APPENDIX B. Descriptive statistics for microsatellite analysis of 16 populations of *Desmognathus fuscus* sampled throughout the Washington D.C.–Baltimore Metropolitan Area.

B.1 Methods

B.1.1 Data Quality

Microsatellite genotypes were checked for the presence of null alleles, stutter products, or allelic dropout using MICRO-CHECKER (v2.2; Van Oosterhout et al. 2004). We tested for significant heterozygote deficiencies at each locus and for linkage disequilibrium and deviations from Hardy-Weinberg expectations among all loci at each sampling locality. All tests were performed in Genepop (v4.2; Raymond and Rousset 1995) using exact tests, with *P*-values estimated using a Markov chain method (Guo and Thompson 1992; 10,000 dememorizations, 1000 batches, 10,000 iterations each). Significance was assessed using a sequential Bonferroni correction to account for multiple comparisons (Holm 1979).

B.1.2 Genetic Summary Statistics

Allele frequency data were used to estimate summary genetic diversity indices for each population. GenAlEx (v.6.5; Peakall and Smouse 2006, 2012) was used to calculate the average number of alleles per locus (N_A), the effective number of alleles per locus (N_E), the number of private alleles, and observed heterozygosity (H_o). The inbreeding coefficient (F_{is}) was calculated using FSTAT (v2.9.3; Goudet 1995). Allelic richness (Petit et al. 1998) and expected heterozygosity (Nei and Roychoudhury 1974) were calculated in order to estimate genetic diversity while correcting for small sample sizes ($n < 50$). Allelic richness and expected heterozygosity were calculated using the programs FSTAT (v2.9.3; Goudet 1995) and GenAlEx (v6.5; Peakall and Smouse 2006, 2012), respectively.

B.2 Results

B.2.1 Data Quality

Significant deviations from Hardy-Weinberg equilibrium were observed at 5.2% of the loci by stream site combinations. Possible null alleles were identified at two loci for five populations, with null allele frequencies ranging from 0.164 to 0.351 (Table B1). Significant heterozygote deficiencies were observed among individuals from one sampling locality (Bynum Run 2; $P = 0.003$; $\alpha = 0.0083$) and globally at the *Dau*8 locus ($P < 0.001$; $\alpha = 0.0031$). No significant linkage between loci was observed for any population. Heterozygote deficiencies and deviations from the expectations of neutrality were rare and not expected to significantly impact the degree of genetic population structuring observed, so all individuals and loci were included in the subsequent analyses.

TABLE B1. Summary table of possible null alleles and their estimated degree of homozygote excess (F_{is}) for 6 microsatellite loci from 16 *Desmognathus fuscus* populations in the Washington D.C. – Baltimore Metropolitan area, with null allele frequencies estimated using the Van Oosterhout (2004) method.

Stream Reach	Locus	Null Allele Frequency	F_{is}
Winter 1	Dau8	0.2639	0.530
Jones 2	Dau8	0.3514	0.769
Gwynn 2	Dau8	0.3086	0.701
Patuxt 1	Doc1	0.3407	0.877
Patuxt 2	Dau8	0.1639	0.355

TABLE B2. Sample size (N), number of private alleles (P), and mean diversity indices across 6 microsatellite loci from *Desmognathus fuscus* sampled at 16 stream locations in the Washington D.C. – Baltimore Metropolitan area. N_A–average number of alleles per locus; N_E–effective number of alleles per locus; H_o–observed heterozygosity; H_e–unbiased expected heterozygosity; AR–allelic richness; F_{is}–inbreeding coefficient.

Watershed	Reach	N	P	N_A	N_E	H_o	H_e	AR	F_{is}
Bynum Run	Bynum 1	11	1	3.167	2.034	0.500	0.499	3.167	0.061
	Bynum 2	20	0	3.167	2.088	0.367	0.415	2.757	0.139
Winters Run	Winter 1	20	0	3.500	2.400	0.508	0.479	3.310	-0.027
	Winter 2	20	1	4.500	2.707	0.525	0.565	4.043	0.072
Little Gunpowder Falls	Little 1	20	0	4.167	3.110	0.658	0.660	4.052	0.029
	Little 2	20	1	4.167	2.494	0.517	0.508	3.762	0.066
Gunpowder Falls	Gun 1	20	0	4.833	3.580	0.675	0.708	4.542	0.083
	Gun 2	20	0	5.333	3.169	0.692	0.638	4.634	0.035
Jones Falls	Jones 1	20	1	4.833	2.913	0.692	0.618	4.266	-0.110
	Jones 2	20	0	5.167	3.213	0.625	0.672	4.593	0.098
Gwynns Falls	Gwynn 1	22	1	5.167	3.481	0.720	0.671	4.594	-0.040
	Gwynn 2	20	0	4.500	3.433	0.617	0.675	4.326	0.102
Patuxent River	Patuxt 1	20	0	3.333	2.465	0.533	0.554	3.133	0.038
	Patuxt 2	20	1	4.833	2.838	0.500	0.513	4.205	0.026
Seneca Creek	Seneca 1	20	0	4.000	2.892	0.533	0.532	3.712	0.003
	Seneca 2	20	1	4.333	2.876	0.567	0.601	3.890	0.059

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