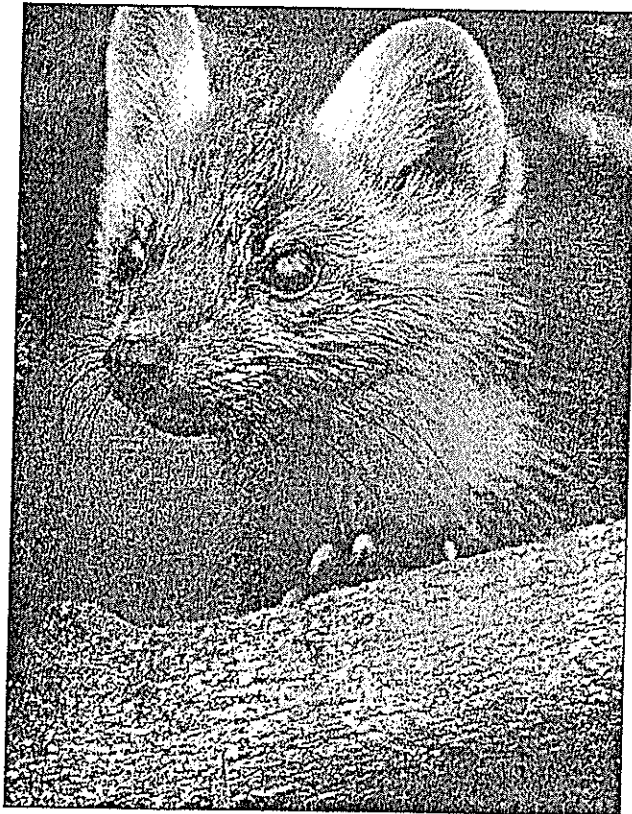
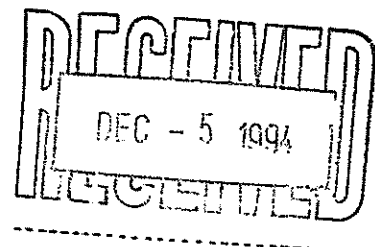


# A Genetic Analysis of Pine Marten (*Martes americana*) in the Western Newfoundland Model Forest.

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## SUMMARY

The population genetics of a western Newfoundland pine marten population was investigated using a RAPD (randomly amplified polymorphic DNA) analysis. The specific goals of this project were a) to establish base line genetic data for comparison with future studies or studies from different regions, b) to determine the level of genetic variability within the western Newfoundland marten population, c) to determine if there is any genetic differentiation between geographically isolated groups of pine marten and d) develop genetic markers which might be useful in captive breeding programs or to monitor family relationships and dispersal patterns.

A total of 23 pine marten were sampled from three locations and analyzed using six different random primers. Of 44 loci scored, 36 were polymorphic, however 35 of these were for rare alleles occurring in only one or two individuals. In general, the RAPD analysis revealed very low levels of genetic variation in the pine marten population of western Newfoundland. There is no evidence of genetic differentiation between marten from the three sample locations. The pine marten in the western Newfoundland model forest can probably be managed as a single population.

The low level of genetic variation in this population may be the result of a contracting population size or a founder effect caused by limited and infrequent colonisation of the island from the mainland. In either case, the paucity of genetic variability may limit the animal's ability to adapt to a changing environment. Comparison of this data set with samples from Labrador and the rest of Canada would broaden our current picture of pine marten genetics.

The RAPD procedure does not appear to be suited for use in a pine marten captive breeding program. A different molecular technique, such as microsatellite markers, might provide the type of information necessary for such a project.

