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Cover Page Footnote
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POPULATION GENETIC DIVERGENCE OF ISLE ROYALE PEARL DACE, *Margarita margariscus* (CYPRINIDAE)

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ABSTRACT

In 1949, Hubbs and Lagler described morphological variation among pearl dace (*Margariscus margarita*) of inland lakes on Isle Royale, Michigan. For Harvey Lake, Hubbs and Lagler proposed that pearl dace were sufficiently morphologically distinct to warrant subspecific status. They argued that divergence of the Harvey Lake pearl dace was due to allopatric differentiation in isolation from lower elevation lakes. Harvey Lake has been isolated by elevation from lower elevation lakes for approximately 10 to 15 thousand years. No genetic studies have been done on Isle Royale pearl dace to evaluate this hypothesis to date. Here we report the analysis of *Margariscus margarita* populations using a limited battery of microsatellite loci to assess the extent of and genetic divergence among the Harvey Lake and other Isle Royale lowland lake populations. Microsatellite loci were analyzed using PCR primers developed for the non-congener, longnose dace (*Rhinichthys cataractae*). Statistical analyses of allele frequency data indicate genetic differentiation among all Isle Royale pearl dace populations inclusive of both Harvey Lake and lowland populations concurring with Hubbs and Lagler's hypothesis that the Harvey Lake population is genetically divergent. Results also indicate that the lowland Isle Royale populations are apparently equally divergent from one another.

Keywords: pearl dace, microsatellites, population divergence, Isle Royale, *Margarita margariscus*

INTRODUCTION

In 1946, during one of the very few formal comprehensive surveys of ichthyofauna of Isle Royale, Michigan, Hubbs and Lagler (1949) identified a population of pearl dace, (*Margariscus margarita*) in the elevationally isolated Harvey Lake. They described unique morphological differences (such as in head shape and fin ray counts) for the population that distinguished them from populations of other lowland lakes on Isle Royale and the mainland. Hubbs and Lagler (1949) further hypothesized that morphological differentiation of Harvey Lake pearl dace was sufficiently distinct to warrant designation of the Harvey Lake pearl dace as a unique subspecies. Other than a small sample collected during a brief and partial faunal survey of the island in the early 1960s (Hubbs and Lagler 1964), no further work was done on the Harvey Lake pearl

dace population. Its designation as a subspecies depended solely on Hubbs and Lagler's initial 1949 description. Bailey and Smith (1981) argued that there is not enough data to appoint subspecific status to the Harvey Lake pearl dace. The taxonomic status of the Harvey Lake pearl dace population has remained questionable since that time (Kallemeyn 2000). Until now no genetic analyses have been done to determine if the degree of genetic differentiation of Harvey Lake from other Isle Royale populations might support Hubbs and Lagler's subspecies hypothesis largely due to the inaccessibility of the island lakes and the difficult logistics of ichtyofaunal survey on Isle Royale.

This study used polymerase chain reaction (PCR) based DNA analysis of highly polymorphic microsatellite loci markers to assay genetic variation to estimate gene flow and population genetic differentiation among Isle Royale populations as well as to estimate the degree of isolation of the Harvey Lake population from other Isle Royale and mainland populations. This study took advantage of the fortuitous sampling of Isle Royale fishes done during the first systematic faunal survey since the 1940s conducted in 1995 by the U.S. Biological Service. Pearl dace samples were collected from Harvey Lake and other Isle Royale lowland lakes for genetic analyses. *Margariscus margarita* samples were frozen and archived tissue samples held at -80 ^{o}C for future analysis.

Isle Royale

Isle Royale National Park was created by President Franklin Roosevelt in 1940. Due to its unique location and characteristics Isle Royale National Park was later inducted into the National Wilderness Preservation System under the Wilderness Act of 1976. In 1981, Isle Royale was identified as a United Nations International Biosphere Reserve in recognition of its global significance for scientific research and education. Isle Royale is perhaps most famous ecologically for being the site of the longest ongoing wildlife research project into predator/prey dynamics of the grey wolf (*Canis lupus*) and the moose (*Alces alces*) ever conducted (Micheal et al. 2008; Peterson et al. 1998).

