

Table S1. Collection locations for the parents used to generate offspring for the common garden study.

Species	Drainage	Latitude	Longitude
Strawberry darter	Strawberry River	36.304214	-91.927684
Current darter	Spring River	36.250560	-91.359318
Brook darter	Black River	37.146415	-90.907459
Orangethroat darter	Vermillion River	40.089035	-88.143440

Table S2. Collection locations for the male fish used in the ACD colour pattern study.

Species	Geography	Drainage	Latitude	Longitude
Orangethroat darter	Allopatric	Sangamon River	40.027663	-88.577180
	Sympatric	Vermillion River	40.089035	-88.143440
Rainbow darter	Sympatric	Vermillion River	40.089035	-88.143440
	Allopatric	Kalamazoo River	42.426825	-85.428370

Table S3. Common garden study results from of two-factor nested ANOVAs on each colour variable with species and family (nested within species) included as factors. R = red colouration, B = blue colouration, r = R value, g = G value, b =B value, Prop = proportion, DF = dorsal fin.

R_r			
	Df	F	P
family	1	8.80	<0.01
species	3	60.67	<0.00001
family*species	3	3.38	<0.05
Residuals	63		
R_g			
	Df	F	P
family	1	6.66	<0.05
species	3	47.16	<0.00001
family*species	3	3.34	<0.05
Residuals	63		
R_b			
	Df	F	P
family	1	5.96	<0.05
species	3	13.83	<0.00001
family*species	3	6.36	<0.001
Residuals	63		
B_r			
	Df	F	P
family	1	6.77	<0.05
species	3	7.90	<0.001
family*species	3	1.63	0.19
Residuals	63		
B_g			
	Df	F	P
family	1	0.98	0.33
species	3	8.51	<0.0001
family*species	3	1.99	0.12
Residuals	63		

B_b	Df	F	P
family	1	1.30	0.26
species	3	9.59	<0.0001
family*species	3	2.21	0.1
Residuals	63		

DF1_Prop_B	Df	F	P
family	1	11.45	<0.01
species	3	43.07	<0.0001
family*species	3	1.29	0.29
Residuals	63		

DF1_Prop_R	Df	F	P
family	1	0.44	0.51
species	3	28.49	<0.0001
family*species	3	0.47	0.7
Residuals	63		

DF2_Prop_B	Df	F	P
family	1	8.38	<0.01
species	3	1.63	0.19
family*species	3	0.47	0.71
Residuals	63	3.00	

DF2_Prop_R	Df	F	P
family	1	0.48	0.49
species	3	23.13	<0.0001
family*species	3	3.44	<0.05
Residuals	63		

Body_Prop_B	Df	F	P
family	1	27.49	<0.0001
species	3	22.93	<0.0001
family*species	3	1.62	0.2

Residuals	63
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Body_Prop_R

	Df	F	P
family	1	7.80	<0.01
species	3	24.10	<0.0001
family*species	3	0.82	0.49
Residuals	63		

Table S4. Pairwise post-hoc Tukey's tests comparing scores for (A) LD1 and (B) LD2 from LDA on colour pattern in fish from the common garden study. This analysis included four allopatric species in the orangethroat darter clade (i.e., orangethroat, strawberry, current, and brook darters).

(A) LD1					
Species 1	Species 2	Estimate	Std. Error	t-value	P
Strawberry	Brook	8.33	0.95	8.74	<0.001
Orangethroat	Brook	0.21	0.96	0.22	0.996
Current	Brook	5.56	0.98	5.66	<0.001
Orangethroat	Strawberry	-8.11	0.98	-8.25	<0.001
Current	Strawberry	-2.76	1.01	-2.75	0.038
Current	Orangethroat	5.35	1.01	5.29	<0.001
(B) LD2					
Species 1	Species 2	Estimate	Std. Error	t-value	P
Strawberry	Brook	0.74	0.93	0.80	0.855
Orangethroat	Brook	-3.18	0.93	-3.41	<0.01
Current	Brook	-5.01	0.95	-5.25	< 0.001
Orangethroat	Strawberry	-3.92	0.96	-4.10	< 0.001
Current	Strawberry	-5.75	0.98	-5.89	< 0.001
Current	Orangethroat	-1.83	0.98	-1.86	0.254

Table S5. Results of ANOVAs comparing scores for (A) LD1 and (B) LD2 from LDA on male colour pattern in wild-caught and lab-reared fish from four allopatric species in the orangethroat darter clade (i.e., orangethroat, strawberry, current, and brook darters). Species and rearing environment (i.e., lab-raised or wild-caught; nested within species) were factors in these analyses.