## INTRODUCTION

The Newfoundland pine marten (*Martes americana atrata*) has been listed as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Information concerning the genetic status of any animal population can be helpful when shaping management decisions and is particularly important when dealing with threatened or endangered species. If genetic variability is limited, a small population will be vulnerable to extinction in a far shorter period of time than freely mixing populations (Ballou and Cooper 1992). The identification of subpopulations within a region can help in determining the most efficient unit of management. Polymorphic genetic markers can be used in captive breeding populations to keep track of families, avoid full sibling matings and prevent inbreeding depression (Ashworth and Parkin 1992). They can also be used to determine the amount of variability within a captive population such that levels similar to those in the wild can be maintained (Ballou and Cooper 1992). Genetic markers have been used in behavioral ecology studies to establish maternal and paternal relationships as well as investigate dispersal patterns (Marinelli et al. 1992).

Recently developed techniques in molecular biology are beginning to be used more often in population genetics as well as population and behavioral ecology. Many of these new technologies can be used to analyze small quantities of tissue which are sampled non-invasively from live trapped animals. The polymerase chain reaction (PCR) can be used to amplify DNA extracted from a small number of hair root cells pulled out of an animal's tail. This permits uncomplicated sampling of tissue in the field, with minimal stress to the animal. Randomly amplified polymorphic DNA (RAPD) analysis is a PCR-based technique which has been used extensively in the study of plant and animal populations (Dawson et al 1993,

Gibbs et al. 1994). It is an extremely efficient method for detecting a large quantity of DNA polymorphisms which can be used as genetic markers (Grattapaglia and Sederoff 1994).

The Western Newfoundland Model Forest has the long term goal of protecting biodiversity and, more specifically, increasing the pine marten population to a level beyond their current threatened status. With this objective in mind, RAPD analysis has been used to investigate the population genetics of pine marten in western Newfoundland. The aims of the project were;

- a) To establish base line genetic data for comparison with future studies or with studies from different regions.
- b) To determine the level of genetic variability within the western Newfoundland marten population.
- c) To determine if there is any genetic differentiation between geographically isolate groups.
- d) Develop genetic markers which might be useful in captive breeding programs or to monitor family relationships and dispersal patterns.

## MATERIALS AND METHODS

*Sample collection*

Pine marten hair samples were collected from three locations in western Newfoundland during the fall of 1993 and winter of 1994 (Figure 1). Hair, along with its root, was pulled from the tail of live-trapped animals and stored dry, at ambient temperature, in plastic test tubes. Samples were kept this way until DNA extraction.

*DNA extraction*

DNA was isolated from hair using a protocol developed by the RCMP forensics division. For each sample, eight to ten hair roots were cut approximately 5 mm from the end of the shaft and placed in a 1.5 ml microcentrifuge tube. The tissue was digested for 16 hours (overnight) at 56 °C in 0.5 ml of DNA extraction buffer (10 mM Tris-HCl pH 8, 10 mM EDTA, 2% SDS, 0.1 M NaCl and 40 mM DTT) with 10 µl of proteinase K solution (20 mg/ml). Three successive organic extractions were performed using 0.5 ml of phenol, phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1) respectfully. The purified DNA was precipitated with two volumes of cold (-20 °C) 95% ethanol and left at -20 °C for one hour before centrifugation at 10,000 x g for 20 min. The DNA pellet was washed with cold 70% ethanol then dried and dissolved in 20 µl of TE buffer and used directly for PCR.

*Amplification conditions and electrophoresis*

RAPD reactions were carried out in 25 µl volumes of 50 mM KCl, 10 mM Tris-HCl pH 9, 0.1% Triton X-100, 3 mM MgCl, 0.2 mM each of dATP, dTTP, dCTP and dGTP (Pharmacia), 1 unit of Taq DNA polymerase (Promega), 0.4 µM of 10-base random primer (Biotechnology Laboratory and Dept. of Forestry, University of British Columbia,

see Table 1) and 1  $\mu$ l of pine marten DNA (due to the small amount of marten DNA available, this volume was determined empirically). A negative control (no template DNA) was run with each set. Reactions were overlaid with a drop of mineral oil and run in a Perken Elmer Cetus DNA Thermal Cycler programmed for 5 min at 95 °C, then 34 cycles of 45 sec at 94 °C, 45 sec at 37 °C and 1 min 30 sec at 72 °C, followed by five minutes at 72 °C. Completed reactions were stored at 5 °C until subjected to electrophoresis.

