8-2002

Systematics of Northeastern Meadow Vole (Microtus pennsylvanicus) Subspecies, with Empasis on the Island Endemic (M. P. Shattucki, Howe 1901) in Penobscot Bay, Maine

Jennifer Marie Lowry

SYSTEMATICS OF NORTHEASTERN MEADOW VOLE (*MICROTUS PENNSYLVANICUS*) SUBSPECIES, WITH EMPHASIS ON THE ISLAND ENDEMIC (*M. P. SHATTUCKI*, Howe 1901) IN PENOBSCOT BAY, MAINE

By

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B.S. University of Florida, 1998

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Wildlife Ecology)

The Graduate School

The University of Maine

August, 2002

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An Abstract of the Thesis Presented
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The Penobscot meadow vole (*Microtus pennsylvanicus shattucki*) (PMV) is an insular subspecies of meadow vole (*M. pennsylvanicus*) inhabiting the islands of North Haven, Islesboro, and Tumbledown Dick in Penobscot Bay, Maine. It is one in a suite of island meadow vole subspecies which has been described from southern New England through eastern Canada. The subspecific recognition of *M. p. shattucki*, along with the others in this group, was solely based on a univariate analysis of a few morphological characters, which has fostered debate about the validity of the subspecies. Despite this uncertainty, the taxonomy is widely applied and conservation issues have been raised: *M. p. shattucki* was listed as a Species of Special Concern in the state of Maine when that listing was in use. The U.S. Fish and Wildlife Service did not propose *M. p. shattucki* for listing at the federal level because of lack of information on the subspecies. Concern about losing unique island taxa such as the PMV is warranted because another subspecies in this group, *M. p. nesophilus*, which was found on Gull Island, NY has already gone extinct.

To clarify the taxonomic status of *M. p. shattucki* for conservation purposes, I used multivariate discriminant function analysis (DFA) to examine historical and recent morphological differences in 14 cranial and three external characters. Historical differentiation was quantified through DFA of museum specimens. To study recent morphological differentiation, extant populations were sampled from the type localities of *M. p. shattucki* (Islesboro and North Haven), as well as populations of *M. p. pennsylvanicus* on another island in Penobscot Bay (Isle au Haut), the closest mainland coastal populations to Islesboro and North Haven (Northport and Rockport, respectively), and an inland mainland site, Orono. To further clarify distinctiveness of *M. p. shattucki*, genetic differentiation of extant populations was investigated by genotyping seven microsatellite loci and doing a phylogenetic analysis of the mitochondrial DNA control region.

M. p. shattucki is morphologically and genetically distinct from the mainland nominant populations of *M. p. pennsylvanicus*. Museum specimens were classified correctly at a 90% rate, while extant specimens had an 80% correct classification rate. Overall, *M. p. shattucki* individuals are larger in cranial and external morphology than mainland *M. p. pennsylvanicus*,. Mitochondrial DNA analysis indicated that *M. p. shattucki* formed a monophyletic lineage. Microsatellite analysis supported this result with the highest genetic distances being between *M. p. shattucki* and populations of *M. p. pennsylvanicus*. All populations of meadow voles appeared to have high levels of inbreeding, heterozygote deficiency and departure from Hardy-Weinberg equilibrium. This is most likely due to the social structure of meadow vole populations and/or non-amplifying (null) alleles that contribute to high estimates of homozygosity.

The morphological and genetic data in this study support the subspecific status of *M. p. shattucki*. In terms of uniqueness, or exchangeability (whether an individual of one population can be placed in the second population), *M. p. shattucki* is historically and recently distinct both morphologically and genetically and while this evidence is suggestive of *M. p. shattucki* as an Evolutionary Significant Unit (ESU), additional study of *M. p. shattucki* is warranted before this conclusion can be made. The naming of a population as an ESU has possible political ramifications that need to be considered in conjunction with the biological data.

DEDICATION

My scientific curiosity came from two professors who recently passed on to better things: Dr. Larry McEdward and Dr. Michael Humphrys-Beher. I dedicate this scientific research to these two men who not only taught me to question everything, especially myself, but to not sacrifice myself for the research, and to live life to the fullest. The scientific community has lost two great scientists.

Additionally, I would like to dedicate this work to my family: to my father and mother, who always taught me I could do anything, my sister, who taught me by example to go after everything I desire, and my brother, who is protecting our freedoms every day. I would also like to dedicate this work to my nephew, Jake, and my two nieces, Sarah and Caleigh, all who still believe that I "chase rats".

Lastly, there are two people who are responsible for the completion of this degree. Tara Henrichon provided me the support and friendship I needed while at the University of Maine. Brad Cayer was eternally patient with me during the past two years. I owe them both my sanity and my appreciation.

ACKNOWLEDGEMENTS

Funding for this project was provided by Maine Outdoor Hertiage Fund, Maine Inland Fisheries and Wildlife Endangered Species Group, Penobscot County Conservation Association, and from the University of Maine: McIntire-Stennis, the Association of Graduate Students and the Green Endowment Fund. I would especially like to single out Dr. Mark McCollough, whom first directed me to this project and showed interested throughout the progress of my research.

Judith Rhymer was instrumental in the completion of this work, as were the other members of my committee. I especially would like to thank Bill Glanz, without whom I would have not caught many voles, nor measured them, nor cleaned them. His help made this thesis possible. Additionally, Fred Servello was always willing to help with ideas for sites and for encouragement.

I would like to thank the following curators and institutions for smoothing the loan process of the museum specimens: Bob Randall, from the American Museum of Natural History, Robert Fisher from Smithsonian National Museum of Natural History, Michel Gosselin from the Canadian Museum of Nature, and Maria Rutzmoser and Terri McFadden from the Harvard Museum of Comparative Zoology. Robert Fisher particularly helped me when I visited the Smithisonian's mammal collection in January, 2000.

Many volunteers helped me in the field, especially Joe Tomazin, my pack mule, and Jon McClosky, whom listened to me worry about whether we were going to get off of Isle au Haut.

Stephen Miller from the Islesboro Land Trust was instrumental in finding sites for trapping on Islesboro, and I'd like to recognize the landowners on Islesboro: Joan Lillie, Charlie and Kathy Bernier, Langhorne Smith, Mrs. Douglas Auchincloss, Mary Homans, and Ed Lawrence. Mr. and Mrs. James Carpenter provided an excellent trapping site on North Haven. Acadia National Park and the two rangers, John and Wayne, were extremely helpful in transportation around Isle au Haut and the use of the cabin. Additionally on Isle au Haut, Mr. and Mrs. Wilson provided a picturesque view as well as an excellent field for trapping. On the mainland, I'd like to thank the KFC corporation for use of their field at Rockport and Mary Gilman and the Edna Drinkwater Elementary School at Northport.

I would like to thank my fellow graduate students for their day to day support, especially Tara Henrichon, who provided me constant support and an endless supply of coffee. I would also like to thank Brad Cayer, whose love, support, and patience allowed me to prioritize my research endeavors. And last but never least, I would like to thank my family for their ability to help me keep everything in perspective.

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CHAPTER 1. MORPHOLOGICAL DIFFERENTIATION

Introduction

Island populations have a natural barrier from mainland source populations. Given enough time and distance from the mainland, island populations may differentiate from the nominate mainland species into novel subspecies and species (Berry 1998). The majority of research concerning species on islands has concentrated on the effect of isolation and area of the island on species assemblages and richness (i.e. MacArthur and Wilson 1963, Crowell 1986), although it is possible that an island population could be on a different evolutionary path than its mainland counterpart. Primary indication of differentiation has historically been quantified through morphological analyses, and there are several theories that exist to explain the tendency for small mammals to be larger on islands. Climate may be a factor: larger body size in small mammals could be due to a less variable climate which would not physiologically restrict an animal's body size as a more variable climate would (Case 1978). Faunal changes may be a factor: a smaller or more isolated island would have less predator and competitor species, which may allow larger animals to survive in the absence of size selective predators or interspecific competition (MacArthur and Wilson 1963, Heaney 1978). Lastly, the niche theory also may explain the trend in larger body size: because of a lack of species on an island, niches are vacant, and small mammals will move to fill that niche, which may lead to an increase in body size (Case 1978). It is further suggested that one can predict body size of small mammals if the body size of an absent competitor is known (Case 1978). Anderbjorn (1986) studied these theories with data from insular rodents of Britain and

concluded that the two main factors influencing an increase in body size are competition from other rodents, most likely because of the importance of body size in interspecific competition, and the lack of size selective predators that remove larger rodents from a population. The trend for small mammals to be larger on islands is known as the island rule (Foster 1964). Because of these factors, morphological differences between island and mainland populations may not be evolutionary change due to different selection pressures in the new island environment (Mayr 1963), but to the island effect. Additionally, cline variation needs to also be considered in this type of study (Mayr 1963).

A suite of island meadow vole (*Microtus pennsylvanicus*) subspecies has been described from southern New England through eastern Canada: *M. p. nesophilus,* Gull Island, NY, *M. p. provectus,* Block Is., RI, *M. p. breweri,* Muskeget Is. MA, *M. p. shattucki,* Islesboro, North Haven, ME, *M. p. copelandi,* Grand Manan, New Brunswick, *M. p. magdalenensis,* Magdalen Is., Québec, *M. p. acadicus,* Nova Scotia, and *M. p. terranovae,* Newfoundland (Youngman 1967, Figure 1.1). Sub-specific status of these populations was primarily based on univariate analysis of a few morphological variables comparing island and mainland populations (Tamarin 1985).

Chamberlain (1954) used univariate analysis of a few skull morphological variables to argue against raising *M. p. provectus* (the Block Island vole) to specific status. Although pelage color and tail length differed between mainland and island populations, there was no significant difference in skull characteristics (greatest length of skull, zygomatic breadth, interorbital construction, nasal length, and maxillary tooth row). He believed that differences in pelage color between the two populations were

attributable to seasonal variation, and that a previous species-level designation was based on specimens that did not include morphological intermediates in populations.

Moyer et al. (1988) performed a complete multivariate statistical analysis of 19 cranial variables and 42 dental variables to study differentiation of insular *M. p. breweri* (Muskeget Island, MA) from mainland *M. p. pennsylvanicus*. Stepwise discriminant function analysis indicated separation of *M. p. breweri* from the rock vole (*M. chrotorrhinus*) and from eight populations of *M. p. pennsylvanicus*. They concluded that *M. p. breweri* should be elevated to full specific status as *M. breweri*.

Taxonomic history of Penobscot meadow vole

Reginald Howe first described the Penobscot field mouse from the islands of Islesboro, North Haven and Tumbledown Dick, in Penobscot Bay, ME (Howe 1901). He named this new subspecies of *M. pennsylvanicus, M. p. shattucki*, based on its larger body size, longer tail, prominent ears, large and globular bulla, broad and bottle-shaped palatine foramina, and darker pelage. However, he did not measure mainland *M. p. pennsylvanicus* from specimens, but used data from a mammalian reference book (Elliot, D. J. 1872 in Howe 1901). Wyman (1953) refuted Howe's (1901) claim, remeasured Howe's specimens, and compared them to specimens from the mainland and other island *M. pennsylvanicus* populations. Using size, ears, coloration, audital bullae, palatine foramina, and tail length, he found that measurements of the *M. p. shattucki* specimens were within the range of *M. pennsylvanicus*, and concluded that the island populations of *M. pennsylvanicus* were not a distinct subspecies.

In another study of island meadow vole populations, Youngman (1967) used univariate analysis of more morphological variables than previous studies. Comparisons

of lambdoidal breadth, zygomatic breadth, length of maxillary tooth row, condylobasal length, length of hind foot, least interorbital breadth, total length and nasal length supported the separation of *M. p. shattucki* as a subspecies, along with two new subspecies: *M. p. magdalenensis* from the Magdalen Islands, Québec and *M. p. copelandi* from Grand Manan Is., New Brunswick. Despite debate on the validity of *M. p. shattucki*, this subspecies has not been further studied, although the taxonomic distinction has been widely used.

The Penobscot meadow vole is not currently listed as threatened or endangered due to a lack of information (U.S.F.W.S. 1994), although it was listed as a Species of Special Concern in Maine when that listing was used (Dr. Mark McCollough, pers. comm.). Given that detailed analysis of another of these island taxa, *M. p. breweri*, indicated that it deserved specific status (Moyer et al. 1988) and that the Gull Island, NY population has gone extinct (Tamarin 1985), this study of the Penobscot meadow vole is warranted. The first step to investigating listing status is to determine if the island populations are distinct from mainland populations. To address the taxonomic status of this subspecies, I reanalyze morphological variation of museum specimens of all the island subspecies in this group, with particular emphasis on differentiation between *M. p. shattucki* and *M. p. pennsylvanicus* populations on mainland Maine and other islands in Penobscot Bay. I also examine the morphological characters of extant populations of *M. p. shattucki* from North Haven and Islesboro and nearby coastal mainland populations and I included a population of *M. p. pennsylvanicus* from another large island in Penobscot Bay (Isle au Haut) to control for variation in traits in isolated island populations.

Methods

Sampling - Museum Specimens

I examined 355 specimens of *Microtus pennsylvanicus* subspecies from island, peninsular, and mainland populations from southern New England through eastern Canada; 21 *M. p. provectus*, Block Is., RI, 25 *M. p. breweri*, Muskeget Is., MA, 21 *M. p. shattucki*, North Haven and Islesboro islands, ME, 34 *M. p. copelandi*, Grand Manan Is., New Brunswick, 91 *M. p. acadicus*, Nova Scotia (peninsula), 45 *M. p. magdalenensis*, Magdalen Is., Québec, 66 *M. p. enixus*, Labrador, Canada (mainland) and 52 *M. p. terranovae*, Newfoundland (Figure 1.1). In addition, 150 specimens of *M. p. pennsylvanicus* from mainland and other island populations from Maine were examined for comparison; 22 specimens from other islands (island Maine), 37 from mainland populations (mainland Maine), 61 from the Gaspé Peninsula in Québec (Gaspe Peninsula), and 30 from the New Brunswick mainland (New Brunswick) (Figure 1.1). Specimens were borrowed from the American Museum of Natural History, Smithsonian National Museum of Natural History, Canadian Museum of Nature, and Harvard Museum of Comparative Zoology.