Isle Royale National Park and Wilderness Area lies roughly 25 miles (40.2 km) offshore of Minnesota in Lake Superior. The island is approximately 40 miles (64.4 km) long by 9 miles (14.5 km) wide at its broadest point and is characterized by about 35 small inland, low elevation lakes. Isle Royale is 98% wilderness and has a total acreage of about 571,000 (231,076 ha), 133,000 of which is considered land-based. Isle Royale's wilderness ecosystem and fauna are of special interest to scientific research because the area is considered to be only minimally disturbed by anthropomorphic activity even though the island is heavily visited by fishermen from Lake Superior and primitive campers during the summer months (Michael et al. 2008; Peterson et al. 1998).

Isle Royale lies approximately halfway between the North Pole and the equator in Lake Superior and as a result has a temperate climate with a high diversity of habitats, ranging across streams, the bog ponds, small lakes, and deep shore waters (Hubbs and Lagler 1964). As a result the fish fauna of Isle Royale is relatively diverse as is that of the whole Great Lakes region (Hubbs and Lagler 1964).

Modern day Lake Superior and Isle Royale were heavily influenced by recession of the Pleistocene Wisconsin Ice Age glaciers in North America. As the glaciers melted and Lake Superior water levels dropped, new opportunities for fish dispersal developed in the Great Lakes region. As Isle Royale emerged many small, inland lakes formed due to a decreasing water level in Lake Superior (Hubbs and Lagler 1964). Those inland

lakes with higher elevations (Harvey Lake) were formed before those of lower elevations. Harvey Lake appears to be unique among Isle Royale lakes in that its 168 ft (51.2 m) elevation has resulted in its presumed relative biological isolation from lower surrounding waters for approximately the last 15,000 years. The end of the last ice age is the last time Lake Superior experienced a high water event of a magnitude reaching Harvey Lake. Although Harvey Lake does, and historically has, drained into Lake Superior, the drainage consists of a 168 ft (51.2 m) waterfall presumed to prevent any upstream migration by fishes from Lake Superior proper or from nearby Isle Royale lowland lakes (Hubbs and Lagle 1964).

The island fish fauna is known to consist of some 41 species from 31 genera representing 14 families (Hubbs and Lagler 1949). The Harvey Lake population of pearl dace (*Margariscus margarita*) is of especial interest because it is presumed to have descended from a relic population left behind by the last recession of the Pleistocene Lake Superior (Hubbs and Lagler 1949). Harvey Lake pearl dace were assumed to have diverged as a result of limited or no gene flow since isolation to the extent that Hubbs and Lagler declared this population to warrant the assignation of unique subspecific status (Hubbs and Lagler 1949). However, due to the relative inaccessibility of Isle Royale inland lakes, as demonstrated by the lack of faunal surveys in the park for the last 70 years, Harvey Lake minnows have remained virtually unstudied since the limited survey conducted in 1964 by Hubbs and Lagler. The most comprehensive survey of the Isle Royale fish fauna was done by Koelz in 1925 (Koelz 1929) followed by partial surveys of only a few Isle Royale lakes since that time (Hubbs and Lagler 1964; Kallemeyn 1995).

To date no attempts have been made to use genetic techniques to assess the degree of gene flow and genetic divergence among the Harvey Lake population and those of adjacent Isle Royale lakes in order to evaluate Hubbs and Lagler's hypothesis.

