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On Irish sticklebacks: morphological diversification in a secondary contact zone

APPENDIX

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APPENDIX

S1: Number and frequencies of haplotypes with assigned lineages

		Number				Frequency			
Number	Code	IR	EU	TA	Total	IR	EU	TA	Assigned
1	AIB	0	7	0	7	0.00	1.00	0.00	EU
2	ANN	0	7	0	7	0.00	1.00	0.00	EU
3	BAN	7	0	0	7	1.00	0.00	0.00	IR
4	BON	4	3	0	7	0.57	0.43	0.00	Admixed
5	BUR	7	0	0	7	1.00	0.00	0.00	IR
6	BWL	0	8	0	8	0.00	1.00	0.00	EU
7	CAM	0	10	0	10	0.00	1.00	0.00	EU
8	CCO	1	0	0	1	1.00	0.00	0.00	IR
9	CRN	0	4	4	8	0.00	0.50	0.50	Admixed
10	CUR	10	0	0	10	1.00	0.00	0.00	IR
11	DER	0	0	3	3	0.00	0.00	1.00	TA
12	DRG	0	8	0	8	0.00	1.00	0.00	EU
13	FEE	0	7	0	7	0.00	1.00	0.00	EU
14	FER	0	4	0	4	0.00	1.00	0.00	EU
16	FUR	0	10	0	10	0.00	1.00	0.00	EU
17	GCR	0	7	0	7	0.00	1.00	0.00	EU
18	GIL	0	7	0	7	0.00	1.00	0.00	EU
19	GLC	1	6	0	7	0.14	0.86	0.00	EU
20	GLN	0	2	0	2	0.00	1.00	0.00	EU
21	GMO	0	6	0	6	0.00	1.00	0.00	EU
22	LEN	7	0	1	8	0.88	0.00	0.13	IR
23	LIL	3	4	1	8	0.38	0.50	0.13	Admixed
25	LWR	4	2	0	6	0.67	0.33	0.00	IR
28	NAM	0	0	8	8	0.00	0.00	1.00	TA
30	NWR	0	5	1	6	0.00	0.83	0.17	EU
29	NAO	7	1	0	8	0.88	0.13	0.00	TA
31	ROB	0	5	1	6	0.00	0.83	0.17	EU
32	SFD	0	4	6	10	0.00	0.40	0.60	Admixed
33	SWI	1	1	6	8	0.13	0.13	0.75	TA
34	TAC	0	4	4	8	0.00	0.50	0.50	Admixed

35	TAL	12	1	0	13	0.92	0.08	0.00	IR
36	TUL	6	2	0	8	0.75	0.25	0.00	IR
37	TYS	0	4	1	5	0.00	0.80	0.20	EU
38	UBD	0	0	4	4	0.00	0.00	1.00	TA

S2: Amplification protocols for microsatellite and mitochondrial markers

Mitochondrial markers, *cytochrome B* (forward 5' ATGAAACTTTGGTTCCCCTCC 3', reverse 5' CGCTGAGCTACTTTTGCATGT 3') and a partial *control region* sequence (forward 5' CCTTTAGTCCTATAATGCATG 3', reverse 5' CCGTAGCCCATTAGAAAGAA 3', were taken from Mäkinen and Merilä (2008), although it should be noted that we did not use the nested primer method they applied. For both primer sets, PCR was carried out in 50 μl volume as follows; 2.5 mM MgCl2, 1 x PCR Buffer (Invitrogen), 200 µM dNTPs, 1 μl of each primer (10 picomoles), 2U *Taq* polymerase (Invitrogen) and approximately 20 ng stock DNA. For *cyt B*, thermocycling conditions consisted of one denaturation step at 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 30 s at 62°C, 60 s at 72°C, and then a final extension step at 72°C for 45 min. Similar conditions were used for control region, although the annealing temperature was set to 52°C. Products were purified and then sequenced using the Big Dye v3.1 cycle sequencing kit (Applied Biosystems Inc) prior to analysis on a 96 capillary ABI 3730xl DNA Analyzer (Applied Biosytems Inc). Sequence base calls were obtained using SEQUENCE ANALYZER v5.4 (Applied Biosystems Inc) and were manually checked, edited and aligned using ChromasPro v1.42.

