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Genetic Polymorphisms in Fishers (Martes pennanti)

ABSTRACT.—Genetic polymorphisms were investigated in fishers (*Martes pennanti*) using horizontal starch gel electrophoresis of allozymes. During the 1996–1997 fisher trapping season muscle and liver samples were collected from animals in New Hampshire, New York, Wisconsin, and West Virginia, whereas blood samples were collected from New Hampshire and New York animals only. A total of 30 gene loci were resolved from muscle, liver and blood samples. For the entire pooled sample, the mean multilocus heterozygosity (direct count) was 0.047 and the percentages of loci polymorphic (0.95 criterion and 0.99 criterion) were 20.8 and 45.8, respectively.

INTRODUCTION

The importance of preserving and maintaining genetic variation is well established and in the past few decades hundreds of species have been studied using biochemical genetic techniques to assess levels of genetic diversity (Nevo, 1978; Mitton and Grant, 1984; Leberg, 1993). In recent years, many studies have been involved in assessing and comparing levels of genetic variation in carnivores, mustelids in particular (Hartl *et al.*, 1988; Mitton and Raphael, 1990; Lidicker and McCollum, 1997; Serfass *et al.*, 1998). However, despite its status as being one of the most commonly reintroduced furbearers in North America (Berg, 1982), there are no data available on levels of gene diversity in the fisher (*Martes pennanti*). The purpose of this study was to identify informative gene loci in fishers and to provide the first report on levels of genetic variation present in this species.

MATERIALS AND METHODS

Blood, liver and muscle collection was facilitated through cooperative arrangements with biologists in four states during the 1996–1997 trapping season. Muscle and liver samples (n = 49) were collected from fishers in Wisconsin (n = 33), New York (n = 4), New Hampshire (n = 4), and West Virginia (n = 8). An additional 39 blood samples were collected from live-trapped fishers from New York (n = 10) and New Hampshire (n = 29). Muscle and liver samples taken in the field were temporarily stored on wet ice until they could be transferred to freezers (−20 C or lower). Blood samples taken from live animals were quickly put onto wet ice, centrifuged, separated out into serum, white blood cells, red blood cells and then frozen at the lowest temperature possible until shipment to Purdue University. All samples were stored in an ultracold freezer (−75 C) until analysis.

Horizontal starch-gel electrophoresis (Selander *et al.*, 1971; Manlove *et al.*, 1975) was performed on blood, liver and muscle samples to detect genetic variation. After screening 47 enzyme systems surveyed in other mustelids (Simonsen, 1982; Hartl *et al.*, 1988; Serfass *et al.*, 1998), 21 enzymes encoded by 30 presumptive gene loci were selected and surveyed for genetic variation in fishers (Table 1). Six of the 30 loci were found in blood samples only and were not resolvable in other tissues. Polymorphisms were determined through direct side-by-side comparison of the migrations of proteins relative to the most common allele. Overall calculations of mean multilocus heterozygosity, mean number of alleles per locus and percent polymorphism were based on the 24 loci resolved in muscle and liver (n = 49) from the four pooled populations. Genotypic data were analyzed using the genetic analysis software package BIOSYS-1 (Swofford and Selander, 1981).

RESULTS AND DISCUSSION

Allele frequencies and single locus heterozygosities (direct count, h) were calculated for each population sampled (Table 2). Single locus heterozygosities (h) of the variable loci for the four populations ranged from 0.030 to 0.750 (Table 2). Across the four populations there were 12 loci that exhibited polymorphisms (Table 2). For the entire sample of fishers, the direct count mean multilocus heterozygosity (H) was 0.047 (SE 0.017), the mean number of alleles per locus was 1.63 (SE 0.16) and the percent polymorphism was 20.8 (0.95 criterion) and 45.8 (0.99 criterion), respectively.

The mean multilocus heterozygosities of fishers are similar to mean heterozygosities estimated for other mustelid species. For example, Lidicker and McCollum (1997) calculated the mean multilocus

TABLE 1.—A list of 30 loci studied in 88 fishers (*Martes pennanti*) from New Hampshire, New York, Wisconsin and West Virginia. Locus acronyms, enzyme commission numbers (E.C.), tissue types and gel buffers with respective pHs are presented

Loci		E.C.	Tissue[a]	Buffer[b]
AAT-1	Aspartate aminotransferase-1	2.6.1.1	L	TC 8.0
AAT-2	Aspartate aminotransferase-2	2.6.1.1	L	TC 8.0
ACP-2	Acid phosphatase-2	3.1.3.2	L	AC 6.1
ALB	Albumin		B	LiOH 8.1
CK-1	Creatine kinase-1	2.7.3.2	M	AC 6.1
CK-2	Creatine kinase-2	2.7.3.2	M	AC 6.1
DIA-2	Diaphorase-2	1.8.1.4	L	TC 8.0
EST-1	Esterase-1	3.1.1.1	M	TC 8.0
EST-2	Esterase-2	3.1.1.1	M	TC 8.0
FH	Fumerate hydratase	4.2.1.2	M	TC 8.0
GLUDH	Glutamate dehydrogenase	1.4.1.2	L	TC 8.0
βHB	β-Hemoglobin		B	THCL 9.0
IDDH	L-iditol dehydrogenase	1.1.1.14	L	AC 6.1
IDHP-1	Isocitrate dehydrogenase-1	1.1.1.41	M	TC 8.0
IDHP-2	Isocitrate dehydrogenase-2	1.1.1.41	M	TC 8.0
LDH-1	Lactate dehydrogenase-1	1.1.1.27	M	TC 8.0
LDH-2	Lactate dehydrogenase-2	1.1.1.27	M	TC 8.0
MDH-1	Malate dehydrogenase-1	1.1.1.37	L	AC 6.1
MDH-2	Malate dehydrogenase-2	1.1.1.37	L	TC 8.0
ME-1	Malic enzyme-1	1.1.1.38	M	PK 8.2
MPI	Mannose-6-phosphate isomerase	5.3.1.8	L	TC 8.0
NP	Nucleoside phosphorylase	2.4.2.1	M	TM 7.4
PEP-1	Peptidase-1	3.4.11.–	M	TC 8.0
PEP-2	Peptidase-2	3.4.11.–	B	THCL 9.0
PEP-3	Peptidase-3	3.4.11.–	B	THCL 9.0
PGDH	6-Phosphogluconate dehydrogenase	1.1.1.44	M	TM 7.4
PGM-1	Phosphoglucomutase-1	5.4.2.2	L	TC 6.7
PGM-3	Phosphoglucomutase-3	5.4.2.2	L	TC 6.7
PPT-B	Plasma protein-B		B	LiOH 8.1
TF	Transferrin		B	LiOH 8.1