Genetic and Statistical Analysis

This study assayed microsatellite loci which are one class of variable number tandem repeats (VNTR) sequence variation. These particular sequences exist at many different loci, distributed throughout eukaryotic genomes, as strings of repeated simple DNA sequences (for example: dinucleotide repeats ATATATAT... and trinucleotide repeats ATCATCATC...) and most of the allelic variation is in the form of repeat number differences at any given locus. The development of PCR based techniques that allow the scoring of allelic variation for single individual microsatellite loci has made microsatellites the VNTRs of current preference used to detect gene flow and population differentiation. The sequences have a high mutation rate (10^{-3} per locus per generation [Edwards et al. 1992; Jeffreys et al. 1988; Kelly et al. 1991]) which makes them highly variable and therefore providing sufficient genetic variation for the discrimination of population level questions. High levels of Mendelian allelic differences usually provide dozens of alleles at each locus (Rico et al. 1993). This translates into the presence of large amounts of relatively easily assayed population genetic variation. Since allelic differences are easily scored, frequency data gathered using these markers are readily analyzed for population structuring and interconnectedness using accepted population genetic methods such as Hardy-Weinberg exact tests (Guo and Thompson 1992; Haldane 1954; Raymond and Rousset 1995b; Rousset and Raymond 1995; Rousset 2008; Weir 1996), genotypic differentiation (Fisher 1935; Goudet et al. 1996; Raymond

and Rousset 1995a; Raymond and Rousset 1995b; Rousset 2008), and F_{st} and Rho_{st} statistics (Cockerham 1973; Michalakis and Excoffier 1996; Raymond and Rousset 1995b; Rousset 2008; Rousset 1996; Weir and Cockerham 1984; Wright 1978).

MATERIALS AND METHODS

Sampling

Population samples were collected by National Park Service and U.S Geological Survey personnel, under the supervision of Larry Kallemeyn (2000), during the faunal survey of Isle Royale fishes conducted in the summer of 1995. Specimens were collected by seine and funnel traps and transported frozen to the Department of Biology at the University of North Dakota. Archived frozen tissue samples were shipped on dry ice to Valdosta State University for genetic analysis. Since several loci were used, each having large amounts of allelic variation, population samples of individual fish need not have been prohibitively large to have generated some confidence in findings. Since our samples were irreplaceable samples of convenience taken during the 1995 survey we were forced to settle on sample sizes of 20 individual specimens from each population in order to achieve equal sample sizes among populations. While these numbers are low per location they proved sufficient to achieve some level of confidence in our analyses. Populations were collected from Harvey Lake, Hatchet Lake, and Forbes Lake on Isle Royale. The mainland population was collected from Scabbard Lake and Marsh Lake, in Northern Minnesota.

DNA Isolation/Extraction

Fish tissues were stored in a -80 ^{o}C freezer until DNA extraction was performed. Standard guanidinium isothiocyanate (GIT) extraction of nucleic acids was performed on all samples (Turner et al. 1989). Individual specimens were processed on ice to prevent DNA decomposition during the procedure. Approximately 0.5 g of muscle tissue was dissected away, skinned, and minced with a razor blade. Samples were suspended in 1.5 mL microfuge tubes with approximately 600-700 µL of GIT and incubated for 24-48 hours. Samples were gently homogenized by hand pestle in the microfuge tube and allowed to stand for another 24-48 hours to ensure complete tissue lysis. After incubation lysed homogenates were extracted twice with equal volume of 1:1 phenol/chloroform mixture to remove protein and purify nucleic acids. Samples were centrifuged for 20 min at 5000 rpm in a Hermle (Wehingen, Germany) Z180M table top centrifuge to separate organics, proteins, and cell debris from the aqueous supernatant containing nucleic acids. Supernatants were subsequently extracted three times with equal volumes of 1:1 chloroform:isoamyl alcohol. DNA was recovered using standard precipitation with two volumes of cold 95% ethanol in the presence of 0.2 volumes of 10 M ammonium acetate. Samples were precipitated overnight at -20 ^{o}C. After centrifugation for 15 min at 5000 rpm at 4 ^{o}C pellets were washed in cold 70% ethanol/ddH_2O. Pelleted DNA was air dried at room temperature and subsequently dissolved in 1 mL of 1 X TE buffer at 37 ^{o}C overnight.