Reaction products were separated according to size using a 2.5% agarose gel in 1 x TBE buffer run at a constant voltage of 56 V for 16 hours (overnight). Gels were stained in ethidium bromide for fifteen minutes then destained in distilled water for up to two hours before visualising bands under U.V. light. A polaroid photo with monochrome photographic negative was taken as a permanent record of the result and was used in subsequent analysis of the RAPD profiles.

#### *Analysis of RAPD profiles*

The monochrome photographic negative was enlarged and stored on disk using an HP DeskScan II. Loci were identified according to their molecular weight and the primer used to generate them (Table 2, see Appendix). Each individual was independently scored by eye.

## RESULTS

A RAPD analysis was performed on hair root samples of 23 pine marten taken from three different locations; 15 from the Marten pond area, 4 from the Glitter pond area and 4 from the Puddle pond area (Figure 1, Table 2).

Eleven random primers were tested and six were selected for the analysis based on the reproducibility and clarity of banding patterns. Amplified products were obtained from the DNA of all samples, however three individuals failed to amplify with three of the primers (Table 2). This may have been the result of poor DNA quality or impurities in the DNA sample which can inhibit the reaction. Analysis of these samples will be repeated at some time in the future.

The RAPD analysis revealed very low levels of genetic variation in the pine marten population of western Newfoundland. Of 44 loci scored, 36 were polymorphic with variant alleles. However, 35 of these were rare alleles occurring in only one or two individuals. The most informative locus was a 400 bp band generated using primer UBC184. The small number of samples collected at Puddle and Glitter ponds and the paucity of informative loci limit the conclusions that can be drawn from this information with respect to population structuring.

One individual, pine marten 53 from Glitter pond, scored consistently different from the rest of the population and possessed 26 of the 36 variant alleles. This animal was trapped a second time during the winter and the analysis repeated with the same results.

## DISCUSSION

There are few equivalent analysis of marten populations from the mainland with which to compare the results presented here. Hicks and Carr (1991) found no difference in the cytochrome b nucleotide sequences of Newfoundland and mainland pine marten, however RAPDs are expected to identify more polymorphic markers than this method. Mitton and Raphael (1990) found high levels of protein electrophoretic variation in a Wyoming population of pine marten. A recent genetic analysis of black bears (*Ursus americanus*) found that populations on the island of Newfoundland have much lower levels of microsatellite DNA variability than those on the mainland (Paetkau and Strobeck 1994). An investigation of mainland pine marten populations using a RAPD or an equivalent type of analysis would help to clarify the current picture of pine marten genetics.

It is difficult to determine the causes and possible consequences of low genetic variability in the western Newfoundland pine marten. Genetic variation may be lost through random drift as an animal's range contracts in size and variant individuals are lost from the population. This size reduction could be the result of habitat losses the marten is currently experiencing or may have occurred naturally at some period in the prehistoric past. Limited colonisation from the mainland and founder effects might also contribute to the paucity of genetic variation observed in this population. Although many mammalian species seem to do well with low levels of genetic variation (Wooten and Smith 1984), such populations may not be under any kind of stress caused by changes to the environmental. A lack of variability may make it difficult for the pine marten to adapt to a radically altered habitat.

## CONCLUSIONS

- 1) Base-line genetic information has been established for future studies involving pine marten on the island of Newfoundland or elsewhere. It is important to broaden this data-base and make it available for comparison with similar studies on pine marten or other organisms.
- 2) It has been determined that the level of genetic variability in the western Newfoundland pine marten population is low. This may limit the animals ability to adapt to the rapid changes in habitat it is currently experiencing.
- 3) There is no evidence of genetic divergence between geographically separated groups of marten within the model forest. These animals can be managed as a single population.
- 4) The RAPD procedure is not providing the type of information that would be of use in a captive breeding program. Other techniques, such as microsatellite analysis (also PCR based), might be better suited to such an application..
- 5) RAPDs provides an efficient means for analysis of small quantities of DNA which can be sampled non-lethally, with minimal stress to an animal in the field.

