*vis-à-vis* the best functional-trait models) implies that geometric morphometrics also captures a substantial portion of the overall component morphology of benthic-limnetic niche divergence in Paxton Lake.

Although adult threespine stickleback exhibit moderate to strong sexual dimorphism in many morphological traits[61,106,141], we found that the extent of sexual dimorphism in the juvenile F_2 hybrids of our mapping population was weak or nearly absent in the traits we investigated. Thus, we did not include sex as a covariate in any of our statistical analyses. In an ontogenetic study of morphometric and meristic variation in several stickleback populations, Kitano *et al.*[158] similarly found a lack of sexual dimorphism in juveniles prior to maturation. Even though we did not use sex as a covariate in any of our analyses, we found that none of the measured morphological traits mapped to linkage group (LG) 19 (Extended Data Fig. 3; Supplementary Table 3). Linkage group 19 corresponds to chromosome XIX[29], the nascent sex chromosome in threespine stickleback, which contains the master sex determination locus[159]. We might have expected to find one or more QTLs on LG 19, rather than none at all (Supplementary Table 3), had there been moderate to strong juvenile sexual dimorphism in some of the measured traits.

7. Removing the 'specimen bending artefact' during shape analysis

The fixation and preservation of fish typically causes a 'specimen bending artefact', represented by a U-shaped displacement of morphometric landmarks that is generally captured by one of the eigenvectors from a PCA of Procrustes-superimposed landmarks[28,61,65,102]. Using the function 'shapepca' in the R package 'shapes'[64], we plotted vectors beginning at the mean position of each of the 19 superimposed landmarks and ending +3 standard deviations along each PC axis. Plots like these were constructed for the first six PC axes recovered from a principal components analysis of superimposed landmarks for all F_2 hybrids sampled from pond no. 4. The resulting plot for the first PC axis (PC1)

revealed upward pointing vectors for landmarks near the tail and head of each fish (e.g., landmark 1 and landmarks 11–15) and downward pointing vectors near the middle of the body (e.g., landmarks 2–7 and landmark 19; see Extended Data Fig. 3 for landmark positions), reflecting an average U-shaped deformation of the preserved specimens. Similar plots for the other PC axes did not reveal any U-shaped displacements of the landmarks. Instead, the other PC axes appeared to represent different aspects of biologically meaningful shape variation. Rather than being associated with natural shape variation, the first eigenvector therefore appeared to represent the common 'specimen bending artefact', which resulted from our fixation of specimens in 7.5% formalin and their subsequent storage in 40% isopropyl alcohol. This eigenvector (PC1) accounted for 23.72% of the total variation in superimposed landmark coordinates. To remove the effect of specimen bending, we transformed the landmark coordinates to their principal components, deleted the first eigenvector and eigenvalue, and then performed an inverse transformation to reconstruct 'unbent' landmark coordinates. Following the approach taken in other studies of threespine stickleback shape variation[28,61,152], we then treated the resulting superimposed and 'un-bent' landmark coordinates as individual shape traits in subsequent analyses.

8. Patterns of body shape variation among the juvenile F_2 hybrids

We begin this supplementary discussion section by explaining a data visualisation technique that we employed to better understand and characterise juvenile shape variation in the F_2 hybrid mapping population in relation to trophic variation. We then discuss the most noteworthy patterns of shape variation, which were revealed by using this using technique in conjunction with reviewing digital photos of the F_2 hybrids and considering measured functional trait variation in the same individuals. Our morphometric data suggest several key features of F_2 body shape that seemed to show coherent variation across the stable isotope landscape towards benthic-like or limnetic-like forms. Importantly, for each body shape feature in the hybrids that could be related to known patterns of shape variation between parental forms, the corresponding benthic–limnetic shape-shift axis in the hybrids proved to be closely aligned with the niche score axis. For these features of shape, detailed below, the 'B' and 'L' hybrids tended to somewhat resemble pure Paxton benthics and limnetics, respectively.