Sampling - Extant Specimens

Meadow voles were live-trapped on the islands of Islesboro and North Haven, type localities of the Penobscot meadow vole. In addition, populations were sampled on Isle au Haut, another large island in Penobscot Bay. For comparison, mainland populations were surveyed at nearby coastal sites; Rockport is closest to North Haven, and Northport to Islesboro (Figure 1.1). An inland population at Orono was also sampled to analyze variation among mainland populations. Large aluminum Sherman live traps

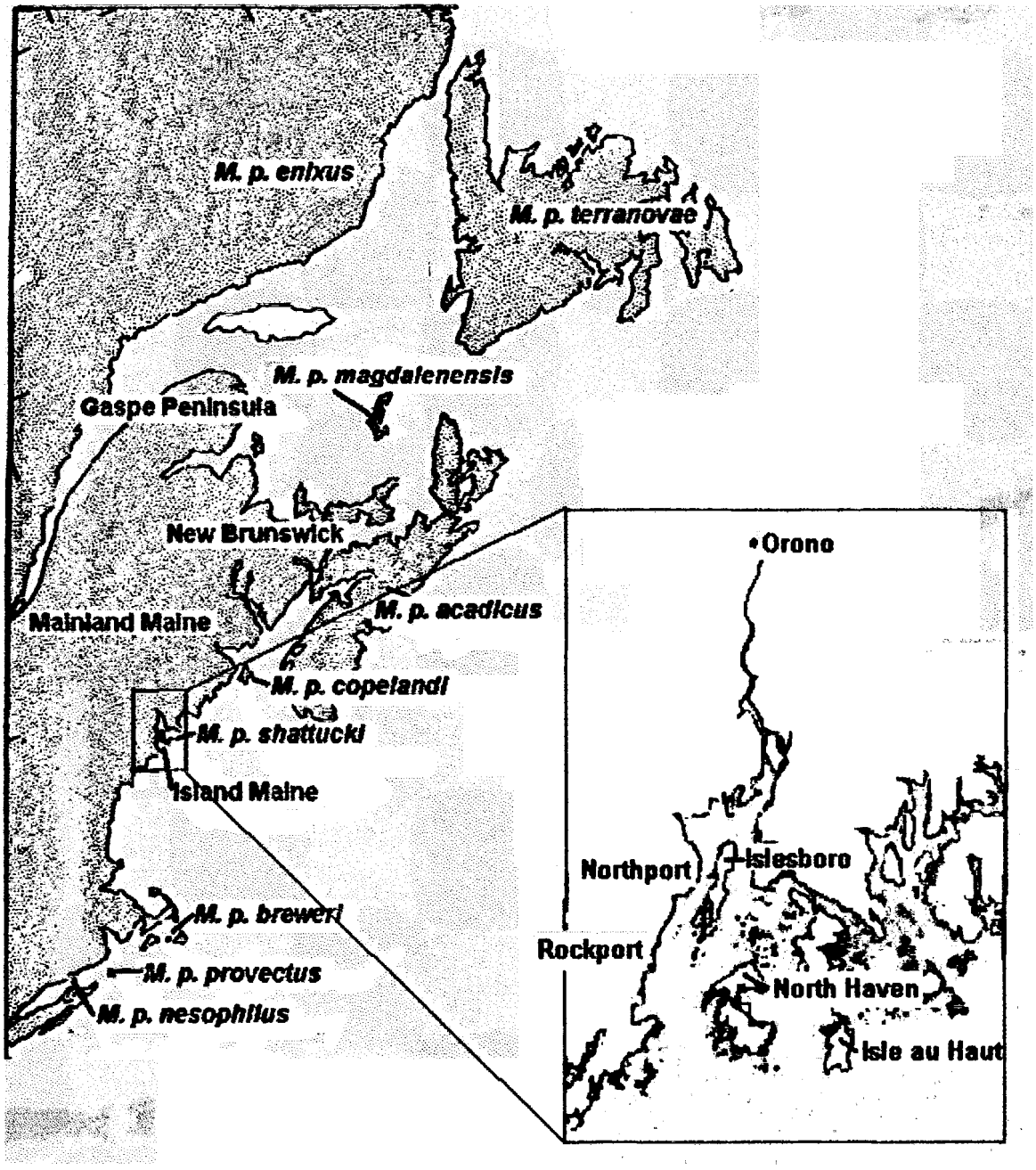

Figure 1.1. Map of northeastern United States and maritime Canada meadow vole (*Microtus pennsylvanicus*) subspecies and extant populations included in this study (insert).

(3x3.5x9") were baited with a mixture of peanut butter and oats, equipped with cotton bedding for warmth, and shaded with natural debris, if needed (Cole 1993). Tall grasslands were identified as possible vole habitat based on the presence of runways (Dr. William Glanz pers. comm.). Traps were placed in the runways to maximize trapping efficiency. Non-target species were released with minimal handling. The last two millimeters of each juvenile vole's tail were collected for genetic analysis (see Chapter 2). These animals were observed for five minutes to ensure healing of the wound and were released in the same area in which they were caught.

For morphological analysis, adult male and non-lactating female meadow voles were sacrificed by cervical dislocation. Specimens were kept on ice until they could be brought back to the lab and stored at -70° C. All specimens were prepared as museum voucher specimens following Anderson (1965). A colony of dermesitid beetles (*Dermestes maculatus*) was used to clean the majority of tissue from the skulls of specimens. After cleaning, skulls were soaked in a 50% hydrogen peroxide solution for 1.5 hours. Any flesh remaining after soaking was manually removed. Skulls were allowed to dry, and were individually stored in glass bottles.

Measurements and Analysis

I used dial calipers to measure 14 cranial morphometric and three external characters (to 0.01 mm) on adult specimens from museum and extant populations, following Moyer et al. (1998); greatest length of skull (GLS), condylozygomatic length (CZL), least interorbital breadth (LIB), palatine foramina (least and greatest breadth (PFLB, PFGB), total length (PFL), length of upper and lower tooth row (LUTR, LLTR), zygomatic breadth (ZB), cranial length (CL), cranial breadth at the squamosals (CBS),

cranial breadth at zygomatic arch (CBZA), nasal length and breadth (NL, NB), hind foot length (HFL), total length of body (TBL), and tail length (TL). For the museum specimens, the three external measurements – HFL, TBL, and TL – were taken from measurements reported on specimen tags because these were taken before skinning and preparation. These three external measurements were measured on extant specimens before skins were prepared. Because the number of specimens was limited for some populations, I allowed the adult age class to include possible sub-adult specimens whose total body length exceeded 120mm to increase sample size. Sub-adult meadow voles are essentially adults that have yet to reproduce (Tamarin 1985), and for the museum specimens, were labeled as such. Sub-adults from extant populations were indistinguishable from adults.

The distribution of variation for each variable was checked for normality. Multivariate Discriminant Function Analysis (DFA) was used to find the linear combination of variables that best separate populations (Rencher 1995). Although meadow voles are considered a monomorphic species (Tamarin 1985), gender was included as a variable. Stepwise DFA was used to discard any redundant variables. Variables were entered at $P<0.05$ significance level and were removed if $P>0.10$ (Moyer et al 1988). First, untransformed data were used to study overall variation among populations. Secondly, raw measurements were divided by total body length and these ratios were log transformed to control for differences in body size, thus exploring the morphological variation due to variables other than total body length.

Mean and standard deviation were calculated for each variable in each population. Hierarchical cluster analysis, using root mean square (Euclidean) distance as the

clustering option and the average distance between population pairs as the linkage option, was employed to study the taxonomic relationships of these 12 populations.

For the museum specimens, an initial DFA including all 12 populations was done to study morphological relationships of meadow vole subspecies of the northeast United States and maritime Canada. An additional comparison focusing on the subspecies of interest - *M. p. shattucki*, *M. p. pennsylvanicus* on other islands in Penobscot Bay, and *M. p. pennsylvanicus* of mainland Maine - was also done. Extant populations were initially compared as individual populations using DFA. This overall comparison was followed by an additional comparison: *M. p. shattucki* (combining North Haven and Islesboro populations) versus the other Penobscot Bay island (Isle au Haut), coastal Maine (Rockport plus Northport), and inland Maine (Orono). All statistics were performed using Systat IX (SPSS Inc, Chicago, IL) or the Statistical Analysis Software (SAS Institute 1995).

Results

Museum Specimens

Overall comparison

Significant differences were found in morphological characters among populations (Wilks Lambda = 0.193, $p<0.001$). Specimens of *M. p. shattucki* were correctly classified at a much higher percent (90.5%) than any of the other populations (Table 1.1). Those individuals of *M. p. shattucki* that were incorrectly classified were placed with the mainland Maine population (9.5%). Other island classification values were far lower. For instance, specimens of *M. p. breweri*, now considered a valid full species (Moyer et al. 1985) were classified correctly only 64.0% of the time,

Table 1.1. Percent (%) of time each population was classified as each other population in the discriminant function analysis with 12 museum populations of island subspecies, mainland subspecies, and mainland populations of *M. p. pennsylvanicus* for untransformed (top number) and log ratio data (bottom number). For example: *M. p. breweri* is classified as itself 64% (68%) of the time while classified as *M. p. provectus* 4.0 (8.0) percent of the time

	M. p. provectus	*M. p. breweri*	*M. p. shattuckl*	*M. p. pennsylvanicus* (Island Maine)	*M. p. pennsylvanicus* (Mainland Maine)	*M. p. copelandi*	*M. p. pennsylvanicus* (New Brunswick)	*M. p. pennsylvanicus* (Gaspe Peninsula)	*M. p. magdalenensis*	*M. p. acadicus*	*M. p. anixus*	*M. p. terranovae*
M. p. provectus (n=21)	**61.9**	0.0	14.3	4.8	0.0	4.8	0.0	4.8	0.0	0.0	2.7	9.5
	61.9	0.0	14.3	0.0	4.8	4.8	0.0	0.0	4.8	0.0	0.0	9.5
M. p. breweri (n=25)	4.0	**64.0**	4.0	0.0	8.0	0.0	0.0	4.0	8.0	0.0	4.0	4.0
	8.0	**68.0**	4.0	0.0	4.0	4.0	0.0	0.0	12.0	0.0	0.0	0.0
M. p. shattuckl (n=21)	0.0	0.0	**90.5**	0.0	9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	**85.7**	0.0	4.8	0.0	0.0	0.0	0.0	4.8	4.8	0.0
M. p. pennsylvanicus (Island Maine) (n=22)	4.6	0.0	0.0	**72.7**	4.6	0.0	0.0	4.6	0.0	9.1	4.6	0.0
	13.6	0.0	0.0	**54.6**	4.6	0.0	4.6	0.0	4.6	9.0	4.6	0.0
M. p. pennsylvanicus (Mainland Maine) (n=37)	10.8	0.0	0.0	13.5	**35.1**	2.7	5.4	2.7	5.4	18.9	2.7	2.7
	8.1	0.0	2.7	8.1	**43.2**	2.7	8.1	0.0	5.4	13.5	0.0	8.1
M. p. copelandi (n=34)	2.9	2.9	17.7	0.0	5.9	**35.3**	8.8	2.9	0.0	2.9	8.8	11.8
	2.9	2.9	17.7	0.0	2.9	**35.3**	5.9	11.8	2.9	2.9	8.8	5.9
M. p. pennsylvanicus (New Brunswick) (n=30)	0.0	0.0	0.0	0.0	0.0	0.0	**66.7**	10.0	3.3	16.7	3.3	0.0
	0.0	0.0	6.7	0.0	6.7	0.0	**43.3**	6.7	16.7	6.7	13.3	0.0
M. p. pennsylvanicus (Gaspe Peninsula) (n=61)	3.3	0.0	1.6	1.6	19.7	3.3	13.1	**31.1**	3.3	6.6	4.9	1.6
	3.3	0.0	4.9	13.1	11.5	13.1	8.2	**31.1**	3.3	6.6	4.9	0.0
M. p. magdalenensis (n=45)	2.2	15.6	2.2	2.2	0.0	6.7	2.2	0.0	**55.6**	2.2	4.4	6.7
	2.2	15.6	4.4	4.4	4.4	6.7	4.4	4.4	**33.3**	0.0	6.7	13.3
M. p. acadicus (n=91)	4.4	1.1	1.1	4.4	2.2	0.0	29.8	12.1	5.5	**39.6**	6.6	3.3
	5.5	6.6	3.3	3.3	4.4	0.0	15.4	9.9	14.3	**24.2**	7.7	5.5
M. p. anixus (n=66)	3.0	0.0	3.0	7.6	10.6	7.6	4.6	6.1	6.1	13.6	**34.9**	3.0
	3.0	7.6	3.0	7.6	7.6	4.6	6.0	9.0	7.6	12.1	**27.3**	4.6
M. p. terranovae (n=52)	0.0	0.0	0.0	7.7	7.7	11.5	9.6	3.9	9.6	1.9	1.9	**46.1**
	5.8	0.0	3.9	7.8	3.9	1.9	3.9	9.6	11.5	0.0	11.5	**41.4**

M. p. copelandi 35.3% of the time and the remaining populations anywhere from only 31.1% (Gaspé Peninsula) to 66.7% (New Brunswick). The correct classification of *M. p. shattucki* was also high for transformed variables correcting for overall body length (85.7%) (Table 1.1). In other words, *M. p. shattucki* was significantly differentiated in variables such as GLS, LIB, CL, ZB, and CBZA – to name a few – when overall body length was taken into account (Table 1.2).

