DEFINITIONS

Assignment test – An analysis that uses genetic information to determine the probability (likelihood) of each individual occurring in any given population. Every individual in a sample is assigned to a population in which its genotype has the greatest likelihood of occurrence. A cross-assigned (or missasigned) individual is one that was sampled from one population but was assigned to a different population. A cross-assigned individual can occur by chance, or could be due to immigration from the population in which that individual was assigned into the populations from which it was sampled. The assignment test can also be used to identify individuals that may have come from a population other than one that was sampled, if the likelihood of that individual occurring in the sampled population is very low.

Dendrogram – A branching diagram (sometimes referred to as a "tree") used to illustrate genetic relationships among populations, individuals, or genetic units (genotypes, alleles, haplotypes, or amplitypes). This is a hierarchical organization, in which closely related populations, individuals, or genetic units are connected by a branch, and closely related pairs are connected to another branch, and so on. An example of a hypothetical dendrogram among amplitypes is represented in Fig. S1.

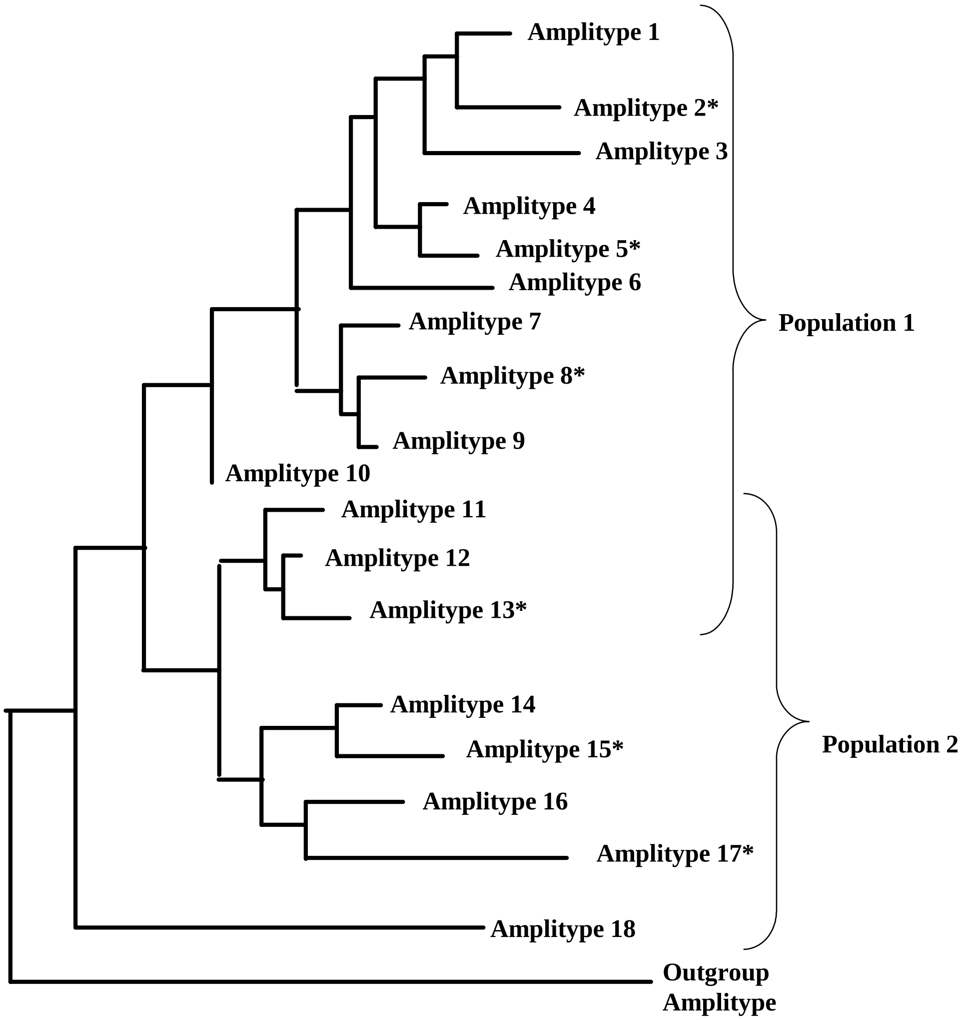

Figure S1. An example of a dendrogram of 17 amplitypes from 2 hypothetical populations. Several things are inferred from this tree: (1) Amplitypes 11-13 are found in both populations, suggesting that there has been gene flow between them; (2) the age of the amplitypes can be inferred from the position on the tree: e.g., haplotypes1 & 2 are terminal branch amplitypes, while amplitype 18 is deeply rooted in the tree, so 18 is much older than 1 &2; (3) Amplitype 1 is more similar to 2 than it is to 3; (4) amplitype 10 is probably the ancestral amplitype to all other amplitypes unique to population 1; (5) Amplitypes 8 and 9 share a common ancestor, and 9 is more similar to this common ancestor than 8 (8 is more "derived"). The "Outgroup" is a closely related species used to "root" the tree. Terminal branch amplitypes are indicated by an asterisk (*).

Effective population size - The size of an ideal population which acts the same as the real population in question. An "ideal population" is one in which there in which 1) there is no selection, 2) every individual has the same chance of producing offspring, 3) each offspring has a random chance of having a particular parent. The effective population size is lower than the total number of individuals present (census size) if there is 1) skewed sex ratio, 2) a mating structure in which a few males (or females) do most of the mating, 3) a large number of non-reproducing individuals.