The nine microsatellite loci (from Lagardier et al (1999) and Peichel et al (2001); see table below) were amplified in two multiplex reactions after Kalbe *et al*. (2009). PCR amplification was carried out using Top-Bio PPP mastermix (Top Bio, Czech Republic); total reaction volume was 3.5 μl with 1.5 μl mastermix, 1 μl template DNA (1-5 ng), and 0.035 μl (10 pM) of each primer with the remainder volume made up with ddH_20. Identical thermocycler conditions were used for both multiplexes, 110°C heated lid, denaturation at 95°C for 15 min and then 20 cycles of 95°C for 30 s, 57°C for 1.5 min and 72°C for 1.5 min, with a final extension of 60°C for 30 min. Fragment analysis was then performed on a 96 capillary 3730xl DNA Analyzer (Applied Biosystems Inc). Raw fragment profiles for each individual were then manually genotyped using GENEMAPPER v4.1 (Applied Biosystems Inc).

Locus	Dye
Multiplex A	
Gac5196	VIC
Gac4170	FAM
Gac1125	FAM
Gac1097	NED
Gac7033	NED
Multiplex B	
STN18	VIC
STN32	FAM
STN75	VIC
STN84	NED

Kalbe, M., Eizaguirre, C., Dankert, I., Reusch, T. B. H., Sommerfield, R. D., Wegner, K. M. and Milinski, M. 2009. Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. *Proceedings of the Royal Society B*, **276**: 925–934.

Largiader, C. R., Fries, V., Kobler, B. and Bakker, T. C. M. 1999. Isolation and characterization of microsatellite loci from the three-spined stickleback (Gasterosteus aculeatus L.). *Mol Ecol*, **8**: 342–344.

Mäkinen, H. S. and Merilä, J. 2008. Mitochondrial DNA phylogeography of the three-spined stickleback (*Gasterosteus aculeatus*) in Europe - Evidence for multiple glacial refugia. *Molecular Phylogenetics and Evolution*, **46**: 167–182.

Peichel, C. L., Nereng, K. S., Ohgi, K. A., Cole, B. L. E., Colosimo, P. F., Buerkle, C. A., Schluter, D. and Kingsley, D. M. 2001. The genetic architecture of divergence between threespine stickleback species. *Nature*, **414**: 901–904.

S3: Univariate GLMs on shape variation within habitat classes, using population as a grouping variable.

	R^2	d.f.	F	P-value
PC1				
Lake	0.51	13, 478	40.76	< 0.0001
River	0.25	14, 311	8.67	< 0.0001
Marine	0.41	9, 164	14.21	< 0.0001
PC2				
Lake	0.36	13, 478	22.29	< 0.0001
River	0.32	14, 311	12.15	< 0.0001
Marine	0.34	9, 164	11.09	< 0.0001