All variables were retained in this model however, those variables with a canonical coefficient less than 0.100 in CV1 and CV2 were considered less important. The first canonical variate (CV1) explained 38.5% of the variation, with GLS (coefficient = 0.795) and LLTR (0.714) loading most heavily out of 15 total variables on this axis (Table 1.2). Other significant variables on CV1 included LUTR (0.586), ZB (0.553), and TBL (0.538), while the other ten variables did not load as significantly, ranging from PFLB (0.453) to CZL (0.227) (Table 1.2). The second canonical variate (CV2) explained 20.2% of the variation with LIB (0.618) the most significant variable out of 13 total variables. Additional important variables were PFL (0.429), PFGB (0.364), CL (0.253), and PFLB (0.231) with the remaining variables ranging from GLS (0.188) to TLB (0.102). The additional 41.3% of the variation in the model was explained by CV3 (13.6%), CV4 (7.4%), CV5 (6.8%), CV6 (5.1%), CV7 (3.5%), CV8 (2.3%), CV9 (1.5%), CV10 (0.7%) and CV11 (0.4%). When the data were transformed to control for the effect of body size, all variables were retained in the model. The same dominant variables as in the raw data model loaded most significantly on CV1, which accounted for 30.0% of the variation and CV2, which accounted for 21.9% of the variation (GLS and LIB respectively) (Table 1.2). Variables such as LUTR, NL, HFL, and CZL that were

Table 1.2. Variables retained in two DFA models with museum populations, with variable loadings (in parentheses) on the first and second canonical variates (CV1 and CV2).

	Untransformed Data		Log Ratio Data	
	CV1	CV2	CV1	CV2
All Populations	GLS (0.795)	LIB (0.618)	GLS (1.818)	LIB (1.198)
	LLTR (0.714)	PFL (0.429)	LIB (0.768)	ZB (0.821)
	LUTR (0.586)	PFGB (0.364)	CL (0.650)	CBZA (0.585)
	ZB (0.553)	CL (0.253)	ZB (0.593)	PFL (0.521)
	TBL (0.538)	PFLB (0.231)	CBS (0.378)	PFGB (0.457)
	PFLB (0.453)	GLS (0.188)	LLTR (0.347)	CL (0.436)
	NL (0.451)	CZL (0.170)	CBZA (0.303)	CBS (0.420)
	HFL (0.409)	ZB (0.134)	PFL (0.285)	LLTR (0.394)
	CL (0.397)	NL (0.126)	PFLB (0.247)	TL (0.124)
	LIB (0.373)	LUTR (0.117)	PFGB (0.138)	Gender (0.121)
	TL (0.356)	TL (0.104)	TL (0.135)	
	PFGB (0.283)	LLTR (0.104)	NB (0.109)	
	PFL (0.277)	TBL (0.102)		
	CBS (0.237)			
	CZL (0.227)			
Maine Populations	GLS (0.767)	PFLB (0.667)	CZL (1.197)	HFL (0.866)
	CZL (0.753)	TBL (0.557)	TL (0.764)	LIB (0.814)
	TBL (0.616)	LIB (0.454)	LUTR (0.550)	NB (0.633)
	LUTR (0.550)	CL (0.325)	LLTR (0.512)	CL (0.594)
	CL (0.348)	CZL (0.295)	CL (0.362)	CZL (0.511)
	LIB (0.215)	LUTR (0.213)	LIB (0.219)	LUTR (0.276)
	PFLB (0.106)	GLS (0.035)	NB (0.162)	TL (0.226)
			HFL (0.056)	LLTR (0.141)

significant in the raw data model were replaced by CBZA, CBS, and Gender in the transformed model (Table 1.2). The additional 48.1% of the variation in the model was explained by CV3 (14.0%), CV4 (9.5%), CV5 (8.1%), CV6 (6.7%), CV7 (3.6%), CV8 (3.0%), CV9 (1.8%), CV10 (1.0%) and CV9 (0.4%).

Delineation of each population into multivariate space indicates that the five subspecies from small islands (*M. p. provectus, M. p. magdalenensis, M. p. copelandi, M. p. shattucki,* and *M. p. breweri*) separate along CV1 from three mainland populations (New Brunswick, the Gaspé Peninsula, and mainland Maine) and the mixed island sample of *M. p. pennsylvanicus*, as well as from the mainland (*M. p. enixus*), peninsular (*M. p. acadicus*) and large island (*M. p. terranovae*) (Figure 1.2). As mentioned, GLS was the primary variable discriminating among populations on CV1. Average skull length ranged from 26.90 mm (*M. p. provectus*) to 29.10 mm (*M. p. breweri*) in the five small island vole populations, compared to 25.50 – 26.69 mm in the other group of populations. CV2 dramatically separated the five subspecies from small islands by least interorbital breadth (LIB). *M. p. magdalenensis*, *M. p. copelandi*, and *M. p. shattucki* had similar average LIB (3.93, 3.98, and 3.98 respectively) and were clustered together, while *M. p. provectus*, with the largest average LIB (4.07 mm) and *M. p. breweri*, with the smallest average LIB (3.62 mm) were separated from the cluster in opposite directions. The separation of these populations indicated that there is no cline variation, as exemplified by mainland Maine and *M. p. enixus*, which are grouped together although geographically distant (Figure 1.2). If a cline was present, the Gaspe Peninsula population should be intermediate between mainland Maine and *M. p. enixus* from Labrador.

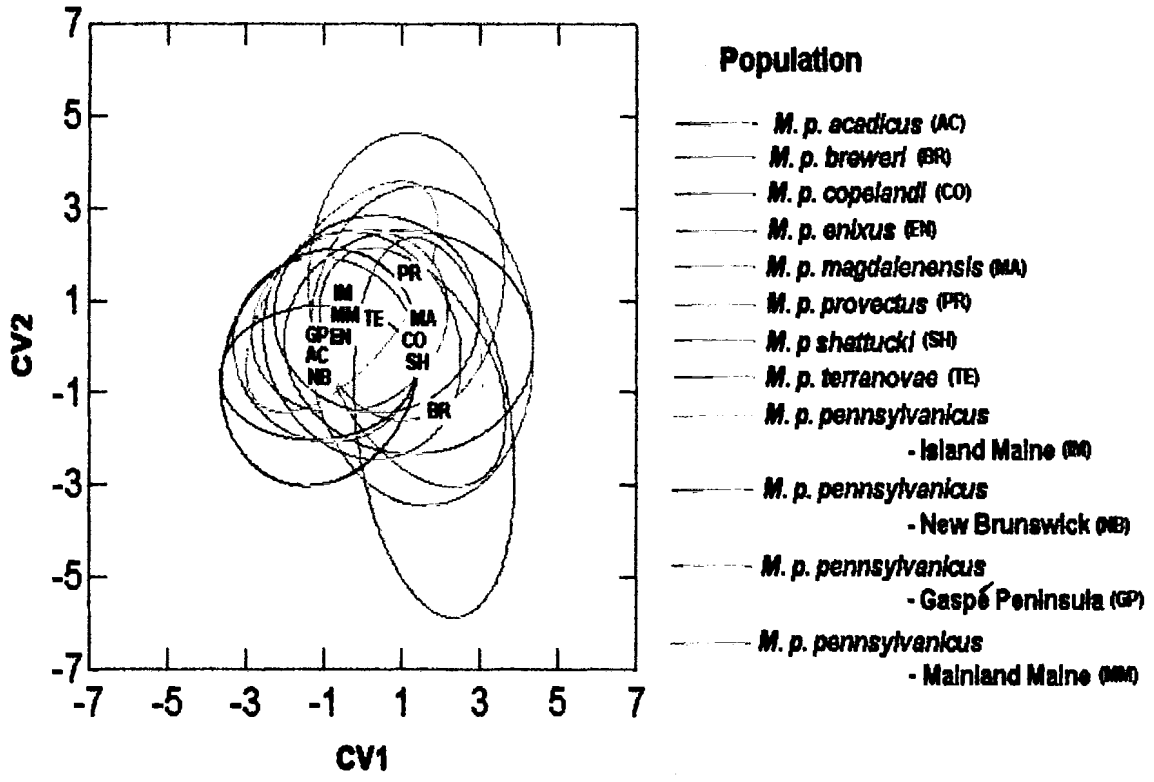

Figure 1.2. Canonical scores graph of the 9 subspecies (12 populations) of meadow voles measured from museum collections.

Penobscot meadow vole comparison

Comparison of *M. p. shattucki* with *M. p. pennsylvanicus* populations from mainland Maine and island Maine showed significant morphological differences among them (Wilks Lambda = 0.192, $p<0.001$). *M. p. shattucki* was correctly classified 86.4% of the time, being misclassified as mainland Maine 13.6% of the time (Table 1.3). Seven variables were retained in the model. Describing 92.5% of the variation in the model, CV1 was influenced mostly by GLS (coefficient = 0.767) and CZL (0.753) (Table 1.2). Total body length (0.616), LUTR (0.550), CL (0.348), LIB (0.215) and PFLB (0.106) were also important variables separating these populations. CV2 accounted for 7.5% of the variation and was driven by PFLB (0.667). Other variables retained in the model for CV2 were TBL (0.557), LIB (0.454), CL (0.325), CZL (0.295), and LUTR (0.213). With the untransformed data, TBL was a significant contributor to the model. When the data were transformed to control for the effect of TBL on all other variables, correct classification was higher than for untransformed data (Table 1.3). Thus, both size (CV1) and shape (CV2) significantly differentiate *M. p. shattucki* from *M. p. pennsylvanicus* in Maine. Eight variables were retained in the model, with CZL (1.197) controlling separation along CV1, which accounted for 89.6% of the variation and HFL (0.866) along CV2, accounting for the remaining 10.4%. The model based on transformed data differed from that with untransformed data by deletion of GLS and PFLB and addition of TL, LLTR and NB.

The canonical scores graph shows that *M. p. shattucki* separated from Island Maine and Mainland Maine along the CV1 axis, which, as stated previously, was influenced by GLS and CZL. Average GLS of *M. p. shattucki* (28.40 mm) was larger

Table 1.3. Percent (%) of time each population was classified as each other population in the discriminant function analysis with museum specimens of *M. p. shattucki* and *M. p. pennsylvanicus* from mainland Maine and other islands in Maine for untransformed (top number) and log ratio data (bottom number).

	M. p. pennsylvanicus (Island Maine)	*M. p. pennsylvanicus* (Mainland Maine)	*M. p. shattucki*
M. p. pennsylvanicus	**68.8**	27.3	4.5
(Island Maine)	**73.3**	18.2	4.5
M. p. pennsylvanicus	29.7	**62.2**	8.1
(Mainland Maine)	20.0	**70.0**	10.0
M. p. shattucki	0.0	13.6	**86.4**
	0.0	4.5	**95.5**

than mainland Maine (26.54 mm) and island Maine (26.39 mm) however average CZL of *M. p. shattucki* (10.94) was smaller than both mainland Maine (11.58 mm) and island Maine (11.71 mm). Along the CV2 axis, mainland Maine was separated from Island Maine and *M. p. shattucki*. This delineation was influenced mostly by PFLB, which was larger on average in *M. p. shattucki* (2.24 mm) and island Maine (2.00 mm) than in mainland Maine (1.89 mm) (Figure 1.3).

Cluster analysis

Hierarchical cluster analysis of the untransformed measurements indicated the 12 populations separate into 2 major groups (Figure 1.4). The first included the Nova Scotia (*M. p. acadicus*), Labrador (*M. p. enixus*), and New Brunswick and Gaspé Peninsula populations of *M. p. pennsylvanicus* – all essentially in mainland maritime Canada. The second grouping split into 3 sub-groups: the first including Newfoundland (*M. p. terranovae*), Grand Manan, NB (*M. p. copelandi*) Block Island, RI (*M. p. provectus*), and mainland Maine (*M. p. pennsylvanicus*) and the second, including

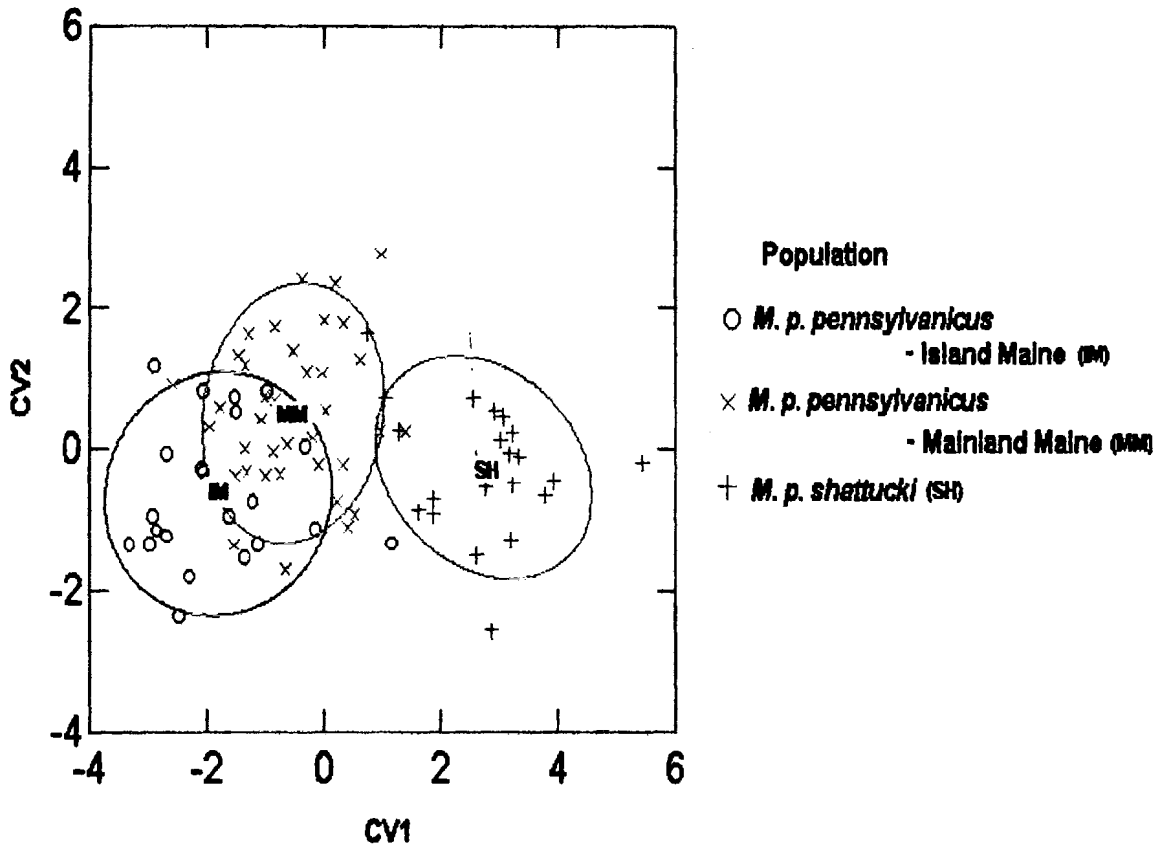

Figure 1.3. Canonical scores graph of the Penobscot meadow vole (*M. p. shattucki*), and *M. p. pennsylvanicus* populations on other islands in Maine and mainland Maine from museum specimens.