Microsatellite Primer Development

Microsatellite primers from non-conspecifics longnose dace (*Rhinichthys cataractae*) were obtained from Integrated DNA Technologies® (IDT) (Waltham, Massachusetts) based on sequences derived from Girard and Angers (2006). These

microsatellite primers were previously shown to cross-amplify loci from the genomes of five other cyprinid minnow species; *Rhinichthys atratullus* (blacknose dace), *Margariscus margarita* (pearl dace), *Exoglossum maxillingua* (cutlips minnow), *Phoxinus eos* (northern redbelly dace), and *Pimephales promelas* (fathead minnow). Of 10 microsatellite loci developed for the longnose dace, nine of them were reported to cross amplify loci for the pearl dace. Only five of these primer pairs proved to cross amplify informative polymorphic loci in our samples. PCR primers were commercially prepared at 100 µM by Integrated DNA Technologies and labeled with IR 700 fluorescent dye for use in the LiCOR® (Lincoln, Nebraska) 4300 infrared genotyping system. Primer pair and locus specifics for *M. margariscus* are given in Table I.

Table I. Longnose dace primer pairs derived from Girard and Angers (2006) that reliably amplified informative loci in *M. margariscus*. Locus designations are those given by Girard and Angers. Rhca designates forward and DQ designates reverse. Ann. = Annealing, T = Temperature, All. = Alleles, No. = number.

Locus / primers	Primer sequence	Repeat Motif	Ann. T C^{o}	No. all.	bp range of All.	He
Rhca 7	GTCCACCTCATACAAATTCC		64	20	86-164	0.81(0.50)
DQ106911	ATGAGGCAACCACTGGAGC	(CA)*n*				
Rhca 15b	CTCACAGACTACCTGCCC		64	16	108-324	0.87(0.55)
DQ106913	CAGAGGTCAAACAGTAGTAGG	(CTAT)*n*				
Rcha 20	CTACATCTGCAAGAAAGGC		64	5	94-130	0.85(0.59)
DQ106915	CAGTGAGGTATAAAGCAAGG	(GA)*n*				
Rhca 24	GTGGTGTTAGCAGAAACCG		54	14	229-453	0.94(0.76)
DQ106917	CTGCTGTTAATATGTCAC	(GA)*n*				
Rhca 52	TTAATGCGAATCTTTGGG		59	12	128-310	0.75(0.10)
DQ106921	CAATGAGACAGATTCGATTC	(CT)*n*				

Polymerase Chain Reaction

PCR was conducted using a Bio-Rad (Hercules, California) MyCycler™ thermal cycler. The PCR protocols were similar to those of Girard and Angers' (2006). PCR was done in 25 µL reactions using New England BioLabs® (Ipswich, Massachusetts) reagents. Reaction components were as follows: NEB's 1X standard *Taq* reaction buffer, 200 µM dNTPs, 0.5 units of *Taq* polymerase, and 1 µM each forward and reverse microsatellite primers. Final reaction volumes were adjusted to 25 µL by adding 17.9 µL of PCR grade H_2O. Thermal gradient PCR was performed to optimize annealing temperatures for each of the microsatellite primer pairs ranging between 54 ^{o}C and 64 ^{o}C (see Table I). Thermal cycles included an initial denaturation at 95 ^{o}C for 2:00 min followed by 30 cycles of 92 ^{o}C; 30 s, annealing temperature (as per Table I) for 30 s and 72 ^{o}C for 30 s. Samples were held at 4 ^{o}C after thermocycling.

Gel Electrophoresis

Gel electrophoresis and microsatellite allele scoring was conducted using standard protocols for the LiCOR® (Lincoln, Nebraska) 4300 sequence analyzer/genotyping system. Twenty-five centimeter long, 0.25 mm thick, 4.5% polyacrylamide gels in 1 X TBE buffer were used to differentiate microsatellite allele

fragment lengths fluorescently labeled with IR 700 primers. Samples were electrophoresed at 2000 V for 2 hours or until standard markers indicated completion. Three different standard LiCOR® fluorescent labeled sizing standards were used to calibrate allele size scoring for each gel.

Statistical Analysis

Allele fragments were identified and scored using the Saga$^{GT™}$ software developed for microsatellite analysis provided with the LiCOR® 4300 DNA analyzer. Saga$^{GT™}$ digitalizes electrophoresis results, finds lanes, locates standards, calibrates band sizes, and scores alleles (LiCOR® Biosciences 2011b). All allele fragments detected by the LiCOR system were also visually checked and confirmed before inclusion in the data set.