## FUTURE PLANS

Currently, we would like to use dried skins from Labrador to expand the current data-base and investigate differences that might exist between mainland and Newfoundland pine marten. We would also like to investigate the applicability of microsattelites to a pine marten captive breeding program.

## ACKNOWLEDGMENT

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Figure 1: Areas where pine marten were trapped and sampled. A) Marten pd., B) Puddle pd. and C) Glitter pd. (1 cm = 4 km).

Table 1: Random primers selected for analysis

<u>Primer</u>	<u>Sequence</u>	<u>Observed loci</u>
UBC184	CAAACGGCAC	12
UBC196	CTCCTCCCCC	5
UBC195	GATCTCACGC	6
UBC197	TCCCCGTTCC	4
UBC193	TGCTGGCTTT	8
UBC198	GCAGGACTGC	9





APPENDIX: EXAMPLES OF RAPD PROFILES

Primer UBC184

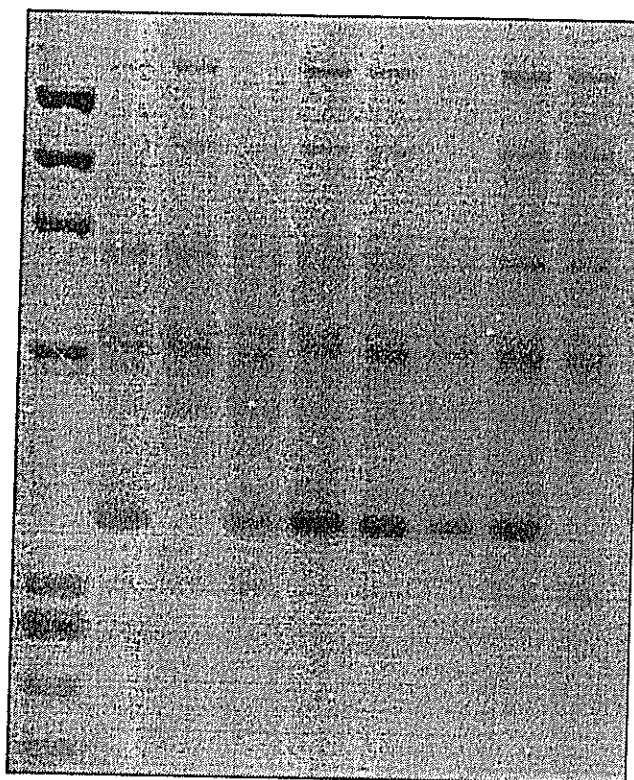