Fst – A ratio of the amount of genetic variation between populations to the total amount of genetic variation (total genetic variation is the average amount of variation among all individuals from all populations). The value of the Fst is used as a reflection of the amount of genetic distinction between populations: the higher the Fst, the more genetically distinct the populations. If the Fst between two sampling sites is not statistically significantly different from 0, the populations are regarded as being from the same genetic population (i.e., the amount of genetic variation among individuals from separate sampling sites is no greater than among individuals from the same sampling site).

TABLES

Table S1 – RAPD primer name[a], DNA sequence of the primer, and molecular length (bp) of polymorphic bands for the RAPD markers used in this study.

Primer name	Primer sequence (5'-3')	Band #	Band Molecular Length
OPA-01	CAGGCCCTTC	1	1700
		2	1240
		3	1030
		4	980
		5	920
OPA-02	TGCCGACCTG	1	1210
		2	1180
		3	400
		4	370
OPA-04	AATCGGGCTG	1	1050
		2	600
OPA-07	GAAACGGGTG	1	1140
		2	880
OPA-09	GGGTAACGCC	1	1150
		2	1000
		3	940
		4	890
OPA-11	CAATCGCCGT	1	2200
		2	1850
		3	840
OPA-15	TTCCGAACCC	1	2000
		2	1850
		3	1500
		4	1050
		5	620
OPA-16	AGCCAGCGAA	1	1400
		2	1200
		3	760
		4	590
		5	390
OPA-18	AGGTGACCTG	1	1070
		2	960
		3	620
		4	580
OPA-20	CTTGCGATTC	1	1530
		2	870
OPD-02	GGACCCAACC	1	1770
		2	1670
		3	670
		4	620

Table S1 (Continued)

OPD-03	GTCGCCGTCA	1	1450
		2	1300
		3	700
		4	650
OPD-05	TGAGCGGACA	1	2100
		2	1200
OPD-08	GTGTGCCCCA	1	1000
		2	820
		3	770
OPD-10	GGTCTACACC	1	1550
		2	800
OPD-11	AGCGCCATTG	1	1520
		2	1260
		3	1180
		4	1140
OPD-12	CACCGTATCC	1	1520
		2	1160
		3	1030
		4	670
OPD-15	CATCCGTGCT	1	1040
OPD-16	AGGGCGTAAG	1	2000
		2	1000
OPD-20	ACCCGGTCAC	1	2000
		2	1200
		3	700
UBC-05	CCTGGGTTCC	1	1020
UBC-06	CCTGGGCCTA	1	1080
		2	920
UBC-09	CCTGCGCTTA	1	2000
UBC-12	CCTGGGTCCA	1	1100
UBC-14	CCTGGGTTTC	1	840
UBC-30	CCGGCCTTAG	1	1500
		2	1200
UBC-31	CCGGCCTTCC	1	1090
		2	890
UBC-34	CCGGCCCCAA	1	1550
		2	1240

[a]"OP" primers were purchased from Operon Technologies (Alameda, CA). "OPA-01" refers to primer #1 from RAPD primer kit A, "OPD-08" refers to primer #8 from kit D, etc. "UBC" primers were purchased from University of British Columbia, Nucleic Acid and Protein Services Unit, Vancouver, BC, Canada.