A straightforward way to explore, understand, and illustrate patterns of body shape variation across the bivariate isotope landscape was to simultaneously compare F_2 hybrids among regions 'L', 'B', and 'A' (Fig. 1a; Supplementary Discussion Section 3). Our technique for visualising body shape variation among these landscape regions started with the 19 Procrustes-superimposed and 'un-bent' morphometric landmarks[63,102] (Methods; Supplementary Discussion Section 7; also see the inset key in Extended Data Fig. 3). The resulting 38 x- and y-coordinates were then used as individual variables in pairwise discriminant function analyses (DFAs) of F_2 hybrids from each of two partitions of the dataset, per DFA. We performed pairwise DFA using MORPHOJ ver. 1.04a[160]. In each analysis, all F_2 hybrids from one of the delineated landscape regions ('L', 'B', or 'A') composed the focal data partition, containing n = 91–93 individuals per region with complete morphometric and isotope data. This focal group was analysed together with a second data partition, consisting of all individuals with complete data (n = 335) that fell outside the three focal regions. An overall 'reference shape' was derived from this larger data partition in each DFA, allowing the average shape of each focal group to be compared to a standard reference. In turn, this approach allowed us to draw inferences on shape variation among all three focal groups simultaneously. To accomplish this, we again used MORPHOJ to produce and overlay two wireframe diagrams (per pairwise comparison) based on resulting discriminant function (DF) scores for the two groups considered in each DFA. Each resulting pair of wireframes represented the mean shape of individuals in one of the three focal groups in relation to the mean reference shape, after

amplifying the between-group shape difference by a specified factor. An amplification factor of eightfold was used for Extended Data Fig. 4, for example. We only used DF scores to visualise shape variation in the manner described above; DF scores were not used for generating shape traits, identifying component traits of niche use, or QTL mapping.

Based on results of these wireframe renderings, we found that average head shape of the F_2 hybrids in region 'L' was noticeably limnetic-like in several respects, albeit only moderately so. The average position of the orbit (i.e., eye socket) in these individuals was ventrally shifted on the head with respect to the 'B' individuals (Extended Data Fig. 4). This difference was apparent in the shorter vertical separation (i.e., distance between y-coordinates) of landmark 11 (or 12) and landmark 17 in the 'L' individuals, on average. Similarly, the overall size of the orbit, which was captured in the relative positions of landmarks 15–17, was slightly larger in the 'L' individuals than the 'B' individuals. These differences in eye position and size between 'L' and 'B' appeared to be in the same direction as those observed between wild limnetics and benthics from Paxton Lake. According to McPhail's classic illustration of the species pair[16], for example, the limnetic species exhibits a smaller vertical distance than the benthic species between the ventral margin of the orbit (landmark 17 in our study) and the corner of the mouth (landmark 12 essentially). McPhail's illustration also depicts a smaller vertical separation in the limnetic species between landmark 17 and the anterior-most extent of the preopercle along the ventral silhouette (landmark 11). In addition, both McPhail[16] and Matthews *et al*.[17] provide data implying that Paxton limnetics have a larger average eye width (relative to head length) than Paxton benthics. Our results suggest that such a pattern was recapitulated among the F_2 hybrids in pond no. 4, to some extent, along the niche score axis. Interestingly, however, threespine stickleback populations of the Cook Inlet Basin (Alaska)[155] showed a pattern of orbit size variation that differs from the findings reported for benthic-limnetic species-pair lakes in British Columbia[16,17,123,161]. Solitary-lake populations in the Cook Inlet Basin with more benthic-like head shapes exhibited larger residual mean eye sizes (from linear regression of vertical eye diameter against within-population mean centroid size of the skull) than solitary limnetic-like populations in the same region of Alaska[155].

In the present study, F_2 hybrids in region 'L' also exhibited a larger average distance between the x-coordinates of landmarks 13 and 15 than hybrids from other regions of trophic space. A review of digital images of the F_2 hybrids hinted that, overall, 'L' individuals also tended to have a more elongated ventral head profile rostrally (i.e., along their lower 'jaw lines') than other individuals in the F_2 mapping population. Upon investigating this suggested difference more formally, we found that, on average, the ventral silhouette of the head in lateral view was elongated in 'L' individuals between the rotation point of the lower jaw — i.e., the point of articulation between the quadrate and articular bones[19,127] (near landmark 11) — and the rostral tip of the closed lower jaw (near landmark 13; Extended Data Fig. 4). This extended 'jaw line' seen in many 'L' individuals was associated with a large average shift (both dorsally and anteriorly) in the relative position of landmark 13 in this F_2 hybrid group, compared to other individuals in the mapping population. In fact, landmark 13 exhibited a greater average shift in relative position on the head, with respect to variation across isotope space, than any other landmark included in our study. This was evident in the large oblique shift of landmark 13 away from the centre of the body form (i.e., centroid) of 'L' individuals, on average, compared both to other landmarks and to other F_2 hybrids from different regions of trophic space (Extended Data Fig. 4).