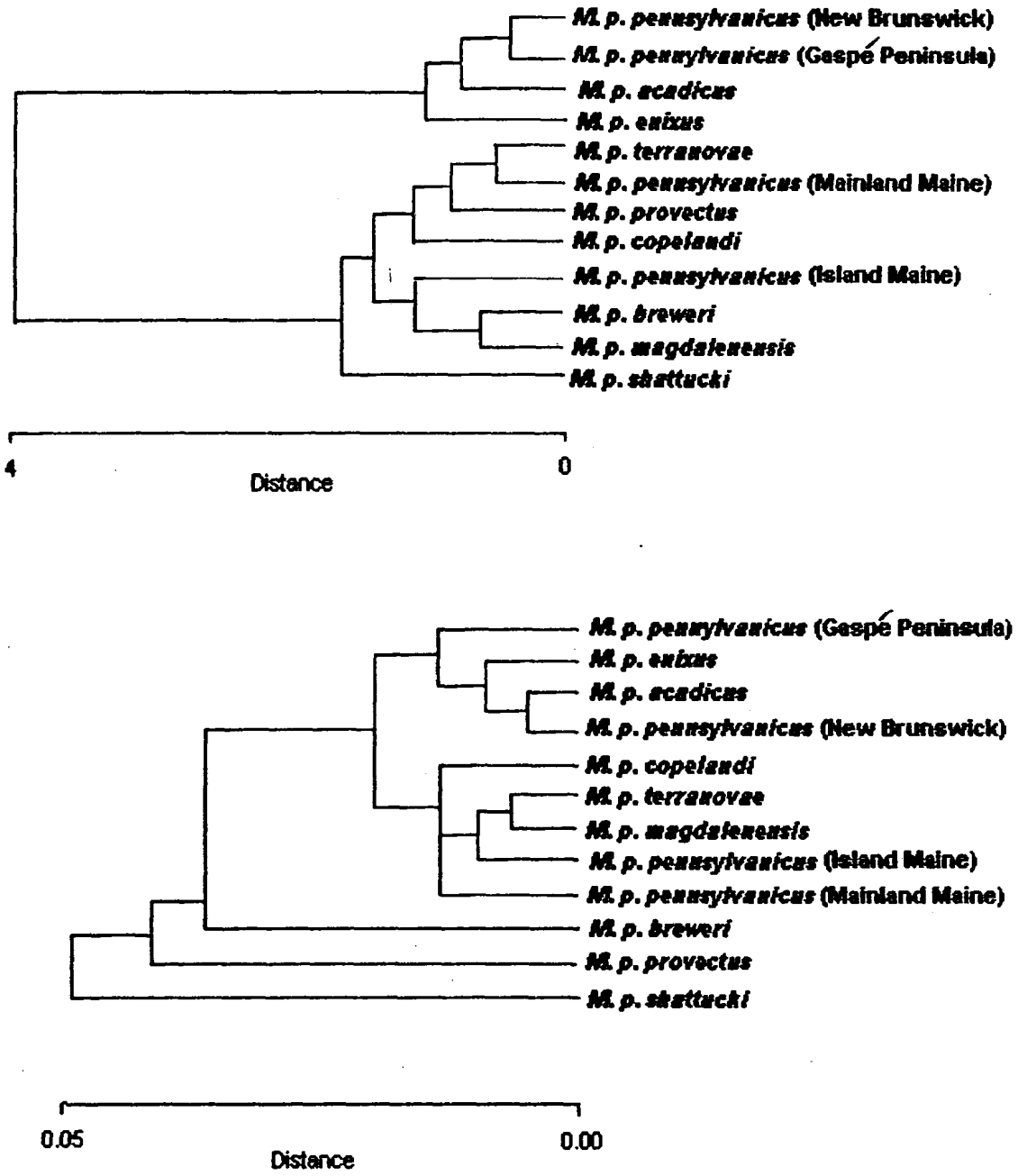

Figure 1.4. Hierarchical cluster analysis of untransformed (top) and log ratio transformed (bottom) morphological measurements of museum populations of *M. pennsylvanicus* subspecies in the northeastern United States and maritime Canada.

M. p. breweri, M. p. magdalenensis, and the island Maine population of *M. p. pennsylvanicus*, and the third solely comprising *M. p. shattucki* (Figure 1.4). The transformed log ratio measurements separated the 12 populations into five clusters, three of which contained a single population from southern New England: one of *M. p. shattucki*, one of *M. p. provectus*, and one of *M. p. breweri* (Figure 1.4). The fourth cluster is comprised of *M. p. acadicus, M. p. enixus, M. p. pennsylvanicus* from the Gaspe Peninsula and New Brunswick, and the last cluster included *M. p. copelandi, M. p. terranovae, M. p. magdalenensis*, Island Maine, and Mainland Maine (Figure 1.4). While there were differences in how populations clustered depending on whether overall size was included or controlled for, *M. p. shattucki* consistently was set apart from the other subspecies.

Extant Populations

Overall comparison

Significant differences were found in morphological characters among extant populations of *M. p. pennsylvanicus* (Northport, Rockport, Orono and Isle au Haut) and *M. p. shattucki* (North Haven and Islesboro) (Wilks Lambda = 0.136, $p < 0.001$). None of the populations were well classified except Isle au Haut (80% correctly classified) (Table 1.4). Specimens from the type localities of *M. p. shattucki*, North Haven and Islesboro, were correctly classified only 45% and 55% of the time, respectively (Table 1.4). Specimens from North Haven were mistakenly classified as Orono and Islesboro (25% each), voles from Islesboro were incorrectly classified as North Haven (20%) while specimens from Isle au Haut were misclassified as Orono and Islesboro (only 10%).

Table 1.4. Percent (%) of time each population was classified as each other population in discriminant function analysis with six extant populations of *M. pennsylvanicus* in (a) comparison of all separate extant populations and (b) comparison of combined geographic areas for untransformed (top number) and log ratio data (bottom number). For example, *M. p. pennsylvanicus* from Orono is classified as itself 29.2% (33.3%) of the time, while classified as Rockport 29.2% (20.8%) of the time.

(a)

	M. p. pennsylvanicus				*M. p. shattucki*	
Population	Orono	Rockport	Northport	Isle au Haut	North Haven	Islesboro
M. p. pennsylvanicus						
Orono (n=24)	**29.2**	29.2	12.5	12.5	12.5	4.2
	33.3	20.8	4.2	16.7	16.7	8.3
Rockport (n=18)	22.2	**44.4**	33.3	0.0	0.0	0.0
	16.7	**44.4**	16.7	0.0	11.1	11.1
Northport (n=20)	10.0	20.0	**55.0**	0.0	5.0	10.0
	5.0	15.0	**65.0**	0.0	10.0	5.0
Isle au Haut (n=10)	10.0	0.0	0.0	**80.0**	0.0	10.0
	10.0	0.0	0.0	**80.0**	0.0	10.0
M. p. shattucki						
North Haven (n=20)	15.0	0.0	5.0	0.0	**55.0**	25.0
	25.0	0.0	5.0	0.0	**45.0**	25.0
Islesboro (n=20)	10.0	0.0	5.0	5.0	20.0	**60.0**
	10.0	0.0	5.0	10.0	20.0	**55.0**

(b)

	M. p. pennsylvanicus			
	Orono	Coastal Maine	Isle au Haut	*M.p. shattucki*
M. p. pennsylvanicus				
Orono (n=24)	**62.5**	12.5	16.7	8.3
	41.7	16.7	29.2	8.3
Coastal Maine (n=38)	21.1	**73.7**	0.0	5.3
	21.1	**63.2**	0.0	13.2
Isle au Haut (n=10)	10.0	0.0	**80.0**	10.0
	10.0	0.0	**80.0**	10.0
M. p. shattucki (n= 40)	12.5	5.0	2.5	**80.0**
	17.5	7.5	7.5	**72.5**

Eight variables were retained in the model (Table 1.5). CV1 accounted for 62.0% of the variation in the model and was influenced primarily by TL (coefficient = 0.854), with other important variables being PFGB (0.482), NL (0.444), TBL (0.431), HFL (0.391), and CBZA (0.195) (Table 1.5). LUTR (coefficient = 1.035) was the most influential variable loading on CV2, which described 19.7% of the variation in the model. The other variables loading on this axis included TBL (0.639), TL (0.354), CBZA (0.292), and PFGB (0.213). The additional 18.7% of variation was explained by CV3 (8.8%), CV4 (6.5%) and CV5 (3.0%). When controlling for effect of body size (TBL), the variable influencing CV1, accounting for 49.1% of variation, was palatine foramina greatest breadth (PFGB), while the variable controlling separation along the CV2 axis, accounting for 29.1% of variation was length of upper tooth row (LUTR), the same as with the untransformed data (Table 1.5). The remaining 21.8% of variation is explained by CV3 (11.0%), CV4 (8.9%), and CV5 (1.9%). Correct classification values in log ratio analyses did not change dramatically from classification values in the using untransformed variables (Table 1.4). The model differed in the deletion of NL and CBZA and addition of LIB and CZL variables loading significantly.

Delineation of North Haven and Islesboro specimens occurred along the CV1 axis from the other populations. The overall pattern of variation in tail length (TL), palatine foramina greatest breadth (PFGB) and the other variables influencing this axis clearly separated *M. p. shattucki* populations of North Haven and Islesboro from *M. p. pennsylvanicus* populations while CV2 separated island populations of *M. p. pennsylvanicus* on Isle au Haut from other *M. p. pennsylvanicus* populations and *M. p. shattucki*.

Table 1.5. Variables retained in two DFA models with extant populations, with variable loadings (in parentheses) on the first and second canonical variate (CV1 and CV2): a) all populations considerable separately and b) some populations combined.

	Untransformed Data		Log Ratio Data	
a.	CV1	CV2	CV1	CV2
M. p. pennsylvanicus	TL (0.854)	LUTR (1.035)	PFGB (0.872)	LUTR (1.428)
Orono	PFGB (0.482)	TBL (0.639)	TL (0.724)	LIB (0.931)
Northport	NL (0.444)	TL (0.354)	HFL (0.605)	TL (0.434)
Rockport	TBL (0.431)	CBZA (0.292)	LIB (0.500)	CZL (0.180)
Isle au Haut	HFL (0.391)	PFGB (0.213)	LUTR (0.415)	HFL (0.116)
M. p. shattucki	CBZA (0.195)	NL (0.059)	ZB (0.220)	ZB (0.099)
North Haven	LUTR (0.076)	ZB (0.053)	CZL (0.114)	PFGB (0.084)
Islesboro	ZB (0.047)	HFL (0.047)		
b.				
M. p. pennsylvanicus	TL (0.887)	LUTR (1.001)	PFGB (0.809)	LUTR (1.416)
Orono	PFGB (0.493)	CBZA (0.550)	TL (0.663)	CBZA (0.627)
Coastal Maine	TBL (0.483)	TBL (0.412)	NL (0.616)	TL (0.416)
(Northport + Rockport	NL (0.438)	TL (0.263)	HFL (0.575)	HFL (0.350)
combined)	HFL (0.411)	ZB (0.161)	LUTR (0.371)	NL (0.125)
Isle au Haut	CBZA (0.193)	NL (0.096)	CBZA (0.139)	PFGB (0.119)
M. p. shattucki	LUTR (0.056)	PFGB (0.087)	ZB (0.082)	ZB (0.006)
(North Haven +	ZB (0.052)	HFL (0.023)		
Islesboro combined)				

Penobscot meadow vole comparison

Significant differences were found in morphology among populations when *M. p. shattucki* (North Haven and Islesboro combined into one population) was compared to *M. p. pennsylvanicus* on another Penobscot Bay island (Isle au Haut), from Coastal Maine (Northport plus Rockport combined) and an inland population of voles (Orono) (Wilks Lambda = 0.201, $p<0.001$). *M. p. shattucki* was incorrectly classified most often as Orono (12.5%) (Table 1.4). CV1 explained 73.4% of variation and was mostly influenced by TL (coefficient = 0.887) followed by PFGB (0.493), TBL (0.483), NL (0.438), HFL (0.411), and CBZA (0.193) (Table 1.5). CV2 accounted for 18.8% of the variation, and was mostly influenced by LUTR (coefficient = 1.001) then CBZA (0.550), TBL (0.412), TL (0.263), and CBZA (0.161). The remaining 7.8% of variation was explained by CV3. Correct classification values dramatically increased from 55% and 60% when North Haven and Islesboro populations were analyzed separately to 80% when these populations were analyzed together as *M. p. shattucki.*

When body size was controlled for by transforming the variables, the seven variables retained in the model were the same as in the raw data analysis, with the obvious exception of total body length (TBL). The controlling variables for each axis were different in this model. CV1, accounting for 73.4% of the variation, was controlled by PFGB (0.809) while CV2, accounting for 18.8% of variation was controlled by LUTR (1.416). Correct classification values between untransformed and transformed models were similar (Table 1.4). CV3 accounting for the remaining 10.1% of the variation.