Table S2 – Wright's Fst values[a] for redbreast sunfish populations collected from contaminated (PRK89, PRK27) and reference sites in the Pigeon River and other rivers.

	PRK 103	PRK 89	PRK 27	PRK 42	Little River	Little Pigeon R.
PRK 103	0	0.007[b]	0.04	0.043	0.075	0.071
PRK 89	0.001[b]	0	0.048	0.047	0.081	0.077
PRK 27	0.098	0.114	0	0.024	0.032	0.032
PRK 42	0.112	0.126	0.044	0	0.056	0.047
Little River	0.179	0.19	0.062	0.147	0	0.032
Little Pigeon R.	0.186	0.207	0.07	0.146	0.064	0

[a] Values above the diagonal are calculated using the program RAPD Fst (*41*), while those below the diagonal are calculated using the program Arlequin (*42*).
[b] Not statistically significantly different from 0.

Table S3 – Analysis of molecular variance[a] data depicting the sum of square, variance components, and percentage of variation attributable to various sources of genetic variation for redbreast sunfish populations collected from contaminated (PRK89, PRK27) and reference sites in the Pigeon River and other rivers.

Source of variation	d.f.	Sum of squares	Percentage of variation
Among groups	1	216.0	7.82
Among sites within groups	4	263.0	7.54
Within Sites	329	3654.7	84.6

[a] "Among groups" refers to Pigeon River vs. Non-Pigeon River (Little and Little Pigeon Rivers). "Sites" refers to sampling sites. These data indicate that 1) there is just as much variation between Pigeon River and Non-Pigeon River sites as there is among Pigeon River sites, and 2) most of the genetic diversity occurs among individuals within sites, rather than among sites (i.e., the amount of genetic divergence between individuals within populations is high compared to the amount of genetic divergence between populations).

Table S4. Assignment test[a] results ("cross-assignments") for redbreast sunfish populations collected from contaminated (PRK89, PRK27) and reference sites in the Pigeon River and other rivers.

	Assigned to				
Sampled from	PRK 89-103	PRK 27	PRK 42	Little River	Little Pigeon R.
PRK 89-103	61**	5*	0	1*	0
PRK 27	0	26**	9*	5*	6*
PRK 42	1	8*	57**	2*	5*
Little River	0	2	0	53**	9*
Little Pigeon R.	0	4*	1	5*	33**

[a] Based upon maximum likelihood procedures that calculate the probability that an individual originated from (is "assigned to") a population other than from where it was sampled. Asterisks denote when the number of cross-assignments is statistically significantly ($P < 0.05$) less than what would be expected by random chance (based upon bootstrap tests with 10,000 subsamples).

Table S5. Maximum likelihood estimates (with 95% confidence intervals)[a] of the value $4N_em$[b] between redbreast sunfish populations in contaminated (PRK89, PRK27) and reference sites in the Pigeon River and other rivers.

Dispersing into	Dispersing from				
	PRK 89-103	PRK 27	PRK 42	Little River	Little Pigeon River
PRK 89-103	-------	9.74 (9.46-10.04)	8.23 (7.97-8.5)	8.51 (8.24-8.79)	7.41 (7.16-7.67)
PRK 27	6.695 (6.47-6.93)	-------	6.18 (5.96-6.40)	5.98 (5.77-6.21)	7.98 (7.73-8.24)
PRK 42	5.85 (5.66-6.05)	2.49 (2.36-2.63)	-------	6.63 (6.42-6.84)	7.06 (6.85-7.28)
Little River	7.99 (7.45-8.20)	5.56 (5.36-5.76)	7.82 (7.58-8.06)	-------	4.34 (4.16-4.52)
Little Pigeon River	6.47 (6.25-6.59)	5.49 (5.12-5.57)	7.69 (7.32-8.07)	6.6 (6.26-6.95)	-------

[a] Calculated using a Markov Chain Monte Carlo approach

[b] N_e is the effective population size, m is the migration rate per generation. Migration rates were calculated asymmetrically, with a different value for migration from population i to j than from j to i.