Many morphological trait differences between Paxton benthic and limnetic sticklebacks breed true in a common laboratory environment[16,122], yet phenotypic plasticity also contributes to variation in a number of traits in this species pair[128]. Phenotypic plasticity has been further documented in several other threespine stickleback populations. When subjected to experimental diet manipulations, for example,

individuals from Alaskan populations exhibited developmental plasticity in several aspects of body shape, including position of the upper lip's anterior tip[97,154], which is located extremely close to our landmark 13. A diet consisting of limnetic prey caused the Alaskan sticklebacks to shift the anterior tip of their upper lip in a direction that was similar to the average shift seen in landmark 13 in 'L' individuals relative to the rest of the mapping population (Extended Data Fig. 4). Intriguingly, over all morphometric landmarks, we found that the x- and y-coordinates of landmark 13 exhibited among the best-supported statistical associations with niche score. Both coordinates (x13 and y13) were present in the overall-best model (i.e., minimum-AIC model) predicting niche score from body shape coordinates. Both of the landmark 13 coordinates were also included as explanatory variables in all other, essentially equally well-supported models ($\Delta AIC \leq 2$; Supplementary Table 2). Accordingly, landmark 13 — anterodorsal extent of the maxilla — appears to be a strong candidate for having acted as one of the many important component traits contributing to niche use and whole-organism feeding performance in the Paxton Lake species pair.

In spite of the strong statistical association between landmark 13 and niche score, we found no QTLs for either x13 or y13 (Extended Data Fig. 3; Supplementary Table 3). This lack of QTLs for landmark 13 is consistent, both with an underlying genetic architecture of divergence in this trait possibly involving multiple genes with effects too small to be detected[162], and with the potential for diet-induced plasticity to produce phenotypic variation in this trait. Given that developmental plasticity has been observed for several morphological characters in a number of threespine stickleback populations[97,128,154], and that opportunity for diet-inducted plasticity certainly occurred in pond no. 4 (Supplementary Discussion Section 3; Extended Data Fig. 1), phenotypic plasticity should be considered an important candidate cause of phenotypic variation among the F_2 hybrids in our study. Phenotypic plasticity is especially important to consider for traits that did not map to any QTLs but were nevertheless strongly associated with niche score, like the Cartesian coordinates of landmark 13. As with other whole-organism performance phenotypes, divergence in the component morphology of niche use in threespine stickleback almost certainly reflects the joint action of heritable genetic effects and phenotypic plasticity. In this regard, it may prove quite interesting to carry out additional investigations that help to: (1) quantify the potential for diet-induced plasticity in landmark 13, specifically in Paxton limnetics, benthics, and their hybrids; and (2) determine the extent to which phenotypic plasticity of landmark 13 affects feeding performance and diet in this species pair.

In the Paxton Lake stickleback system, the genetic architecture of trophic performance (described at length in the main paper) and diet-associated phenotypic variation in the relative position of landmark 13 (Extended Data Fig. 4) suggest a hypothesized process by which the interaction of genotypes underlying certain component traits and phenotypic plasticity in other component traits could accelerate niche divergence. Our main findings illustrate how phenotypic variation in component traits due to underlying genetic factors influence an emergent whole-organism performance phenotype, such as niche use. The specific environmental conditions that individuals in a population find most profitable should often depend on phenotypic variation in whole-organism niche use, which in turn depends on genotypes underlying the associated component traits. In animals, individuals are often able to seek out profitable microenvironments or specific resources within a habitat[115,117] (e.g., preferred foods), producing a potential effect of underlying genotypes on the environmental conditions that are most often experienced during an individual's own development and growth. More broadly, genotypes underlying whole-organism performance in parents will affect which environmental conditions prove most favourable for offspring growth, survival, and/or reproduction. As individuals and their offspring begin exploiting a novel habitat, the new environmental conditions of that habitat can induce new phenotypic variation in plastic traits that may not be directly affected by the genetic architecture of divergence in whole-organism

performance. If divergence in a plastic trait of this kind effectively has no underlying genetic basis, yet the trait is also an important component trait and the induced phenotypically-plastic variation is also directed toward the performance optimum in the new habitat, such a trait may 'boost' performance (and likely fitness) beyond that predicted from the genotypes of all other component traits. As a consequence, adaptation to new habitats and/or niche divergence may proceed more rapidly when such plastic 'booster traits' contribute to an individual's whole-organism performance phenotype in a coherent direction with respect to selection on the genetically-based component trait variation. Owing to the lack of QTLs detected for landmark 13 in the present study, and the finding from other stickleback studies of coherent diet-induced plasticity in the anterior tip of the upper lip[97,154], we hypothesize that ecological divergence between Paxton benthics and limnetics potentially could have been facilitated by this sort of interaction between the genetic architecture of niche use and one or more 'booster traits'. We further speculate that the relative position of landmark 13 with respect to other landmarks on the head could potentially be an example of such a phenotypically plastic booster trait. Additional research is needed to test these hypotheses.