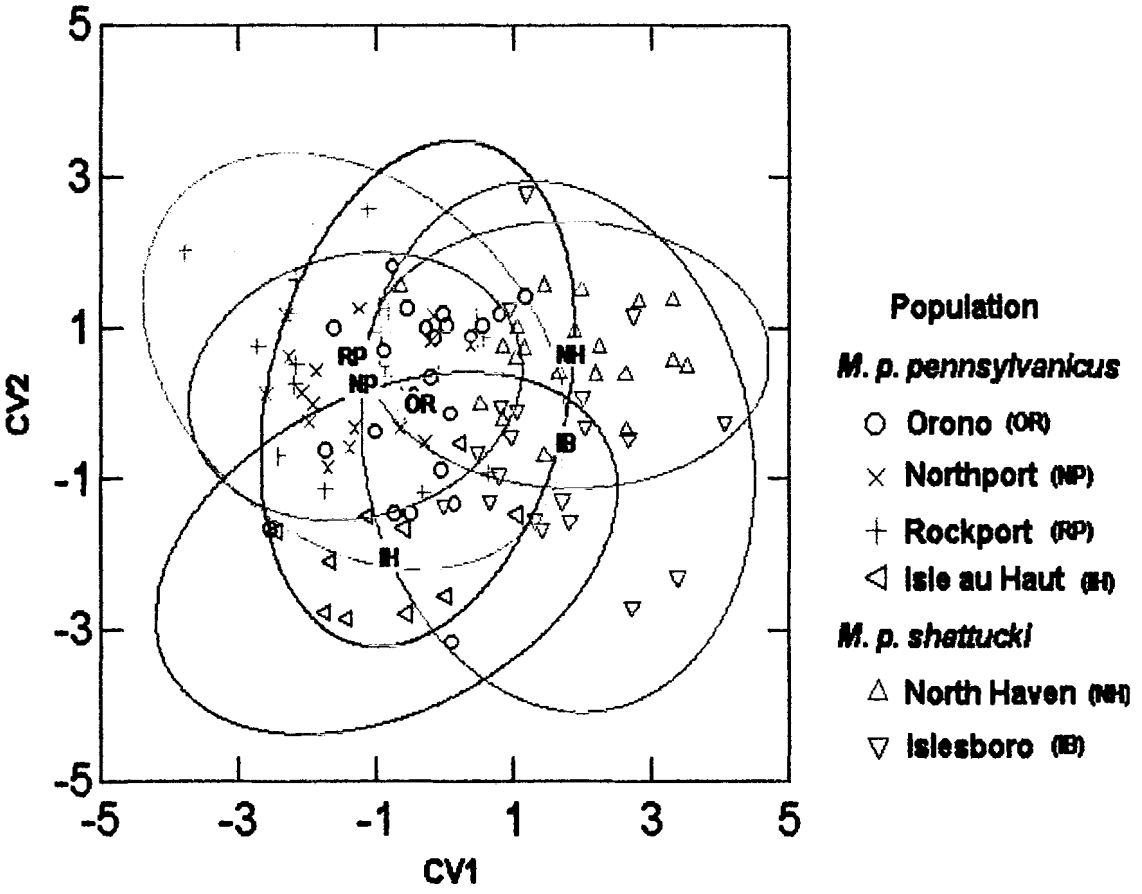

Figure 1.5. Canonical scores graph of extant populations of *M. p. shattucki* (North Haven and Islesboro) and *M. p. pennsylvanicus* from Isle au Haut, coastal Maine (Northport and Rockport) and Orono.

Delineation on the canonical scores graph indicated that *M. p. shattucki* separated from Coastal Maine, Orono and Isle au Haut along the CV1 axis, which was strongly influenced by tail length (TL) (Figure 1.6). *M. p. shattucki* (North Haven plus Islesboro) had on average, smaller TL (42.73 mm) than the *M. p. pennsylvanicus* populations (43.33 – 45.89 mm). This result is surprising: in the original description of *M. p. shattucki*, Howe (1901) described the subspecies with a longer tail. Along the CV2 axis, *M. p. pennsylvanicus* on Isle au Haut was separated from all other populations. The controlling variable, LUTR, was smaller in Isle au Haut voles (6.12 mm) than in the other populations (6.65 – 6.96 mm), as pointed out previously.

Cluster analysis

Hierarchical cluster analysis with the untransformed data clustered the inland population of *M. p. pennsylvanicus* from Orono with the island population of Isle au Haut, and then with *M. p. shattucki*. *M. p. pennsylvanicus* from Coastal Maine was at the base of the cluster (Figure 1.7). Log ratio transformed measurements clustered the inland population of *M. p. pennsylvanicus* from Orono, *M. p. shattucki*, and these two with *M. p. pennsylvanicus* from Isle au Haut. Again, coastal Maine was basal to the major cluster (Figure 1.7).

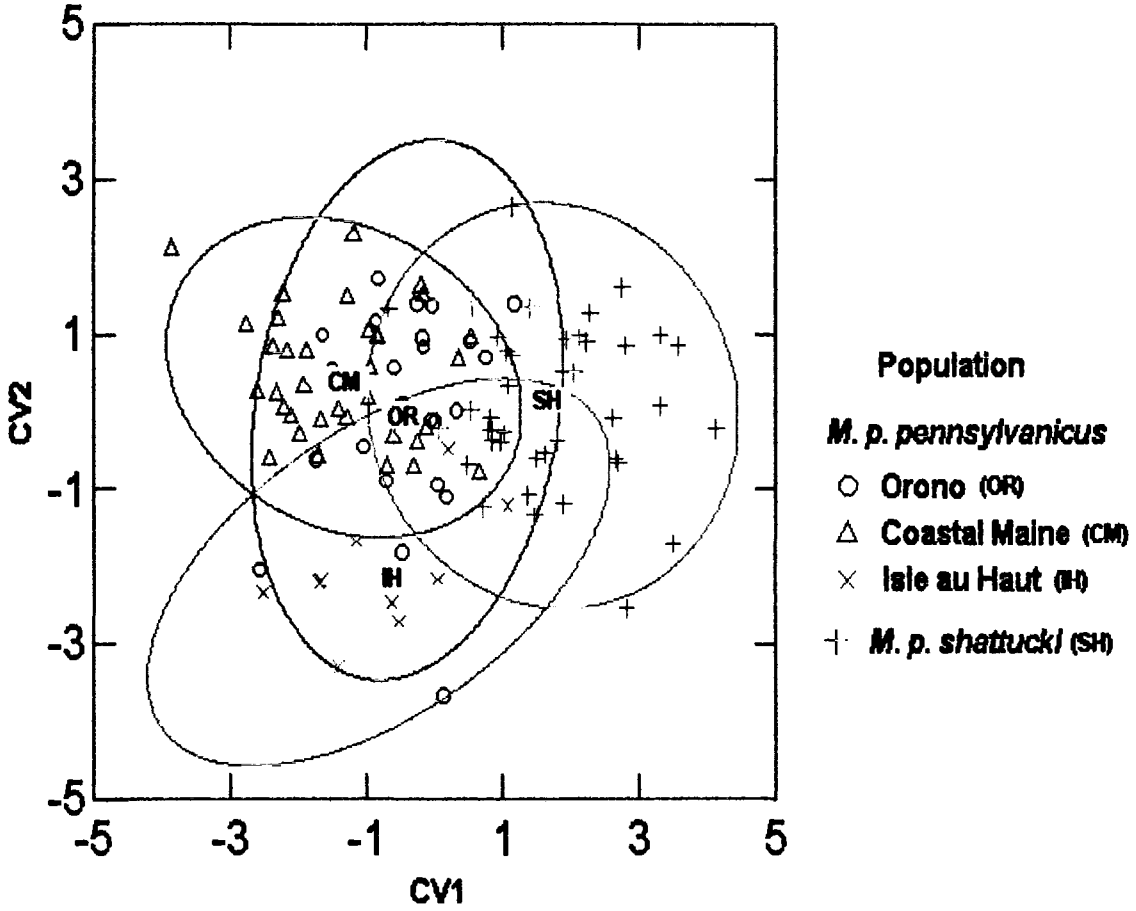

Figure 1.6. Canonical scores graph of *M. p. shattucki* (North Haven and Islesboro combined) and *M. p. pennsylvanicus* from Isle au Haut, coastal Maine (Northport and Rockport combined) and Orono.

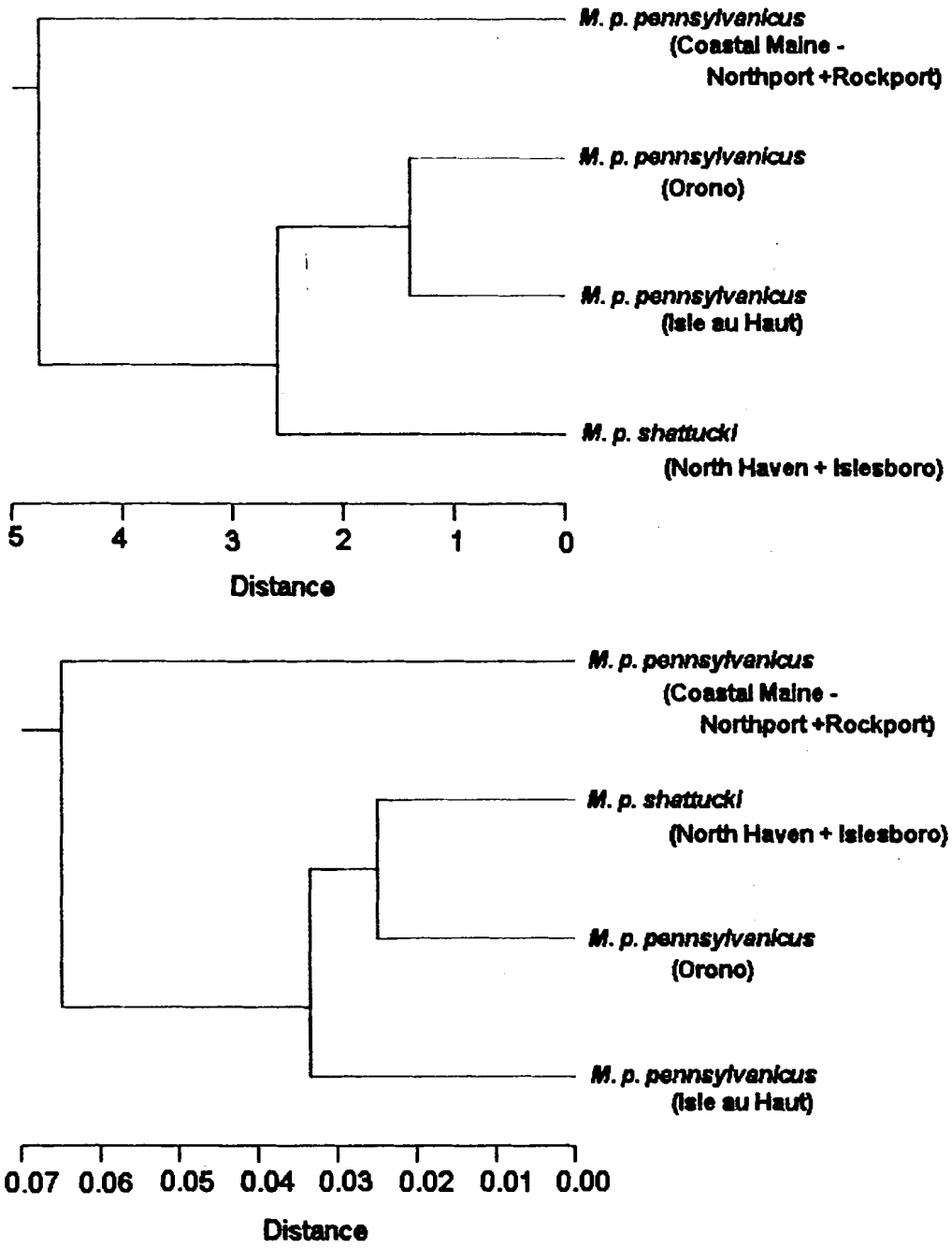

Figure 1.7. Hierarchical cluster analysis of untransformed (top) and log ratio transformed (bottom) morphological measurements of extant *M. p. pennsylvanicus* populations from islands and mainland Maine compared to *M. p. shattucki*.

Discussion

M. p. shattucki is morphologically distinct from mainland meadow vole populations of *M. p. pennsylvanicus*. Measurements of museum specimens indicated that *M. p. shattucki* was differentiated by larger overall cranial morphology. However, the metrics for the back of the skull are generally smaller than their island and mainland counterparts, while the forward structures are larger. Measurements of extant populations indicate that *M. p. shattucki* is distinct by being larger in both cranial and external body measurements, and in all metrics that were included in the models, for example zygomatic breadth, nasal breadth, tail length and total body length.

The New England island subspecies, *M. p. provectus* and *M. p. breweri*, had high correct classification rates, possibly because no direct mainland comparisons were made. The maritime Canada subspecies were not well supported including the small island populations of *M. p. copelandi* on Grand Manan, NB and *M. p. magdalenensis* (Magdalen Island, Québec) and the large island populations of *M. p. acadicus* (Nova Scotia) and *M. p terranovae* (Newfoundland) and the mainland subspecies of *M. p. enixus* (Labrador). Although, the canonical scores graph indicated that the small island populations of *M. p. provectus, M. p. magdalenensis, M. p. copelandi, M. p. shattucki* and *M. p. breweri* were separated from the large island subspecies (*M. p. acadicus* and *M. p. terranovae*), mainland subspecies (*M. p. enixus*) and mainland *M. p. pennsylvanicus* populations along the first axis, only *M. p. shattucki* is highly supported as a separate subspecies (90.5% correct classification when average length is included in the analysis, 85.7% when comparisons are controlled for overall size).

The museum populations of *M. p. shattucki* had a higher morphological differentiation than extant populations from North Haven and Islesboro – correct classification of *M. p. shattucki* dropped to 80%, which is still high. The decrease in morphological differentiation between older museum specimens that were collected from the 1850s and 1950s and the recent extant populations could indicate that gene flow between the islands and mainland populations has been restored. However, the mainland Maine museum population sampled included many Presque Isle specimens from northern Maine. Comparing *M. p. shattucki* to northern Maine specimens could have inflated morphological differences from mainland *M. p. pennsylvanicus* populations. Comparison of *M. p. shattucki* to the extant coastal populations, which are more likely to be the source *M. p. pennsylvanicus* population for the islands of North Haven and Islesboro is probably a more accurate estimation of differentiation of *M. p. shattucki*. Additionally, there may have been sampling artifacts, such as bias toward larger animals that are easier to handle and prepare in the museum specimens that cannot be teased apart from possible genuine change in variation over time.