(A) LD1			
Factor	Df	F	P
Species	3, 103	122.96	<0.000001
Environment	1, 103	1.51	0.222
Species* Environment	3, 103	1.62	0.190
(B) LD2			
Factor	Df	F	P
Species	3, 103	117.98	<0.000001
Environment	1, 103	0.42	0.520
Species* Environment	3, 103	1.61	0.192

Table S6. ACD study results from of two-factor nested ANOVAs on each colour variable with species and geography (nested within species) included as factors. R = red colouration, B = blue colouration, r = R value, g = G value, b =B value, Prop = proportion, DF = dorsal fin, AF = anal fin, CF = caudal fin.

R_r			
	Df	F	P
geography	1	4.19	<0.05
species	1	1.21	0.28
geography*species	1	0.29	0.59
Residuals	36		
R_g			
	Df	F	P
geography	1	0.09	0.77
species	1	0.18	0.68
geography*species	1	2.27	<0.01
Residuals	36		
R_b			
	Df	F	P
geography	1	0.93	<0.01
species	1	1.38	0.25
geography*species	1	0.16	0.7
Residuals	36		
B_r			
	Df	F	P
geography	1	3.55	0.07
species	1	6.30	<0.05
geography*species	1	3.65	0.06
Residuals	36		
B_g			
	Df	F	P
geography	1	5.10	0.03
species	1	0.31	0.58
geography*species	1	2.22	0.15
Residuals	36		

B_b	Df	F	P
geography	1	3.33	0.08
species	1	0.06	0.8
geography*species	1	4.26	<0.05
Residuals	36		

DF1_Prop_B	Df	F	P
geography	1	12.19	<0.01
species	1	2.48	0.12
geography*species	1	8.31	<0.01
Residuals	36		

DF1_Prop_R	Df	F	P
geography	1	0.03	0.87
species	1	7.32	<0.05
geography*species	1	11.67	<0.01
Residuals	36		

DF2_Prop_B	Df	F	P
geography	1	0.09	0.77
species	1	0.87	0.36
geography*species	1	17.83	<0.001
Residuals	36		

DF2_Prop_R	Df	F	P
geography	1	0.81	0.37
species	1	4.12	<0.05
geography*species	1	1.70	0.2
Residuals	36		

AF_Prop_B	Df	F	P
geography	1	0.15	0.7
species	1	37.55	<0.0001

geography*species	1	5.05	<0.05
Residuals	36		

AF_Prop_R

	Df	F	P
geography	1	0.00	0.99
species	1	173.00	<0.0001
geography*species	1	0.00	0.99
Residuals	36		

Body_Prop_B

	Df	F	P
geography	1	0.01	0.93
species	1	7.49	<0.01
geography*species	1	0.02	0.9
Residuals	36		

Body_Prop_R

	Df	F	P
geography	1	12.98	<0.001
species	1	1.07	0.31
geography*species	1	0.85	0.36
Residuals	36		

CF_Prop_R

	Df	F	P
geography	1	10.55	<0.01
species	1	65.34	<0.00001
geography*species	1	10.55	<0.01
Residuals	36		

Table S7. Pairwise post-hoc Tukey's tests comparing scores for (A) LD1 and (B) LD2 from ACD colour pattern analysis on sympatric (SYM) and allopatric (ALLO) orangethroat (O) and rainbow (R) darters.

(A) LD1					
Group 1	Group 2	Estimate	Std. Error	t-value	P
ALLO RB	ALLO OT	3.14	0.45	7.02	< 0.000001
SYM OT	ALLO OT	-4.02	0.45	-9.00	< 0.000001
SYM RB	ALLO OT	6.27	0.45	14.01	< 0.000001
SYM OT	ALLO RB	-7.16	0.45	-16.02	< 0.000001
SYM RB	ALLO RB	3.13	0.45	6.99	< 0.000001
SYM RB	SYM OT	10.29	0.45	23.01	< 0.000001

(B) LD2					
Group 1	Group 2	Estimate	Std. Error	t-value	P
ALLO RB	ALLO OT	6.60	0.45	14.75	< 0.001
SYM OT	ALLO OT	6.06	0.45	13.56	< 0.001
SYM RB	ALLO OT	4.21	0.45	9.42	< 0.001
SYM OT	ALLO RB	-0.53	0.45	-1.19	0.64
SYM RB	ALLO RB	-2.39	0.45	-5.34	< 0.001
SYM RB	SYM OT	-1.85	0.45	-4.14	<0.01