

Table S1 Mitochondrial diversity estimates based on cytochrome *b* gene for Arkansas Darters, *Etheostoma cragini*, and Least Darters, *E. microperca*, partitioned by state

Species/Population	N	H _N	π (s.d.)	<i>h</i> (s.d.)	Θ_k (95% CI)	TD
<i>Etheostoma cragini</i>						
Arkansas populations	16	11	0.000252(0.000345)	0.9083 (0.0633)	14.271 (5.614, 37.590)	-0.448
Spring River, Missouri	6	5	0.007577(0.004761)	0.9333 (0.1217)	11.442 (2.578, 52.031)	0.974
<i>E. microperca</i>						
Arkansas populations	9	7	0.000241(0.000354)	0.9444 (0.0702)	12.564 (3.663, 45.868)	-1.088
Northern populations	56	33	0.003071(0.001829)	0.9740 (0.0084)	32.842 (19.450, 55.748)	-1.877*

N = number of individuals, H_N = number of haplotypes treating missing data as a fifth state, π = nucleotide and *h* = gene diversities with their standard deviations (s.d.), Θ_k = population parameter theta with 95% confidence interval, TD = Tajima's D, *indicates significance

Table S2 Genetic Diversity estimates for Arkansas Darters, *Etheostoma cragini*, from nine nuclear microsatellite loci

Locality/Field No.	N	N _A	A _M	I	H _O	H _E	PA	AR (PAR)
Chamlin/Wise Spring (JSB10-66)	4	10	1.111(0.111)	0.074(0.074)	0.083(0.083)	0.060(0.060)	0	1.11(0)
Osage Creek (BKW10-84)	5	13	1.444(0.294)	0.275(0.122)	0.133(0.111)	0.196(0.089)	1	4.69(0.1)
Turentine Spring	7	14	1.556(0.242)	0.294(0.123)	0.206(0.104)	0.210(0.088)	0	1.45(0.03)
Clabber Creek (BKW09-28)	5	15	1.667(0.289)	0.283(0.134)	0.067(0.033)	0.185(0.089)	2	1.50(0.19)
Wilson Spring (BKW09-29)	3	15	1.667(0.236)	0.365(0.121)	0.185(0.113)	0.333(0.120)	0	1.67(0)
Gailey Hollow (BKW09-32)	5	20	2.222(0.401)	0.468(0.156)	0.244(0.093)	0.291(0.092)	1	1.87(0.07)
Mendez/Myers Spring (BKW10-54)	5	20	2.222(0.547)	0.527(0.195)	0.261(0.102)	0.322(0.117)	0	5.24(0)
Sigmon Farm (BKW10-76)	5	21	2.333(0.441)	0.622(0.175)	0.422(0.143)	0.412(0.110)	0	2.10(0)
Hughes' Pond (BKW10-36)	5	22	2.444(0.626)	0.611(0.214)	0.311(0.130)	0.365(0.122)	1	5.39(0.1)
Little Osage Creek (BKW09-30)	5	22	2.444(0.412)	0.648(0.190)	0.317(0.100)	0.414(0.120)	1	2.20(0.08)
Bolin Farm (BKW10-81)	5	22	2.444(0.444)	0.651(0.178)	0.422(0.127)	0.477(0.128)	1	2.16(0.12)
Benton Spring (BKW09-33)	5	23	2.556(0.338)	0.721(0.148)	0.428(0.112)	0.480(0.097)	0	2.28(0)
Healing Spring (BKW09-31/JSB10-69)	8	25	2.778(0.465)	0.752(0.182)	0.363(0.116)	0.461(0.108)	1	2.27(0.04)
Shoal Creek, Missouri (JSB10-70)	26	45	5.000(1.658)	1.013(0.296)	0.432(0.134)	0.495(0.126)	15	2.61(0.66)
Spring River, Missouri (JSB10-71)	24	50	5.556(1.608)	1.042(0.280)	0.489(0.118)	0.493(0.107)	17	2.63(0.59)

N – Number of genotypes examined, N_A – Total number of alleles, A_M – mean number of different alleles, I – Shannon's Information Index, H_O – observed heterozygosity, H_E – Nei's (1978) unbiased heterozygosity, PA – Number of alleles unique to a single population, AR – Allelic Richness with rarefaction with 6 genes, PAR – Private Allelic Richness with rarefaction with 6 genes

Table S3 Genetic diversity estimates for Least Darters, *Etheostoma microperca*, from seven nuclear microsatellite loci