M. p. pennsylvanicus on Isle au Haut was included in the analysis to investigate the island effect on morphological change. However, there was no difference in correct classification rates between the log ratio analysis that controlled for body size and the analysis with untransformed data, which did not. *M. p. shattucki* also did not exhibit a dramatic change between the two analyses. Therefore, the morphological differences between *M. p. shattucki* and all other populations and between *M. p. pennsylvanicus* on Isle au Haut and all other populations were not due simple to the island effect of increased body size of island populations (MacArthur and Wilson 1963, Case, 1978, Heaney 1978, and Anderbjorn 1986). However, because sample size in this study was

small for the Isle au Haut populations (n=10), further sampling of Isle au Haut is necessary to obtain more support for these findings. While *M. p. shattucki* is larger from *M. p. pennsylvanicus* from mainland populations both cranially and externally, *M. p. pennsylvanicus* on Isle au Haut show no obvious trends compared to mainland populations. For example, *M. p. pennsylvanicus* on Isle au Haut averaged larger greatest length of skull, but smaller nasal length and breadth, while externally had longer tail lengths, however similar hind foot lengths. These differences suggest that populations on the different islands are on different evolutionary paths, keeping in mind that inadequate sampling on Isle au Haut may have biased results.

The statistical approach of Discriminant Function Analysis (DFA) with morphological characters to elucidate taxonomic relationships is similar to those of Galliari and Pardinas (1987) who studied a genus of rodent, *Necromys* from southeastern South America. They used 39 cranial and external measurements in a DFA and cluster analysis of five geographically separate groups. Two geographic groups were concluded to be of the same species due to clustering in the phenogram and correct classification values (100%). Three other geographic groups were determined to be a different species because they clustered together. This species was further split into a subspecies encompassing two geographic groups due to clustering and lower classification values (83% and 94%). These results are very similar to my results of *M. p. shattucki*, and bolsters my conclusion of *M. p. shattucki* as a valid subspecies. Similarly, two species of western chipmunks (*Tamias* spp.) were studied with a DFA of 12 external and cranial characters, along with genital features (Sutton and Patterson 2000). Those authors concluded that only 59% correct classification was sufficient to conclude that coastal

populations were significantly differentiated into a separate subspecies from inland population, substantially lower support than I show for *M. p. shattucki*.

Island populations of meadow voles

In a study of island populations of *M. pennsylvanicus*, Crowell (1973) concluded that meadow voles on islands undergo frequent extinctions and recolonizations. He suggested that meadow voles will first colonize an island (usually via swimming), the population size will then grow dramatically and eventually exceed carrying capacity, after which the population will go extinct. However, Kohn and Tamarin (1978) found that island populations of *M. breweri* did not cycle. My data support Kohn and Tamarin's (1978) findings: if island populations of meadow voles do cycle through recolonizations and extinctions, there should be no morphological differentiation between *M. p. shattucki* and mainland *M. p. pennsylvanicus*, as the meadow voles sampled would be recent colonizers from the closest mainland population. It is interesting to note that during Crowell's (1973) ten-year experiment, meadow vole populations on many of the islands did not go extinct.

Geographic isolation has most likely played a role in evolution of morphological change of *M. p. shattucki*; the closest point from the mainland to Islesboro (negating any current development) is about 3.06 km (1.9 miles) and to North Haven in 10.5 km (6.5 miles). Therefore, these two islands are out of swimming range for voles, which can swim up to 1 km (Crowell 1973). Isle au Haut, although 37.02 km (23 miles) away from the closest mainland, is not so isolated: there is scattering of islands between the mainland and island. Ice bridges, however, may have allowed dispersal of voles to the islands. Lomolino (1989) saw active vole runways under snow over ice and concluded through treadmill experiments that meadow voles can disperse up to 6 km over land.

During the 1800s, Penobscot Bay froze completely twice, allowing for possible movement of meadow voles to the islands. The islands in the Penobscot Bay have been isolated from the mainland since 11,500 years before present (ybp) and human influences on Islesboro and North Haven have been great: from the first Native Americans around 5000 to 6000 ybp to the Europeans during the 17th and 18th centuries and recent human settlement (Conkling 1999). It is possible that meadow voles may have been carried with all of these influences to the islands creating gene flow between mainland and island populations. Despite all these different ways meadow voles may have colonized the islands from the mainland, this study shows that restricted gene flow has lead to morphological differentiation of island populations on North Haven and Islesboro from mainland populations, as well as from another island population on Isle au Haut. These analyses support the subspecific status of *M. p. shattucki* separate from the nominate species. Additional islands should thus be studied to determine if range of this subspecies extend beyond North Haven and Islesboro in the Penobscot Bay.

CHAPTER 2: GENETIC DIFFERENTIATION

Introduction

Island populations have a natural barrier from their mainland nominant populations, and given enough time and distance from the mainland, island populations may differentiate into distinct subspecies and species. Historically, species on islands have been studied in terms of classic island biogeography theory, which is mainly concerned with the community of different species on islands (MacArthur and Wilson 1967, e.g. Crowell 1986). Island biogeography theory predicts the number of species on a given island by its size and isolation from the mainland (MacArthur and Wilson 1967). Genetic variability also exhibits a pattern dictated by area and isolation (Jaenike 1973). Differences between island and mainland populations are greater when there is a small number of founding individuals, low immigration rates, and a large disparity between population size of mainland and islands (Jaenike 1973).

There are many different aspects that, in conjunction, dictate the genetic differentiation, and subsequent speciation, of island populations. First, the number of individuals and their genetic makeup dictates the future genetic structure of the island population (Mayr 1954). This founder effect immediately introduces a genetic bias, as not all alleles in the mainland population are represented in the island population. Additionally, because population size of the initial colonization event is usually small, other evolutionary forces act on this new population, such as genetic drift, to change allele frequencies from one generation to the next (Whittaker 1998). If low population size on the island is sustained, genetic drift can have substantial effects, such as fixation

of alleles or pairing of deleterious recessive alleles which may lower survivorship and fecundity of an individual, or even cause mortality before any reproductive effort. However, should new individuals immigrate to the island, new alleles will be introduced into the existing population, and both founder effects and genetic drift would be counteracted, and genetic differentiation may be precluded. Theoretically, if new individuals are not introduced, extinction may occur due to inbreeding depression and accumulation of deleterious mutations (Frankham 1996). While fruit flies in laboratory experiments exhibit inbreeding depression and accumulation of deleterious mutations, there is little evidence of inbreeding depression or deleterious mutations in natural populations (see Saccheri et al. 1998 for example of inbreeding depression in nature). However, should the population persist, there will be a loss of genetic variation and subsequent adaptation to the island environment (Frankham 1996). Historically, genetic differentiation has been difficult to quantify, however, molecular genetic techniques have been recently developed to more easily quantify genetic variation among and within populations.

The Penobscot meadow vole (*Microtus pennsylvanicus shattucki*) (PMV) is an insular subspecies inhabiting Islesboro, North Haven, and TumbleDown Dick islands in Penobscot Bay, Maine (Howe 1901). Reginald Howe first described *M. p. shattucki* based on its larger body size, longer tail, prominent ears, large and globular bullae, broad and bottle-shaped palatine foramina, and darker pelage. However, he did not measure mainland *M. p. pennsylvanicus* for comparison purposes. Wyman (1953) refuted Howe's (1901) claim, remeasured Howe's specimens, and compared them to specimens from the mainland and other island *M. pennsylvanicus* populations. Using size, ears, coloration,

audital bullae, palatine foramina, and tail length, he found that measurements of the *M. p. shattucki* specimens were within the range of *M. pennsylvanicus*, and concluded that the island populations of *M. pennsylvanicus* were not a distinct subspecies. In another study of island meadow vole populations Youngman (1967) used univariate analysis of more morphological variables than previous studies. Despite debate on the validity of *M. p. shattucki*, this subspecies has not been further studied, although the taxonomic distinction has been widely used.

There are many different techniques that can be used to assess genetic differentiation among and within populations: analysis of microsatellite variation is the current method of choice to assess nuclear DNA variation (e.g. Van de Zande et al. 2000) and direct sequencing of mitochondrial DNA (mtDNA) genes or the non-coding control region is used to analyze mtDNA variation (e.g. Stewart and Baker 1997). Because of differential rates of mutation of each type of genetic assay ($10^{-4} - 10^{-5}$ for microsatellites; 10^{-6} for mtDNA control region), a combination of these methods will be used to determine if differentiation of the PMV from mainland populations can be demonstrated.

The PMV is not currently listed as threatened or endangered due to a lack of information (U.S.F.W.S. 1994), although it was listed as a Species of Special Concern in Maine when that listing was in use (Dr. Mark McCollough, pers. comm.). Morphological analyses indicate that the PMV can be distinguished from the nominate species on the mainland as well as on other islands (Chapter 1). To further clarify taxonomic and subsequent listing status of *M. p. shattucki*, I used both nuclear DNA markers (microsatellites) and mtDNA sequence variation to elucidate the genetic variation within and among these populations.

Methods

Samples and DNA Isolation

Meadow voles were live-trapped on the islands of Islesboro and North Haven, type localities of the Penobscot meadow vole, and Isle au Haut, another large island in Penobscot Bay. Mainland populations were surveyed for comparison to island populations at nearby coastal sites; Rockport is closest to North Haven and Northport to Islesboro. An additional inland population at Orono was also sampled to analyze variation among mainland populations. See Chapter 1, Methods for complete sampling process. Two millimeters of tail were taken from all captured juveniles for genetic analysis. Breast and heart tissue were collected from adults during museum voucher preparation of specimens (see Chapter 1). DNA was isolated from tail tips and breast muscle tissue by standard phenol-chloroform method (Sambrook et al. 1989) and with the QIAamp DNA Minikit (Qiagen, Inc.), DNA was then quantified with a Hoefer DyNA Quant Fluorometer.

Microsatellite Analysis – Loci and Amplification

I attempted to amplify twenty-six microsatellite loci from a variety of sources: five developed by Research Genetics, Inc for *Mus* and previously used with *M. pennsylvanicus* (Moncrief 1997), eight developed for the water vole, *Arvicola terrestris*, of the United Kingdom (Stewart et al. 1998), five for the grey red-backed vole, *Clethrionomys rufocanus bedfordiae*, a subspecies from Hokkaido, Japan (Ishibashi et al. 1995), and eight for the root vole, *M. oeconomus*, of the Netherlands (Van de Zande et al. 2000). Seven of these microsatellite loci were amplified successfully in this study: Mscrb-5 (Ishibashi et al. 1995), Av-4 (Stewart et al. 1998) and Moe-1, Moe-2, Moe-4, Moe-5, and Moe-6 (Van de Zande et al. 2000) (Table 2.1).

For amplification of the seven microsatellite loci, I used an MJ PTC-100 programmable thermal cycler. Twenty-five µl PCR reactions contained 10x buffer (pH = 8.3), varying concentrations of $MgCl_2$ depending on the locus, 0.5 µM of each forward and reverse primer, 0.5 units Taq polymerase (Perkin-Elmer), DNA concentrations of varying amounts, 0.2 mM dNTPs, and 0.26 µM fluorescent dNTPs in one of three dye colors: green (R86), yellow (TAMRA) and blue (R110)(Applied Biosystems) (Table 2.2). The PCR program used for Mscrb-5 was: two minutes initial denaturing at 93°C, and 30 cycles of denaturing at 91°C for 30 seconds, annealing at 54°C for 20 seconds, and extension at 72°C for 20 seconds. For Av-4, the program was two minutes denaturing at 91°C, and 30 cycles of denaturing at 91°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for two minutes. The five Moe- loci were amplified by initial denaturing at 94°C for three minutes, 30 cycles of denaturing at 94°C for one minute, annealing at 52° – 57°C (Table 2.2) for two minutes, extension at 72°C for 1.5 minutes, and a final extension at 72°C for 10 minutes. After amplification, I ran samples on a 1.5% agarose (GibcoBRL) and synergel (Diversified Biotech) gel to visualize PCR products. Samples were purified in CENTRI_SEP (Princeton Separations, Inc.) columns with Sephadex (G-50, Sigma), to remove unincorporated fluorescent dNTPs, and run on an ABI 377 automatic sequencer. Alleles were scored and analyzed with GeneScan™ and Genotyper™ software (Applied Biosystems Inc.) using the red size standard Genotype TAMRA 50-500 DNA ladder (Invitrogen). Histograms of allele frequencies per locus were created with Genotyper to bin alleles (Figure 2.1).

Table 2.1. Primer sequences and characteristics of seven microsatellite loci developed for different species and used for meadow vole (*M. pennsylvanicus*).

						This Study		
Locus (Genbank Accession Number)	Developed For	Allele size range	Number of Alleles	Primer sequence	Core Sequence	Allele size range	Number of alleles	Reference
Mscrb-5 (D37836)	*Clethrionomys rufocanus bedfordiae*	196-214	9	F: ggttggtgtttgcatttagg R: ctcctggtaattttcatcttacc	Mix of CA, ATAC, ATGT repeats	126-194	21	Ishibashi et al. 1995
Av-4 (Y16556)	*Arvicola terrestris*	226	11	F: gaattacacatgggagtctgag R: cacagccacaaggtagaaag	$(GATA)_{14}AG$ $GA(GATA)_{19}$	231-451	47	Stewart et al. 1998
Moe-1 (AF68902)	*Microtus oeconomus*	96-136	20	F: tggttgttctgtggtgaatacag R: acagtaagcagtttatccacaaacc	$(GT)_{18}$	74-143	28	Van de Zande et al. 2000
Moe-2 (AF68903)	"	145-175	13	F: catctgatgagtccctgagg R: gcaaccttcttctgacttttac	$(GT)_{17}$	130-216	31	"
Moe-4 (AF68905)	"	99-111	4	F: accatagcacaatgtacacacattg R: tttgattagttacaccatggcctat	$(GT)_7GA(GT)$	75-177	20	"
Moe-5 (AF68906)	"	119-165	22	F: ggtcatgctccaagaagctc R: aaaaccaagggtgctgctc	$(TC)_7T(TC)_{25}$	105-234	31	"
Moe-6 (AF68907)	"	222-258	19	F: ggttttctgttcggagg R: cctcttctggcctctccag	$(TG)_{25}$	200-271	41	"

Table 2.2. Reaction conditions of microsatellite amplification. T_A = annealing temperature.