Allele frequency data analysis was conducted using the web based software Genepop (Raymond and Rousset 1995b; Rousset 2008). Allelic sizes and frequencies at each locus were scored for all populations. Standard population genetic statistics were calculated using the web-based software Genepop (Raymond and Rousset 1995b; Rousset 2008). The analyses include the following: Rho (Valdes et al. 1993; Michalakis and Excoffier 1996; Raymond and Rousset 1995b; Rousset 2008; Rousset 1996) and F-statistics (Wright 1978) for all populations, genotypic differentiation P-values for exact G tests (Fisher 1929) between pairs of populations, and Hardy Weinberg heterozygote deficiency and excess tests (Chakraborty and Jin 1992) for each locus in each population.

RESULTS

The F statistics (Table II) were calculated to estimate the level of heterozygosity (Table III) within and among individuals, and, within and among populations (Wright 1978).

The maximum likelihood exact G-test was used among population pairs to see if any two populations have the same distribution of genotypes. The exact G-test uses the log-likelihood of how well an observed pair of genotypic distributions fits the expected genotypic distributions. The sum of all the loci probabilities within the contingency tables was used to generate a rejection zone. An impartially estimated P-value of the exact G-test is produced for each population pair based on the best fit of the observed genotype to the contingency table (Goudet et al. 1996; Raymond and Rousset 1995a; Raymond and Rousset 1995b; Rousset 2008). The null hypothesis in this test is that "genotypes are drawn from the same distribution in all populations" (Raymond and Rousset 1995b; Rousset 2008). A low P-value will result in a rejection of this H_o. The estimated P-values are shown in Table III. S. E. is the standard error which is the standard deviation between the observed and expected distributions (Larson and Farber 2006).

Table II. Tabulated estimated F and Rho Statistics. F_{is} compares the genetic variability of an individual to the subpopulation or the chance of inbreeding in a population. F_{st} compares the genetic variability of a subpopulation to the total population. F_{it} compares the genetic variability of the individual against the total population (Wright 1978). Rho (R-statistics) is a less conservative measure that estimates the relatedness of alleles by their size assuming microsatellites evolve in the stepwise mutation model (SSM) (Valdes

et al. 1993). Under SSM models individuals with alleles close in size are more related to one another (Michalakis and Excoffier 1996; Raymond and Rousset 1995b; Rousset 2008; Rousset 1996).

	F-Statistics			Rho-Statistics		
Locus	Fis^	Fst^	Fit^	Rhois^	Rhost^	Rhoit^
Rhca7	-0.171388	0.157509	0.013116	0.376497	0.235838	-0.05187
Rhca15b	0.350877	0.603463	0.742599	0.065527	0.854639	0.864164
Rhca20	-0.108504	0.277192	0.198764	0.005059	0.432304	0.435176
Rhca24	-0.151589	0.364778	0.268485	-0.4044	0.577789	0.407046
Rhca52	-0.054132	0.268485	0.030448	0.162827	0.104696	0.250476
Multi Loci	-0.0800	0.2714	0.2131	-0.1816	0.6678	0.6075

Table IIIa and b. Exact G-test. The maximum likelihood results testing the hypothesis that alleles are drawn from the same distribution for all population pairs.

Table IIIa

Exact G Test	Rhca7		Rhca15b		Rhca20	
Population Pair	P-value	S.E.	P-value	S.E.	P-value	S.E.
Harvey/Forbes	0.000000	0.000000	0.000010	0.000010	0.755950	0.002130
Hatchet/Forbes	0.000000	0.000000	0.000000	0.000000	0.007150	0.000800
Hatchet/Harvey	0.000000	0.000000	0.000610	0.000250	0.030400	0.001500
Scabbard/Forbes	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Scabbard/Harvey	0.000000	0.000000	0.292850	0.001980	0.000000	0.000000
Scabbard/Hatchet	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000