1353 bp

1078 bp

872 bp

603 bp

310 bp



Primer UBC196

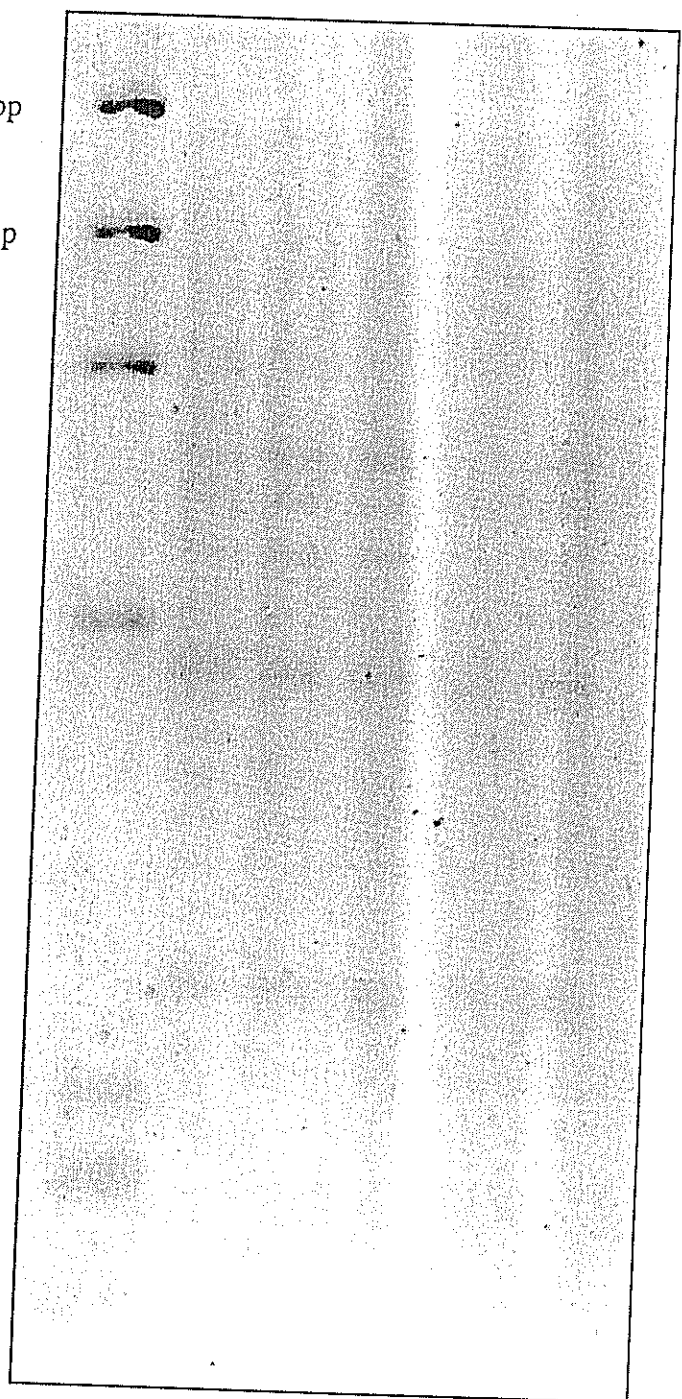
1353 bp

1078 bp

872 bp

603 bp

310 bp



Primer UBC195

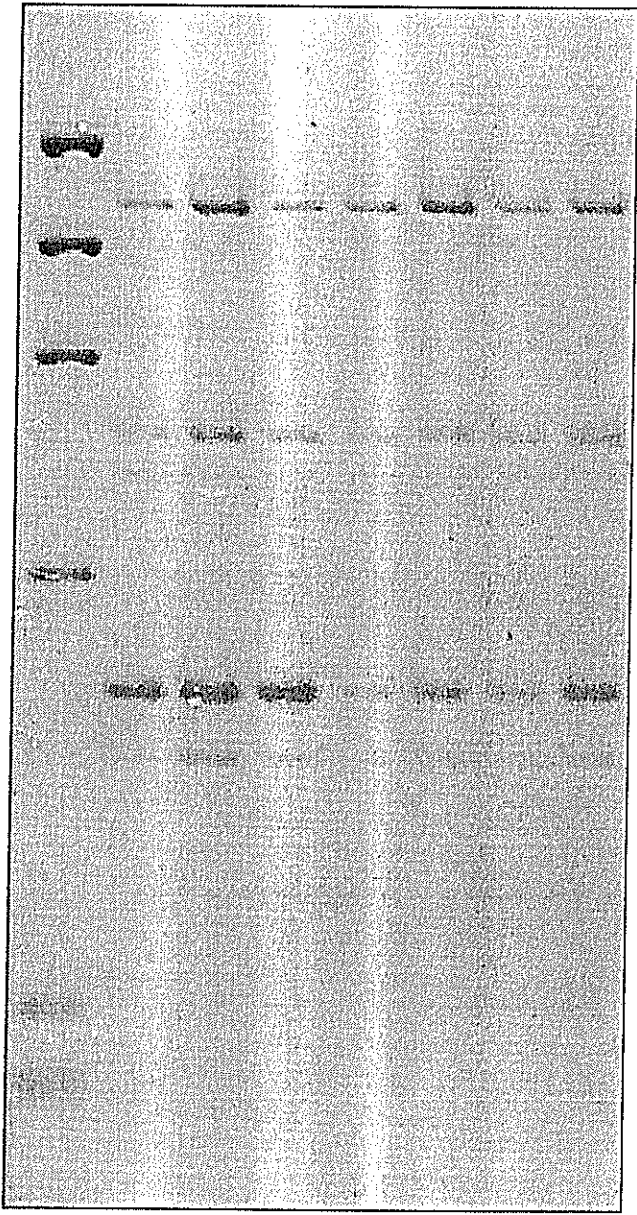
1353 bp

1078 bp

872 bp

603 bp

310 bp



Primer UBC193

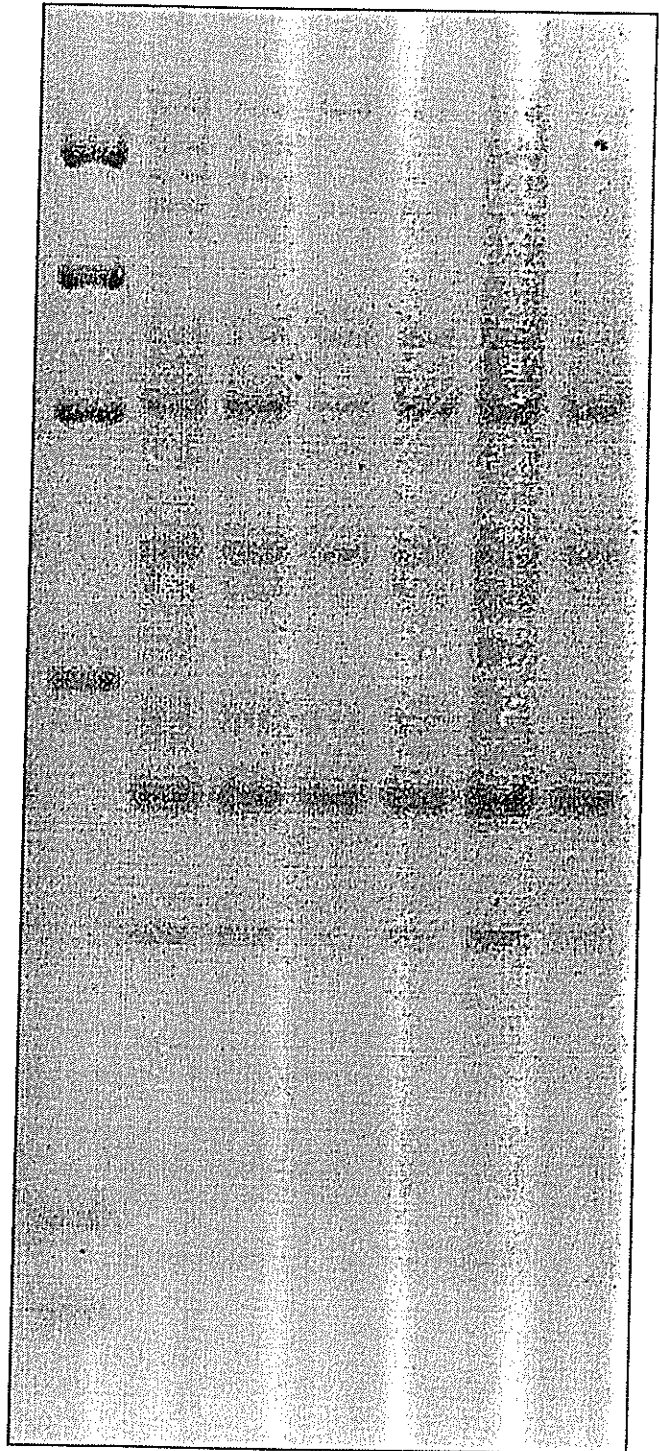
1353 bp

1078 bp

872 bp

603 bp

310 bp



Primer UBC198

1353 bp

1078 bp

872 bp

603 bp

310 bp