Locus Name	DNA Concentration (ng)	$MgCl_2$ Concentration (mM)	T_A (°C)
Mscrb-5	15	1.5	54°
Av-4	10	2.0	59°
Moe-1	15	2.0	57°
Moe-2	10	2.0	57°
Moe-4	15	2.0	55°
Moe-5	15	1.5	57°
Moe-6	30	1.5	52°

Allele binning is a common process used to account for slight differences between genotyping gels and possible inconsistencies in PCR amplification of alleles. In the binning procedure, alleles are placed into base pair segments dictated by their repeat unit and are called as the highest frequency allele to account for differences between genotyping gels. For example, in Moe-2, a dinucleotide repeat, all raw alleles of size 166 to 168 base pairs are called 168, which is the highest frequency allele and is two base pairs away from the next bin of 170 (Figure 2.1). Binned data are used in all subsequent analyses and is not further differentiated from raw data.

Microsatellites Analysis – Statistics

I used Arlequin 2.0 to test allele frequencies for departures from Hardy-Weinberg equilibrium at each locus, and to estimate the standard genetic distance, F_{ST}, between all populations and subsequent number of migrants per generation (*Nm*) (Schneider et al. 2000). An Analysis of Molecular Variance (AMOVA) was used to investigate patterns of genetic variation within and among populations. Variation can be partitioned among user-defined groups of populations, among populations within user defined groups, and

within populations. Three comparisons were made with microsatellite data. First, all populations were clustered into one group to establish a baseline of variation for comparisons. Second, *M. p. shattucki* (North Haven and Islesboro) was compared to all *M. p. pennsylvanicus* populations combined (Orono, Rockport, Northport, and Isle au Haut). Finally, *M. p. shattucki* was compared to the closest coastal *M. p. pennsylvanicus* populations (Northport plus Rockport), *M. p. pennsylvanicus* on another island in Penobscot Bay (Isle au Haut), plus an inland Maine population of *M. p. pennsylvanicus* (Orono). Genepop 3.3 (Rousset 2001) was used to test for pairwise locus linkage disequilibrium, to calculate the number of alleles per locus, number of private alleles (alleles found in one population only) per population , the inbreeding coeffecient, F_{IS}, and to test for isolation by distance. For isolation by distance analysis, genetic distance (F_{ST}) is correlated with the geographic distance (km) between sampling sites. Distance was measured from the center of each site or island from maps in The Maine Atlas and Gazetteer (1999). F_{ST} values were transformed by $F_{ST}/(1- F_{ST})$ and distance values were log transformed for analysis. One thousand permutations were done to assess statistical significance.

Mitochondrial DNA Analysis - Amplification

The 5' variable domain of the mitochondrial control region, or d-loop, was amplified with primers designed for meadow voles from DNA sequences from four congenic species: *M. oeconomus*, *M. agrestis*, *M. arvalis* and *M. epiroticus* (Genbank Accession numbers AJ009888, AJ009884, AJ009883, and AJ009882, Stacy and Ehrich unpub.). The d-loop L primer (5'-ACTACTTCTTGAGTACATAA-3') is in the 5'

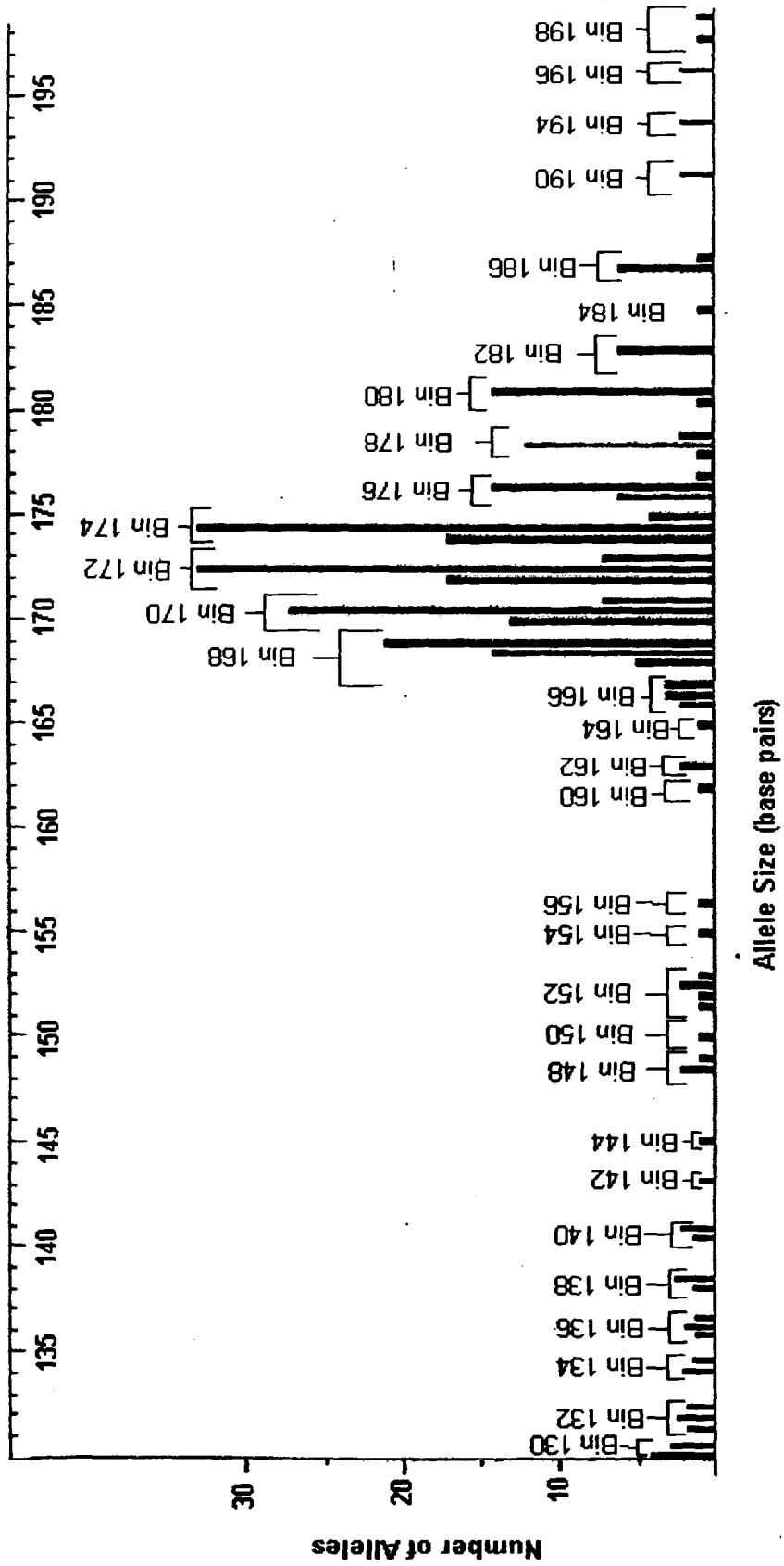

Figure 2.1. Histogram of Moe-2 allele sizes in meadow voles, with labeled bin sizes.

$tRNA^{pro}$ region flanking the control region, and the d-loop H primer (5'-CCGTGAAACCAATCAACCCG-3') is approximately 300 base pairs downstream. PCR reactions were done in a MJ PTC-100 programmable thermal cycler in either 25 or 50 μl reactions containing : 0.2 mM dNTP's, 10x buffer (pH 8.3), 2.5 mM $MgCl_2$, 0.2 μM of each primer, 1.25 units Taq polymerase (Perkin-Elmer) and 30 ng DNA. The PCR amplification program was an initial 5 minutes of denaturing at 94°C, followed by 34 cycles of 94°C for 45 seconds, 1 minute annealing at 50°C and 1 minute extension at 72°C. A final extension of five minutes at 72°C was done. PCR products were run on a 1.5% NuSieve GTG (FMC BioProducts) gel to determine successful amplification. PCR products were cleaned using Nanosep™ microconcentrators (Princeton Separations) and I quantified the DNA concentration in the cleaned PCR products in a Hoefer DyNA Quant Fluorometer.

Direct sequencing was done on an ABI 373 Stretch Automatic Sequencer. Fifteen individuals were sequenced from Orono, Rockport, and Isle au Haut, 13 individuals were sequenced from Northport and Islesboro, and 12 individuals were sequenced from North Haven. Additionally, 10 individuals were sequenced for use as an outgroup from a Newfoundland population of meadow voles, *M. p. terranovae*. Both strands of mtDNA were sequenced for all individuals to confirm the correct sequence and clarify ambiguities.

Mitochondrial DNA Analysis - Statistics

To edit and align sequences, I used Sequence Navigator 1.0 (Applied Biosystems)

with the Clustal algorithm. Phylogenetic analyses were done with PAUP v. 4.08 (Swofford 1999). Maximum likelihood analysis was done using the HKY85 (Hasegawa et al. 1985) model to estimate the gamma shape parameter (α) for the proportion of variable sites. The gamma distribution models mutation rate variation among nucleotide sites by estimating alpha, which is the degree of rate variation. The larger the alpha value, the less the variability between sites are, and when alpha is estimated as infinity there is uniform variation among sites (Hasegawa et al. 1985). Maximum likelihood is a method of phylogenetic tree construction in which trees are estimated by probability calculations that indicate how likely each possible tree is with the given sequence data and chosen substitution model (Felsenstein 1981). Neighbor-joining analysis, which uses an algorithm that sequentially joins taxa that minimizes the number of evolutionary changes on a tree, was done with and without this shape parameter (Saitou and Nei 1987). Neighbor-joining was performed with Tamura-Nei (Tamura and Nei 1993) genetic distance. Bootstrap was used to generate a confidence level at each node of each tree. This technique, in which phylogenetic trees are re-sampled a given number of times, results in a percentage value indicating how many replicates had that certain split in the tree. One hundred replicates were performed.

Genetic structure of these seven extant populations was investigated with the Analysis of Molecular Variance (AMOVA) in Arlequin 2.0 (Schneider et al. 2000). The same three comparisons were made with mtDNA haplotypes as for the microsatellite analysis. Using Arlequin 2.0, pairwise F_{ST}'s for each population and 1000 permutations to determine significance were calculated. Using F_{ST}, the number of migrants per generation (*Nm*) was also estimated as an indirect measure of female gene flow.

Results

Microsatellites

All seven loci were highly polymorphic, varying between 20 (Moe-4) and 47 (Av-4) alleles (2.3). Expected heterozygosity (H_E) varied from a low of 0.70 in one of the *M. p. shattucki* populations (North Haven) to a high of 0.87 in one of the coastal Maine populations (Rockport). North Haven and Islesboro, the main localities of *M. p. shattucki*, had essentially the same H_E (0.70 and 0.71, repectively) and similar observed heterozygosity (H_O) (0.53 and 0.59, respectively). In fact, all populations had substantially lower levels of heterozygosity than expected. All populations were found to not be in Hardy-Weinberg Equilibrium (HWE) over all loci. In Rockport and Islesboro, there were no loci in HWE (Table 2.4). The populations of Northport, North Haven, and Isle au Haut were in HWE for only one locus each (Mscrb-5, Moe-5, and Moe-1, respectively), while Orono was in HWE for three loci: Mscrb-5, Moe-2, and Moe-4 (Table 2.4). Correspondingly, inbreeding coefficients (F_{IS}) over all loci were high and significantly different from zero, ranging from 0.16 (Islesboro) to 0.38 (Isle au Haut) (Table 2.3).

Pair-wise population comparisons of F_{ST} indicated that all comparisons were significantly different from zero ($p<0.001$), and therefore, all populations are genetically distinct from each other (Table 2.5a). This included the two separate populations of *M. p. shattucki* on North Haven and Islesboro ($F_{ST} = 0.16$) (Table 2.5a). The least distinct populations were the two coastal Maine populations in Rockport and Northport ($F_{ST}=0.04$). Corresponding *Nm* values, an indirect estimate of gene flow, showed that gene flow is greatest between voles from Northport and Rockport (about 12 voles per

Table 2.3. Number of alleles per locus, including private alleles in parentheses, for populations of *M. p. pennsylvanicus* and *M. p. shattucki* in Maine.

	M. p. pennsylvanicus				*M.p.shattucki*		
Locus	**Orono**	**Rockport**	**Northport**	**Isle au Haut**	**North Haven**	**Islesboro**	**Total Alleles /Locus**
Mscrb-5	11 (2)	10 (2)	10 (1)	8 (2)	3 (0)	9 (1)	21
Av-4	13 (3)	19 (1)	15 (0)	21 (10)	20 (11)	3 (0)	47
Moe-1	14 (1)	15 (1)	15 (1)	14 (1)	8 (2)	14 (1)	28
Moe-2	15 (2)	13 (2)	15 (6)	11 (2)	9 (0)	10 (2)	31
Moe-4	7 (0)	13 (2)	6 (0)	10 (2)	9 (2)	8 (1)	20
Moe-6	22 (5)	22 (7)	13 (0)	16 (2)	13 (2)	13 (0)	41
Moe-5	13 (6)	14 (2)	13 (2)	15 (0)	9 (0)	16 (4)	31
Total	95 (19)	106 (17)	87 (10)	95 (19)	71 (17)	73 (9)	

Table 2.4. Observed (H_O) and expected heterozygosities (H_E) and departures from Hardy-Weinberg equilibrium (underlined values, $p<0.05$) for all populations and all loci, plus number of individuals (n) genotyped.