Among the most distinctive hallmarks of body shape divergence in the Paxton Lake species pair are the large overall body depth and prominent dorsal hump (immediately posterior to the neurocranium) that characterise the adult benthic form[16,84]. The dorsal hump contributes directly to the elevated capacity that benthics have for suction generation in comparison to limnetics[18,19]. In turn, greater suction forces during feeding, which Paxton benthic stickleback are predicted to be capable of generating, are thought to enhance foraging efficiency in littoral and shallow benthic habitats[14,21,22]. Mean body depth was greatest in the F_2 hybrids of region 'B', as illustrated by the large distance between y5 and y18 in the wireframe diagram for this group of hybrids compared to hybrids from other regions of trophic space (Extended Data Fig. 4). Upon examining digital images of fish specimens collected from pond no. 4, we identified a number of F_2 hybrids that appeared to have a distinctive dorsal hump, and most of these individuals belonged to landscape region 'B'. Unfortunately, the relative height of the dorsal hump was not particularly well captured by our chosen morphometric landmarks, which were based, wherever possible, on homologous bony structures that could be identified reliably in images of stained juveniles. Landmarks like these are generally not well suited to detecting variation in soft, fleshy morphological characters[102]. However, height of the dorsal hump is directly related to anterior epaxial muscle height, which we did measure (Fig. 2f). Indeed, height of the anterior epaxial muscle was greater in 'B' individuals, on average, than any other group of F_2 hybrids (Extended Data Fig. 5b). Moreover, anterior epaxial muscle height was included as an explanatory variable in three of nine well-supported models predicting niche score from F_2 hybrid functional morphology ($0 \leq \Delta AIC \leq 2$; Supplementary Table 1). In addition, we mapped anterior epaxial muscle height to a QTL on LG 14 (Supplementary Table 3).

Use of the data visualisation technique also suggested that the average snout shape of F_2 hybrids in landscape region 'A' was somewhat more similar to individuals in region 'B' than individuals in region 'L' (Extended Data Fig. 4). Conversely, relative eye position of the 'A' individuals appeared to be somewhat more similar to that of 'L' individuals than 'B' individuals (and slightly more similar to that of wild limnetics than wild benthics[16]). Based on body length (i.e., the Euclidean distance between landmarks 1 and 13), the individuals in region 'A' of trophic space were the smallest F_2 hybrids in the mapping population (Fig. 1a). The small body sizes of these juvenile F_2 hybrids, overall, were also apparent in the small values that the 'A' individuals exhibited for functional morphological traits measured as linear distances in and around the head (Fig. 2f). Examples of these traits include neurocranium outlever length (Extended Data Fig. 5d), buccal cavity length (Extended Data Fig. 5e), and gape (Extended Data Fig. 5f). The wireframe diagram for 'A' further suggested that the 'A' individuals

tended to be characterised, in particular, by slightly smaller relative head sizes (as scaled by centroid size for all landmarks) than other F_2 hybrids in the mapping population. In the F_2 hybrids of landscape region 'A', the average positions of many morphometric landmarks on the anterior region of the head generally appeared to be contracted toward the centre of the body form (i.e., centroid) to a slightly greater extent, perhaps, than landmarks located elsewhere on the body (Extended Data Fig. 4).