Table IIIb

Exact G Test	Rhca24		Rhca52	
Population Pair	P-value	S.E.	P-value	S.E.
Harvey/Forbes	0.000000	0.000000	0.001690	0.000410
Hatchet/Forbes	0.000000	0.000000	0.000000	0.000000
Hatchet/Harvey	0.016910	0.002010	0.111570	0.005560
Scabbard/Forbes	0.000000	0.000000	0.000250	0.000230
Scabbard/Harvey	0.000000	0.000000	0.000070	0.000070
Scabbard/Hatchet	0.000000	0.000000	0.000000	0.000000

Hardy-Weinberg (HW) exact tests were performed to analyze deviations from HW equilibrium within and among populations. HW deficiency and excess tests determine if there is a deficiency or an excess of heterozygosity (Guo and Thompson 1992; Haldane 1954; Weir 1996; Raymond and Rousset 1995b; Rousset 2008; Rousset and Raymond 1995). The HW deficiency and excess data for each population is shown in Table IV along with a global U-test for multi-locus Hardy-Weinberg expectation over all populations combined (Raymond and Rousset 1995a; Raymond and Rousset 1995b; Rousset 2008).

The Hardy- Weinberg exact tests use Fisher's (1935) method to calculate a P-value for a X^2 for each population across all loci (Table IV) (Fisher 1935; Raymond and

Rousset 1995a; Raymond and Rousset 1995b; Rousset 2008). Fisher's (1925) method was used to acquire a P-value from X^2 value for each population pair across all of the loci (Table IV).

The maximum likelihood exact G-test (Table V) was used among population pairs to determine if any two populations had the same distribution of genotypes. The exact G-test uses the log-likelihood of how well an observed pair of genotypic distributions fits to the expected genotypic distributions. The sum of all the loci contingency tables' probabilities was used to generate a rejection zone. An impartially estimated P-value of the exact G-test is produced (Raymond and Rousset 1995; Rousset 2008). The null hypothesis in this test is that "genotypes are drawn from the same distribution in all populations" (Raymond and Rousset 1995; Rousset 2008). A low P-value will result in a

Table IV. Hardy-Weinberg exact tests.

Harvey Lake Hardy-Weinberg Exact Test

Locus	Probability P - value	Probability SE	Deficiency /Excess	P value	SE
Rhca 7	0.0165	0.0048	Deficiency	0.3598	0.0202
			Excess	0.6553	0.0200
Rhca 15b	0.1142	0.0017	Deficiency	0.1142	0.0017
			Excess	1.0000	0.0000
Rhca 20	1.0000	0.0000	Deficiency	1.0000	0.0000
			Excess	0.5398	0.0021
Rhca 24	0.0321	0.0088	Deficiency	0.0575	0.0109
			Excess	0.9755	0.0063
Rhca 52	0.0000	0.0000	Deficiency	0.0000	0.0000
			Excess	1.0000	0.0000
Overall	$Chi^2 = \infty$, DF = 10, Probability = Highly significant				

Forbes Lake Hardy-Weinberg Exact Test

Locus	Probability P - value	Probability SE	Deficiency /Excess	P value	SE
Rhca 7	0.052	0.007	Deficiency	1.0000	0.0000
			Excess	0.0017	0.0007
Rhca 15b	1.000	0.000	Deficiency	1.0000	0.0000
			Excess	0.9735	0.0008
Rhca 20	0.273	0.002	Deficiency	0.2725	0.0020
			Excess	0.9880	0.0006
Rhca 24	0.020	0.001	Deficiency	0.0290	0.0016
			Excess	0.9791	0.0014
Rhca 52	0.016	0.003	Deficiency	0.3916	0.0161
			Excess	0.6090	0.0161
Overall	$Chi^2 = 24.62$, DF = 10, Probability = 0.0061				

Table IV. Hardy-Weinberg exact tests. Continued.