[a] L = liver, M = muscle, B = blood

[b] AC 6.1 = Amine-Citrate 6.1, TC 8.0 = Tris-Citrate 8.0, TC 6.7 = Tris-Citrate 6.7, TM 7.4 = Tris-Maleate 7.4, PK 8.2 = Poulik discontinuous Tris-Citrate 8.2, LiOH 8.1 = Lithium Hydroxide 8.1, THCL = Tris Hydrochloric Acid 9.0.

heterozygosity of the California sea otter (*Enhydra lutris*) at 0.049, whereas the mean heterozygosities estimated for populations of North American river otters (*Lontra canadensis*) ranged from 0.018 to 0.032 (Serfass *et al.*, 1998). In addition, a survey of genetic variation in mustelids, by Hartl *et al.* (1988), provided estimates of multilocus heterozygosities consistent with our findings for fishers (*Mustela nivalis,* 0.066; *M. erminea,* 0.033; *M. putorius,* 0.012; *Meles meles,* 0.014; and *Martes foina,* 0.019). Our heterozygosity estimates for fishers also are comparable to the mean heterozygosities for mammals in general estimated by Nevo (1978) at 0.0359 and Wooten and Smith (1985) at 0.039.

TABLE 2.—Allele frequencies and single locus heterozygosities (direct count, h) for the twelve variable loci examined in fishers (*Martes pennanti*) from New Hampshire, New York, Wisconsin and West Virginia

Locus	New Hampshire	New York	Wisconsin	West Virginia
AAT-2				
(N)	4	4	33	8
A	1.000	1.000	0.970	1.000
B	0.000	0.000	0.015	0.000
C	0.000	0.000	0.015	0.000
(h)	0.000	0.000	0.061	0.000
ACP-2				
(N)	4	4	33	8
A	0.625	1.000	0.985	0.750
C	0.375	0.000	0.015	0.250
(h)	0.750	0.000	0.030	0.250
DIA-2				
(N)	4	4	33	8
A	1.000	1.000	0.985	1.000
B	0.000	0.000	0.015	0.000
(h)	0.000	0.000	0.030	0.000
EST-2				
(N)	4	4	33	8
A	1.000	1.000	0.758	1.000
C	0.000	0.000	0.242	0.000
(h)	0.000	0.000	0.303	0.000
FH				
(N)	4	4	33	8
A	1.000	0.750	0.636	1.000
B	0.000	0.250	0.364	0.000
(h)	0.000	0.500	0.303	0.000
GLUDH				
(N)	4	4	33	8
A	0.750	0.625	0.712	0.688
B	0.250	0.250	0.182	0.313
C	0.000	0.125	0.106	0.000
(h)	0.500	0.250	0.212	0.125
IDDH				
(N)	4	4	33	8
A	1.000	0.625	0.939	0.875
B	0.000	0.000	0.030	0.000
C	0.000	0.375	0.030	0.125
(h)	0.000	0.750	0.121	0.250
IDHP-1				
(N)	4	4	33	8
A	1.000	1.000	0.970	1.000
B	0.000	0.000	0.030	0.000
(h)	0.000	0.000	0.000	0.000

TABLE 2.—Continued

Locus	New Hampshire	New York	Wisconsin	West Virginia
MDH-2				
(N)	4	4	33	8
A	1.000	1.000	0.970	1.000
B	0.000	0.000	0.015	0.000
C	0.000	0.000	0.015	0.000
(h)	0.000	0.000	0.061	0.000
MPI				
(N)	4	4	33	8
A	1.000	1.000	0.985	1.000
C	0.000	0.000	0.015	0.000
(h)	0.000	0.000	0.030	0.000
PEP-2[a]				
(N)	28	10	0	0
A	0.768	0.650	—	—
C	0.232	0.350	—	—
(h)	0.036	0.500	—	—
PGM-1				
(N)	4	4	33	8
A	1.000	1.000	0.985	1.000
B	0.000	0.000	0.015	0.000
(h)	0.000	0.000	0.030	0.000

[a] Loci resolved in blood samples only

[1] The remaining 18 loci were monomorphic for all populations: AAT-1, ALB[a], CK-1, CK-2, EST-1, βHB[a], ICD-2, LDH-1, LDH-2, MDH-1, ME-1, NP, PEP-1, PEP-3[a], PGDH, PGM-3, PPT-B[a], TF[a]

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