Locality (Catalog/Field No.)	N	N _A	A _M	I	H _O	H _E	PA	AR(PAR)
Doke Lake, OH (SLUM4224.1)	6	12	1.714(0.360)	0.245(0.132)	0.176(0.099)	0.154(0.083)	1	1.32(0.14)
Tenmile Creek, OH (SLUM4226.1)	12	17	2.429(0.841)	0.458(0.252)	0.19(0.097)	0.246(0.133)	1	1.56(0.06)
Mallet River, ON (JSB10-16)	10	15	2.143(0.634)	0.446(0.229)	0.314(0.155)	0.265(0.132)	1	1.57(0.08)
Lisbon Fork, OH (SLUM4227.1)	5	17	2.429(0.751)	0.52(0.262)	0.286(0.137)	0.302(0.147)	0	1.71(0.12)
Deer Creek, OH (SLUM4228.1)	16	31	4.429(1.716)	0.799(0.362)	0.384(0.168)	0.362(0.158)	2	1.90(0.33)
Raccoon Creek, WI (STL591.7)	3	20	2.857(0.884)	0.702(0.332)	0.429(0.202)	0.41(0.193)	0	2.17(0.16)
Auglaize River, OH (STL538.3)	5	22	3.143(0.911)	0.783(0.306)	0.343(0.167)	0.444(0.163)	0	2.09(0.07)
Spring Creek, IL (JSB09-29)	36	57	8.143(2.558)	1.17(0.448)	0.416(0.178)	0.446(0.165)	11	2.18(0.36)
Moots Creek, IN (STL522.2)	4	24	3.429(0.948)	0.887(0.328)	0.488(0.179)	0.503(0.18)	1	2.33(0.27)
Nichols Lake, MI (JSB09-25)	10	31	4.429(1.587)	0.989(0.328)	0.357(0.143)	0.508(0.136)	8	2.20(0.34)
Little Osage Creek, AR (BKW10-63)	2	12	1.714(0.606)	0.396(0.256)	0.286(0.184)	0.286(0.184)	0	10.57(0.31)
Osage Creek, AR (BKW10-84)	5	14	2.000(0.577)	0.471(0.241)	0.357(0.18)	0.338(0.164)	1	1.80(0.34)
Trudell Spring, AR (JSB10-60)	32	30	4.286(1.507)	0.783(0.336)	0.366(0.144)	0.364(0.146)	3	1.88(0.37)
Flint Creek, AR	5	20	2.857(0.937)	0.667(0.322)	0.314(0.162)	0.365(0.173)	4	1.94(0.40)
Benton Spring, AR (BKW09-33)	5	26	3.714(1.392)	0.785(0.384)	0.371(0.182)	0.381(0.182)	1	2.05(0.30)
Healing Spring, AR (BKW09-31/JSB10-69/ UAIC11693.1)	37	59	8.429(3.598)	1.138(0.492)	0.401(0.189)	0.426(0.173)	11	2.13(0.3)

N – Number of genotypes examined, N_A – Total number of alleles, A_M – mean number of different alleles, I – Shannon's Information Index, H_O – observed heterozygosity, H_E – Nei's (1978) unbiased heterozygosity, PA – Number of alleles unique to a single population, AR – Allelic Richness with rarefaction with 4 genes, PAR – Private Allelic Richness with rarefaction with 4 genes

Table S4 Results from Analyses of Molecular Variance (AMOVA) for Arkansas Darters, *Etheostoma cragini*, from mitochondrial cytochrome *b* gene and nine nuclear microsatellite loci

Source of variation	d.f.	S.S.	% Total variance	Statistic*	P
mtDNA					
Among populations (Arkansas vs Missouri)	1	72.068	90.34	$\Phi_{CT}=0.903$	ns
Among populations within drainages	3	0.550	-3.23	$\Phi_{SC}=-0.334$	ns
Among individuals within populations	17	20.200	12.89	$\Phi_{ST}=0.871$	<0.001
Among populations (Osage Ck vs Clear Ck)	1	0.250	1.18	$\Phi_{CT}=0.012$	ns
Among populations within drainages	2	0.300	18.82	$\Phi_{SC}=0.190$	ns
Among individuals within populations	12	1.200	80.00	$\Phi_{ST}=0.200$	ns
Microsatellites					
Among populations (Arkansas vs Missouri)	1	93.462	23.69	$\Phi_{RT}=0.237$	<0.05
Among populations within drainages	14	120.592	21.31	$\Phi_{PR}=0.279$	<0.001
Among individuals within populations	218	322.912	55.00	$\Phi_{PT}=0.450$	<0.001
Among populations (Osage Ck vs Clear Ck)	1	22.695	28.81	$\Phi_{RT}=0.288$	<0.05
Among populations within drainages	12	54.642	14.99	$\Phi_{PR}=0.211$	<0.001
Among individuals within populations	120	153.327	56.20	$\Phi_{PT}=0.438$	<0.001
Among populations (Clabber/Wilson Sp vs Turentine Sp vs all other localities)	2	33.826	24.12	$\Phi_{RT}=0.241$	<0.05
Among populations within drainages	11	43.511	14.00	$\Phi_{PR}=0.185$	<0.001
Among individuals within populations	120	153.327	61.88	$\Phi_{PT}=0.381$	<0.001