Lastly, we used the data visualisation technique described above to examine average body shape of the 15% of F_2 hybrids that were located closest to the bivariate mode of the stable isotope distribution. Relative to the bivariate mean of the distribution, the $\delta^{13}C$-$\delta^{15}N$ mode was slightly skewed toward landscape region 'B' (Fig. 1a). The geometry of the experimental pond (Extended Data Fig. 1) potentially contributed to this slight skew in the stable isotope data, as the ratio of littoral/benthic to pelagic habitat in pond no. 4 likely supported a higher abundance of benthos and littoral food resources for threespine stickleback, compared to zooplankton and other pelagic food resources for threespine stickleback. Despite the slight skew in isotopes, the modal F_2 hybrids nevertheless exhibited fairly intermediate trophic signatures of past diet (e.g., niche score and diet deviation score; Fig. 1, Extended Data Fig. 2) in relation to the overall mapping population in pond no. 4. In addition, we found that F_2 hybrids near the $\delta^{13}C$-$\delta^{15}N$ mode exhibited an intermediate average body shape (results not shown) relative to F_2 hybrids from each of the three isotopically extreme regions ('B', 'L', or 'A'). Not surprisingly, the modal F_2 hybrids also tended to have intermediate phenotypic values for functional morphological traits (Extended Data Fig. 6). In contrast to this centrally located landscape region around the $\delta^{13}C$-$\delta^{15}N$ mode, regions 'B', 'L', and 'A' represented extreme 'corners' of a roughly triangle-shaped isotope distribution. As we have shown in detail above, F_2 hybrids from regions 'B', 'L', and 'A' tended to exhibit more extreme features of body shape compared to all other F_2 hybrids in the mapping population.

9. Testing for QTL clustering in the *Gasterosteus aculeatus* genome

We were interested in testing whether QTLs were non-randomly distributed either (1) among or (2) within linkage LGs, the numbering of which matches that used for the 21 chromosomes in the threespine stickleback's nuclear genome[29,163] (Supplementary Table 4). To accomplish the first aim, we conducted goodness-of-fit tests on the observed numbers of QTLs per LG (either all QTLs or just those for the morphological 'component traits' of niche use). These tests were performed under a null hypothesis of randomly distributed QTLs, according to a simple proportional model for the expected number of QTLs on each LG. Expectations for the tests were based on either the relative physical sizes of the corresponding chromosomes or the number of genes reported for each chromosome (Extended Data Table 1). Goodness of fit was evaluated using Pearson's chi-squared test, performed by means of the R function 'chisq.test'[51]. Given the small expected cell values in the contingency tables, we computed the *P*-value for each test by Monte-Carlo simulation (10,000 replicates per test). No matter how the test was constructed, the outcome favoured the alternate hypothesis that QTLs were non-randomly distributed in the genome: $\chi^2_{20} = 45.17$, $P = 0.0016$ (for all traits, with an expectation based on chromosome size); $\chi^2_{20} = 55.76$, $P = 0.0002$ (all traits, based on gene number); $\chi^2_{20} = 34.87$, $P = 0.0219$ ('component traits', based on chromosome size); and $\chi^2_{20} = 39.12$, $P = 0.0083$ ('component traits', based on gene number). Under each of these four test constructions, we also computed standardised residuals[164] ('stdres') as follows for each LG:

$$\text{stdres} = (\text{observed count} - \text{expected count}) / (\text{resvar})^{-½},$$

where 'resvar' is the residual cell variance[164]. Based on stdres, we identified which LGs contained more or fewer QTLs than expected by chance. Across groups, stdres values roughly sum up to the χ^2 value of

the corresponding goodness-of-fit test. Thus, stdres for each LG can be thought of as an approximate z-score from a standard normal distribution for that linkage group. Following Agresti[164], we considered extreme values of stdres (i.e., stdres $\geq +2$ or stdres ≤ -2) to be significant at $\alpha = 0.05$. It was thereby revealed that significantly more QTLs than expected for component traits occurred on LGs 16 and 20, and that significantly more QTLs than expected for all traits occurred on LGs 4 and 16 (Extended Data Table 1).

To accomplish the second aim (testing whether QTLs were non-randomly distributed within LGs), we compared the vector of all QTL peak positions within each linkage group (i.e., 'Position' in cM in Supplementary Table 3) to the uniform probability distribution function, using a one-sample Kolmogorov-Smirnov test. Such an approach tests the null hypothesis that each detected QTL on a LG occurred with equal probability at any recombination distance along that LG. Kolmogorov-Smirnov tests for the different LGs were performed using the R function 'ks.test'[51]. Outcomes of these tests for LGs 4 and 16 favoured the alternate hypothesis of a deviation from randomly distributed QTLs along the respective LGs, indicating either clustering or overdispersion of QTLs. For LG 4, D = 0.4446 and two-sided $P = 0.00734$; for LG 16, D = 0.5418 and two-sided $P = 0.00531$. In both cases, it was readily apparent that QTLs were clustered on the LGs rather than overdispersed (Extended Data Fig. 3; Supplementary Table 3).

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