Hatchet Lake Hardy-Weinberg Exact Test

Locus	Probability P - value	Probability SE	Deficiency /Excess	P value	SE
Rhca 7	0.0018	0.0090	Deficiency	0.9478	0.0103
			Excess	0.0599	0.0110
Rhca 15b	0.0000	0.0000	Deficiency	0.0000	0.0000
			Excess	1.0000	0.0000
Rhca 20	0.0358	0.0032	Deficiency	0.9886	0.0022
			Excess	0.0267	0.0033
Rhca 24	0.0000	0.0000	Deficiency	0.3734	0.0188
			Excess	0.6266	0.0188
Rhca 52	0.0041	0.0038	Deficiency	0.3351	0.0357
			Excess	0.6940	0.0348
Overall	$Chi^2 = \infty$, DF = 10 , Probability = Highly Significant				

Scabbard Lake Hardy-Weinberg Exact Test

Locus	Probability P - value	Probability SE	Deficiency /Excess	P value	SE
			Deficiency	0.0423	0.0147
Rhca 7	0.2838	0.0356	Excess	0.9577	0.0145
			Deficiency	0.0000	0.0000
Rhca 15b	0.0000	0.0000	Excess	0.0000	0.0000
			Deficiency	0.3143	0.0042
Rhca 20	0.3817	0.0049	Excess	0.9013	0.0025
			Deficiency	1.0000	0.0000
Rhca 24	0.0000	0.0000	Excess	0.0000	0.0000
			Deficiency	1.0000	0.0000
Rhca 52	0.0000	0.0000	Excess	0.0003	0.0002
Overall	Chi = 45.08, DF = 10, Probability = 0.0000				

Global U-Test.

Population	Deficiency /Excess	P value	SE
Forbes Lake	Deficiency	0.360	0.011
	Excess	0.640	0.011
Harvey Lake	Deficiency	0.000	0.000
	Excess	1.000	0.000
Hatchet Lake	Deficiency	0.026	0.007
	Excess	0.974	0.007
Scabbard lake	Deficiency	0.718	0.018
	Excess	0.282	0.018
All loci for all populations combined	Deficiency	0.002	0.001
	Excess	0.998	0.001
Overall	Chi2 = ∞, DF = 10 , Probability = Highly Significant		

rejection of this H_o. The estimated P-values are shown in Table Va and Vb. S. E. is the standard error which is the standard deviation between the observed and expected distributions (Larson and Farber 2006).

Table Va. The maximum likelihood (exact G-test) among population pairs. Polulation pairs are lakes sampled. A value of 0.0 means four digits following the value 0.0 are all zeros (0).

Genetic differentiation of population pairs based on Exact G-Tests

Locus	Rhca 7		Rhca 15b		Rhca 20	
Population Pairs	P Value	S.E.	P Value	S.E.	P Value	S.E.
Harvey -Forbes	0.0	0.0	0.00001	0.00001	0.75595	0.00213
Hatchet - Forbes	0.0	0.0	0.0	0.0	0.00715	0.0008
Hatchet - Harvey	0.0	0.0	0.00061	0.00025	0.0304	0.0015
Scabbard - Forbes	0.0	0.0	0.0	0.0	0.0	0.0
Scabbard - Harvey	0.0	0.0	0.29285	0.00198	0.0	0.0
Scabbard - Harvey	0.0	0.0	0.0	0.0	0.0	0.0

Table Vb. The maximum likelihood (exact G-test) among population pairs. Continued.

Genetic differentiation of population pairs based on Exact G-Tests

Locus	Rhca 24		Rhca 52	
Population Pair	P Value	S.E.	P Value	S.E.
Harvey -Forbes	0.0	0.0	0.00169	0.00041
Hatchet - Forbes	0.0	0.0	0.0	0.0
Hatchet - Harvey	0.01691	0.00201	0.11157	0.00556
Scabbard - Forbes	0.0	0.0	0.00025	0.00023
Scabbard - Harvey	0.0	0.0	0.00007	0.00007
Scabbard - Harvey	0.0	0.0	0.0	0.0