* Φ_{RT} = Proportion of variance among a priori defined groups relative to the total variance, Φ_{PR} = proportion of variance among populations within each a priori defined group relative to variance both within and among drainages, Φ_{PT} = proportion of variance among all drainages relative to the total

Table S5 Results from Analyses of Molecular Variance (AMOVA) for Least Darters, *Etheostoma microperca*, from mitochondrial cytochrome *b* gene and seven nuclear microsatellite loci

Source of variation	d.f.	S.S.	% Total variance	Statistic*	<i>P</i>
mtDNA					
Among populations					
(Arkansas vs northern populations)	1	506.335	96.09	$\phi_{CT}=0.961$	<0.05
Among populations within drainages	4	55.329	2.51	$\phi_{SC}=0.642$	<0.001
Among individuals within populations	8	22.167	1.40	$\phi_{ST}=0.986$	<0.001
Microsatellites					
Among populations					
(Arkansas vs northern populations)	1	132.812	28.44	$\phi_{RT}=0.284$	<0.001
Among populations within drainages	15	111.065	14.13	$\phi_{PR}=0.197$	<0.001
Among individuals within populations	359	464.046	57.44	$\phi_{PT}=0.426$	<0.001
Among populations					
(Trudell Spring vs all other localities)	1	38.713	28.03	$\phi_{RT}=0.280$	ns
Among populations within drainages	5	4.485	-1.74	$\phi_{PR}=-0.024$	ns
Among individuals within populations	161	208.826	73.71	$\phi_{PT}=0.263$	<0.001
Among populations					
(Osage Creek vs Flint Creek vs Trudell Spring)	2	41.973	28.71	$\phi_{RT}=0.287$	<0.05
Among populations within drainages	4	1.225	-4.42	$\phi_{PR}=-0.062$	ns
Among individuals within populations	161	208.826	75.71	$\phi_{PT}=0.243$	<0.001

ϕ_{RT} = Proportion of variance among a priori defined groups relative to the total variance, ϕ_{PR} = proportion of variance among populations within each a priori defined group relative to variance both within and among drainages, ϕ_{PT} = proportion of variance among all drainages relative to the total

Table S6 Rates of migration (m) among Arkansas populations of the Arkansas Darter, *Etheostoma cragini*, and Least Darter, *Etheostoma microperca*, inferred from a coalescent method (Migrate- n) and a Bayesian assignment method (BayesAss)

Species/Population	Migrate- n (m)	95% confidence interval	BayesAss (m)	95% confidence interval
<i>Etheostoma cragini</i>				
ClabberCk/WilsonSp → TurentineSp	0.0269	0.0163-0.0367	0.0302	-0.0243-0.0846
ClabberCk/WilsonSp → OsageCk	0.0016	0.0003-0.0028	0.0586	-0.0140-0.1312
TurentineSp → ClabberCk/WilsonSp	0.0367	0.0327-0.0453	0.0333	-0.0255-0.0922
TurentineSp → OsageCk	0.0026	0.0008-0.0045	0.0386	-0.0288-0.1061
OsageCk → ClabberCk/WilsonSp	0.0296	0.0228-0.0334	0.0071	-0.0067-0.0208
OsageCk → TurentineSp	0.0375	0.0344-0.0444	0.0104	-0.0086-0.0294
<i>Etheostoma microperca</i>				
TrudellSp → OsageCk/FlintCk	0.0005	0.0000-0.0013	0.0117	-0.0103-0.0337
OsageCk/FlintCk → TrudellSp	0.0322	0.0338-0.0430	0.0157	-0.0088-0.0403