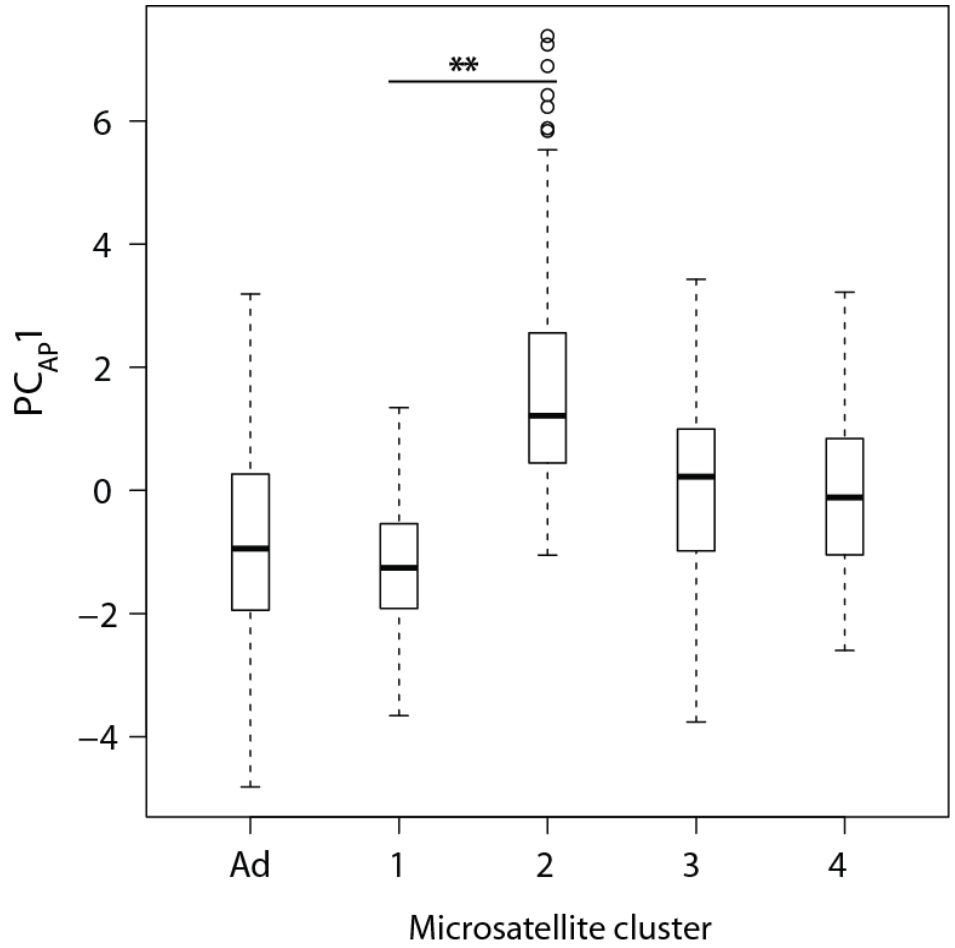
S4: Univariate GLMs on anti-predator and measured body trait variation within habitat classes using population as a grouping variable

	R^2	d.f.	F	P-value
$PC_{AP}1$				
Lake	0.62	13, 404	52.64	< 0.0001
River	0.67	14, 169	27.72	< 0.0001
Marine	0.6	9, 139	25.27	< 0.0001
$PC_{TRAIT}1$				
Lake	0.39	13, 404	21.35	< 0.0001
River	0.25	14, 169	5.307	< 0.0001
Marine	0.33	9, 139	9.03	< 0.0001
$PC_{TRAIT}2$				
Lake	0.23	13, 404	10.41	< 0.0001
River	0.19	14, 169	4.03	< 0.0001
Marine	0.51	9, 139	18.36	< 0.0001
$PC_{TRAIT}3$				
Lake	0.39	13, 404	21.18	< 0.0001
River	0.19	14, 169	4.07	< 0.0001
Marine	0.17	9, 139	4.25	< 0.0001

S5: Correlation tests between lineage-specific haplotype frequencies and mean population values for body shape ($PC_{SHAPE}1$), antipredator traits ($PC_{AP}1$) and measured traits ($PC_{TRAIT}1$)

Haplotype frequency	$PC_{SHAPE}1$	$PC_{AP}1$	$PC_{TRAIT}1$
Irish	r = -0.06, P = 0.73	r = -0.30, P = 0.09	r = -0.03, P = 0.88
European	r = 0.10, P = 0.57	r = 0.21, P = 0.25	r = -0.06, P = 0.73
Transatlantic	r = -0.06, P = 0.74	r = 0.10, P = 0.59	r = 0.11, P = 0.53

S6: Boxplot indicating differences in $PC_{AP}1$ values between inferred microsatellite clusters (Stars indicate significance of P < 0.001.)



S7: Canonical variate loadings for axes of shared divergence between marine and freshwater habitats

Traits	Anti-predator CV	Measured traits CV
1st dorsal spine	0.93	-
2nd dorsal spine	0.94	-
Pelvic spine	0.98	-
Body depth	-	0.66
Head length	-	0.08
Jaw length	-	0.21
Head depth	-	0.60
Dorsal length	-	-0.08
Caudal length	-	-0.17
Caudal depth	-	0.75
Eye diameter	-	-0.62