CONCLUSION & DISCUSSION

For the purpose of discussing the results of F-statistics the populations Forbes Lake, Harvey Lake, Hatchet Lake, and Scabbard Lake are referred to as subpopulations of a total population. Wright's 1978 proposed guidelines were used to interpret resulting F statistics. Under these guidelines F values of 0.0-0.05 indicate little genetic differentiation, 0.05-0.15 indicates moderate genetic differentiation, 0.15-0.25 indicates great differentiation, and F values above 0.25 indicate very great genetic differentiation. High Fst values for each locus among all populations reveal great genetic differentiation for loci Rhca 7, Rhca 15b, Rhca 20, and Rhca 24 (F_{st}=0.16, F_{st}=0.60, F_{st}=0.27, and F_{st}=0.36 respectively) among populations (Table I). Locus Rhca 52 shows moderate genetic differentiation (F_{st}=0.08) among the populations. Overall, the F values for each

locus within populations reveal reduced heterozygosities relative to Hardy-Weinberg expectations. While Rho values tend to be numerically higher than Fst values the overall pattern of differentiation revealed by Rho values is essentially the same as that revealed by F statistic counterparts. Rho values fell within the same Wright's interpretive guidelines as their F statistic counterparts. These Rho values imply that average lengths of the microsatellite alleles per locus differ among populations suggesting weak mutational relationships between alleles among populations.

The pair wise exact G-test also revealed divergence among populations (Table V). A pair wise X^2 was calculated for use in the exact G test to examine relationships over all loci among populations. The H_o for this particular test was that genotypes were drawn from a single distribution in all population samples (Raymond and Rousset 1995b; Rousset 2008). When all loci are considered, resulting P-values (Table V) reject the H_o implying that individual population distributions over all loci were not subsamples drawn from one overall distribution but rather represented genetically divergent, distinct populations. Not unexpectedly some single loci frequencies considered individually were not different for all populations. For example, locus Rhca 15b, Scabbard Lake and Harvey Lake fail to reject the H_o (P-value = 0.29285). For locus Rhca 20, Harvey Lake and Forbes Lake fail to reject the H_o (P-value = 0.75595). For Rhca 52, Harvey Lake and Hatchet Lake fail to reject the H_o (P-value = 0.11157).

Hardy-Weinberg exact tests were performed to evaluate heterozygote deficiency or excess for individual loci within populations and over all loci within populations (Table IV) (Raymond and Rousset 1995b; Rousset 2008). The overall probability was highly significant rejecting the null hypothesis of Hardy-Weinberg equilibrium over all populations and all loci. Table IV shows that there is an overall excess of heterozygote genotypes across all populations for loci.

These results indicate that inland lake populations of *M. margariscus* on Isle Royale, as well as those on the mainland, have diverged from the Harvey Lake population as well as from each other. These data indicate a large amount of genetic variability within and among all Isle Royale populations. It is interesting to note the sharing of genotypic distributions for only some loci between Harvey Lake and other populations. There may be some limited, unidirectional gene flow from Harvey Lake to the other lowland lakes. The 168 ft waterfall that is Harvey Lake's sole outlet might well serve as a unidirectional barrier to dispersal for a species of limited vagility such as *M. margariscus*. Any such migration out of Harvey Lake would necessarily be extremely limited since migrants would be exposed to large numbers of predators in lowland lake drainages and Lake Superior proper which provides the only connectivity among lakes.

Our limited findings are consistent with Hubbs and Lagler's (1949) hypothesis that the *M. margariscus* population in Harvey Lake is divergent. It is evident, however, that microsatellite loci indicate that all other populations on Isle Royale are also significantly divergent from one another. Limited sampling of mainland populations would seem to imply that the degree of differentiation among Isle Royale populations is relatively no greater than that found among mainland populations. This study makes no attempt to correlate the degree of microsatellite distribution divergence with degree of morphological divergence as proposed by Hubbs and Lagler (1949). Bailey and Smith's (1981) claim of insufficient data to support Hubbs and Lagler's proposed morphological divergence of Harvey Lake pearl dace is not directly addressed by our findings. However, our findings do indicate that Harvey Lake and other Isle Royale lake

populations of *M. margariscus* are genetically distinct gene pools that, given the National Wilderness Area status of Isle Royale, may warrant special consideration and management.

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