	Population		Mscrb-5	Av-4	Moe-1	Moe-2	Moe-4	Moe-5	Moe-6	Overall/ Population
M. p.	Orono	H_E	0.58	0.75	0.89	0.41	0.91	0.74	0.86	0.73
pennsylvanicus	n=30	H_O	0.44	0.90	0.50	0.48	0.79	0.40	0.40	0.55
		F_{IS}	0.19	-0.20	0.53	0.43	-0.17	0.45	0.12	0.23
	Rockport	H_E	0.71	0.90	0.88	0.84	0.95	0.90	0.92	0.87
	n=30	H_O	0.44	0.87	0.62	0.55	0.83	0.30	0.79	0.61
		F_{IS}	0.36	0.03	0.13	0.29	0.35	0.67	0.12	0.27
	Northport	H_E	0.80	0.93	0.92	0.67	0.83	0.89	0.93	0.85
	n=30	H_O	0.68	0.33	0.82	0.33	0.64	0.70	0.80	0.60
		F_{IS}	0.15	0.64	0.13	0.11	0.50	0.21	0.21	0.27
	Isle au Haut	H_E	0.73	0.92	0.76	0.84	0.92	0.93	0.86	0.86
	n=30	H_O	0.11	0.77	0.48	0.65	0.54	0.41	0.72	0.50
		F_{IS}	0.85	0.17	0.16	0.37	0.22	0.55	0.42	0.38
M. p.	*shattucki* North Haven	H_E	0.27	0.94	0.81	0.74	0.79	0.71	0.68	0.70
	n=30	H_O	0.10	0.77	0.70	0.37	0.61	0.67	0.50	0.53
		F_{IS}	0.59	0.16	0.27	0.13	0.49	0.06	0.22	0.24
	Islesboro	H_E	0.68	0.54	0.72	0.53	0.81	0.83	0.91	0.71
	n=30	H_O	0.45	0.97	0.73	0.37	0.53	0.50	0.63	0.59
		F_{IS}	0.34	-0.81	0.31	-0.03	0.27	0.39	0.34	0.16
	Total/ Locus	H_E	0.64	0.83	0.86	0.83	0.67	0.82	0.87	
	n=180	H_O	0.35	0.77	0.26	0.63	0.44	0.49	0.63	
		F_{IS}	0.39	0.07	0.25	0.22	0.31	0.40	0.23	

generation) and the least between voles from Islesboro and North Haven (3 voles per generation) (Table 2.5a). F_{ST} values between the two *M. p. shattucki* populations (North Haven and Islesboro) and their closest possible mainland source populations (Rockport and Northport) were both 0.09, with estimated gene flow of 5 individuals per generation. These estimates indicate that the nearby coastal populations are similar but still distinct from *M. p. shattucki* populations.

AMOVA results indicated that most of the variation (>90%) is among individuals within populations regardless of how data are structured (Table 2.6a-c). If no subspecific structure is assumed, only 9% of the variation was accounted for by variation among populations (Table 2.6a). When comparing *M. p. shattucki* (North Haven and Islesboro combined) to populations of *M. p. pennsylvanicus* (inland Maine (Orono), coastal Maine (Northport and Rockport combined), and another island in the Penobscot Bay (Isle au Haut), the majority of the variation was still among individuals within populations (94%), while the proportion of the variation explained by population structure dropped to 6% (Table 2.6b). When populations are grouped by subspecific designation *M. p. shattucki* (North Haven, Islesboro) and *M. p. pennsylvanicus* (Orono, Northport, Rockport, Isle au Haut), results were similar to the nonstructured comparison, with the majority of the variation among individuals within populations (90%), 9% of the variation among was populations, while variation due to subspecies was only 1% (Table 2.6c). In all comparisons, between population variation ranged between 6 and 9%, which is a relatively large proportion of the variation for extremely variable microsatellites, which are essentially individual fingerprints.

Two one-tailed statistical tests were performed to study isolation by distance. The first tested whether the expected correlation between F_{ST} and geographic distance was

Table 2.5. Pairwise population F_{ST} values (lower matrix) and corresponding number of migrants per generation (Nm) (upper matrix) among populations for (a) microsatellite and (b) mtDNA analyses. Underlined values indicate the F_{ST} value is significantly different from 0.00 ($p<0.05$).

a. microsatellites

		M. p. pennsylvanicus				*M. p. shattucki*	
Subspecies	Populations	Orono	Rockport	Northport	Isle au Haut	North Haven	Islesboro
M. p.	Orono	-	7.9	8.3	4.7	3.1	7.1
pennsylvanicus	Rockport	0.06	-	11.7	8.3	5.1	6.5
	Northport	0.06	0.04	-	7.1	3.8	5.0
	Isle au Haut	0.10	0.06	0.07	-	3.4	3.2
M. p. shattucki	North Haven	0.14	0.09	0.12	0.13	-	2.7
	Islesboro	0.07	0.07	0.09	0.13	0.16	-

b. mtDNA

		M. p. pennsylvanicus				*M. p. shattucki*		*M. p. terranovae*
Subspecies	Populations	Orono	Rockport	Northport	Isle au Haut	North Haven	Islesboro	(NewFoundland)
M. p.	Orono	-	0.8	0.7	0.6	0.3	0.2	0.9
pennsylvanicus	Rockport	0.37	-	0.4	5.0	0.4	0.2	0.5
	Northport	0.41	0.58	-	0.3	0.2	0.1	0.3
	Isle au Haut	0.47	0.09	0.60	-	0.3	0.2	0.3
M. p. shattucki	North Haven	0.63	0.59	0.69	0.62	-	0.3	0.2
	Islesboro	0.74	0.74	0.79	0.75	0.65	-	0.1
M. p. terranovae	(Newfoundland)	0.36	0.48	0.61	0.59	0.68	0.86	-

Table 2.6. Analysis of Molecular Variance results for microsatellite and mitochondrial DNA analysis. Underlined values of F_{ST} (genetic distance among populations among given groups), F_{SC} (among populations within given groups) and F_{CT} (populations among groups) are significantly different from zero ($p<0.05$).

		Microsatellites			Mitochondrial DNA		
Groups	Populations	Source of Variation	Percent of Variation		Source of Variation	Percent of Variation	
a) All Populations (No Structure)	Orono Rockport Northport Isle au Haut North Haven Islesboro	Among Populations Within Populations	9.13	Φ_{ST} = 0.091	d) Among Populations Within Populations	62.53 37.47	Φ_{ST} = 0.625
b) Geographic Structure	Inland Maine (Orono)	Among Populations	5.96	Φ_{ST} = 0.060	e) Among Populations	42.40	Φ_{ST} = 0.424
	Coastal Maine (Northport, Island Maine North Haven	Within Populations	94.04		Within Populations	57.60	
c) Two Groups: *M. p. pennsylvanicus* *M. p. shattucki*	Orono Rockport Northport Isle au Haut North Haven Islesboro	Between Groups (subspecies)	0.99	Φ_{CT} = -0.010	f) Between Groups (subspecies)	34.63	Φ_{CT} = 0.346
		Among Populations within Groups	8.56	Φ_{SC} = 0.097	Among Populations within Groups	33.88	Φ_{SC} = 0.518
		Within Populations	90.45	Φ_{ST} = 0.096	Within Populations	31.48	Φ_{ST} = 0.685

greater than the observed correlation, while the second test investigated if the expected correlation was less than the observed correlation. Both tests indicated that the expected correlation between genetic and geographic distance was neither greater ($p=0.70$) nor less than ($p=0.30$) the observed correlation. Therefore, genetic differentiation between populations was not due to the geographic distance between localities.

Significant linkage disequilibrium between loci is an indication that loci may not be inherited independently, and may bias results. Comparisons between loci for each population indicated that Orono was the only population in which linkage disequilibrium was found for Moe-4 and Moe-1 ($p<0.001$). For Moe-6 and Moe-1, Isle au Haut was the only population in which linkage disequilibrium was found ($p<0.03$). Because disequilibrium was found in only two populations and in only two comparisons of loci, I concluded that these were probably due to sampling issues, and further concluded that all seven loci were assorting independently.

MtDNA

Of the 299 base pairs amplified from the mtDNA control region, 35 sites were variable. There were 32 haplotypes among the 93 individuals sequenced. North Haven had the most haplotypes (8), followed by Northport and Isle au Haut (6 each), Rockport (5), and lastly Orono, Islesboro, and Newfoundland (3 each). Isle au Haut shared haplotypes with both Rockport and Northport, and Islesboro shared haplotypes with North Haven. Orono and Newfoundland had no shared haplotypes with any other population. The transition/transversion ratio was 1.5. The shape parameter, α, of the gamma distribution, was 0.005. Because this value was essentially zero, all subsequent phylogenetic analyses were done without considering the gamma distribution.

Both maximum likelihood (ML) and neighbor-joining (NJ) analyses estimated phylogenetic trees that agree in their topology (Figures 2.2, 2.3 respectively). The ML tree was unrooted, and indicated a distinct separation of North Haven and Islesboro (*M. p. shattucki*) from all other populations sampled. There was no structuring of the other five populations sampled, including Isle au Haut, which could possibly harbor a population of *M. p. shattucki* (Figure 2.2). NJ analysis with *M. p. terranovae* as outgroup also indicated a distinct separation of *M. p. shattucki* with significant bootstrap support (91%). Two lineages were found within *M. p. shattucki*, both of which were found in populations from both North Haven and Islesboro. There was no structuring among *M. p. pennsylvanicus* populations or, surprisingly, between *M. p. pennsylvanicus* and *M. p. terranovae*.

Hypothesizing no genetic structure in the AMOVA provided a base comparison which indicated that a large amount of the variation is due to variation among populations (about 63%), while variation due to individuals within populations was about 37% (Table 2.6d). When geographically close populations are grouped so that the comparison is now *M. p. pennsylvanicus* from inland Maine (Orono), coastal Maine (Northport and Rockport combined), and island Maine (Isle au Haut) versus *M. p. shattucki* (North Haven and Islesboro combined), variation among populations dropped to 42%, while variation among individuals within populations increased to 58% (Table 2.6e). When the primary comparison is between subspecies: *M. p. shattucki* (North Haven, Islesboro) versus *M. p. pennsylvanicus* (Orono, Northport, Rockport, Isle au Haut), variation due to subspecies was found to be about equal to variation found within populations and among populations

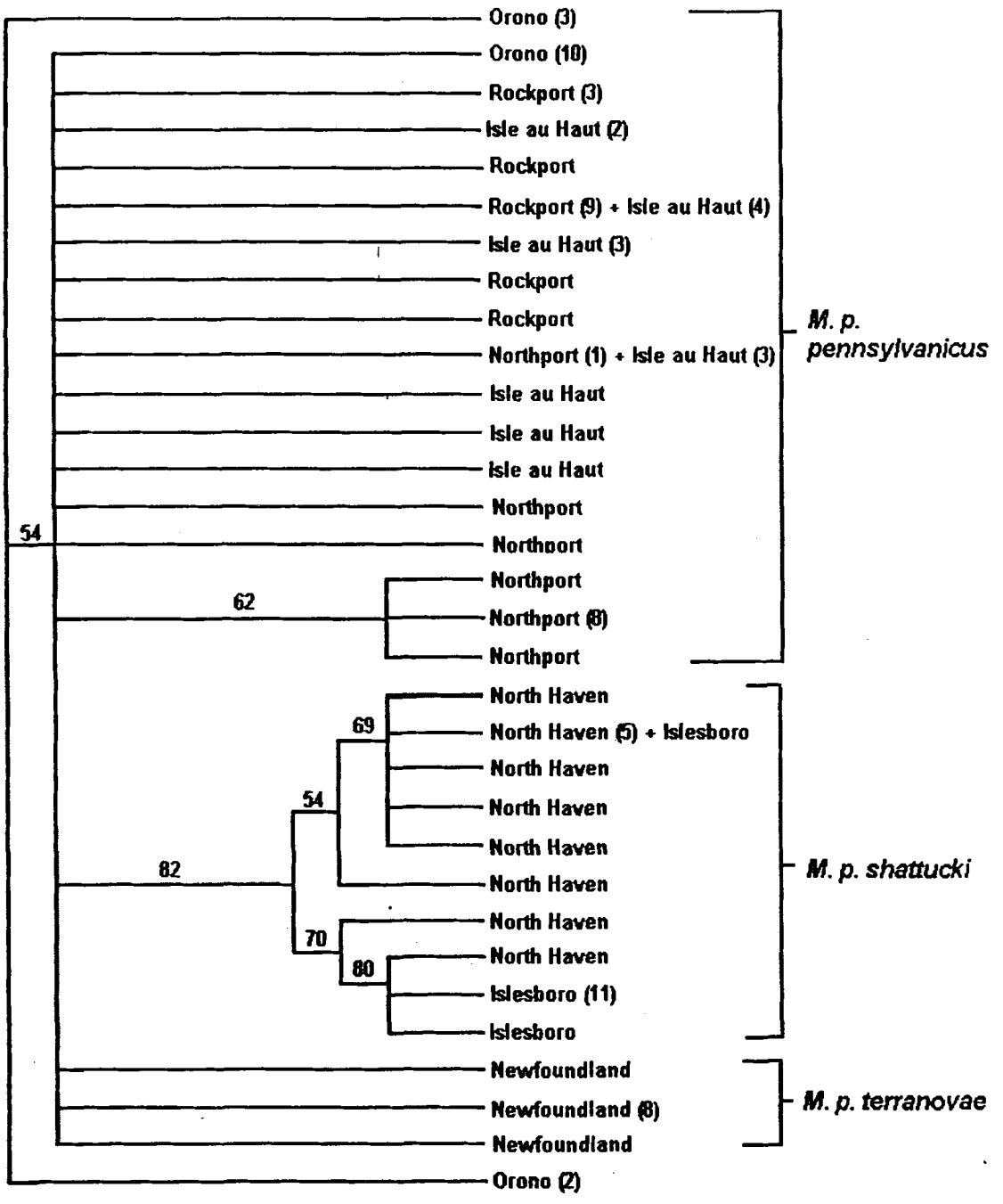

Figure 2.2. Maximum Likelihood tree using HKY-85 genetic distances of four populations of *M. p. pennsylvanicus* (Orono, Northport, Rockport, and Isle au Haut), two populations of *M. p. shattucki* (North Haven and Islesboro) and *M. p. terranovae* from Newfoundland. Numbers in parenthesis indicate number of individuals represented and numbers on branch lengths represent bootstrap score out of 100 replications.

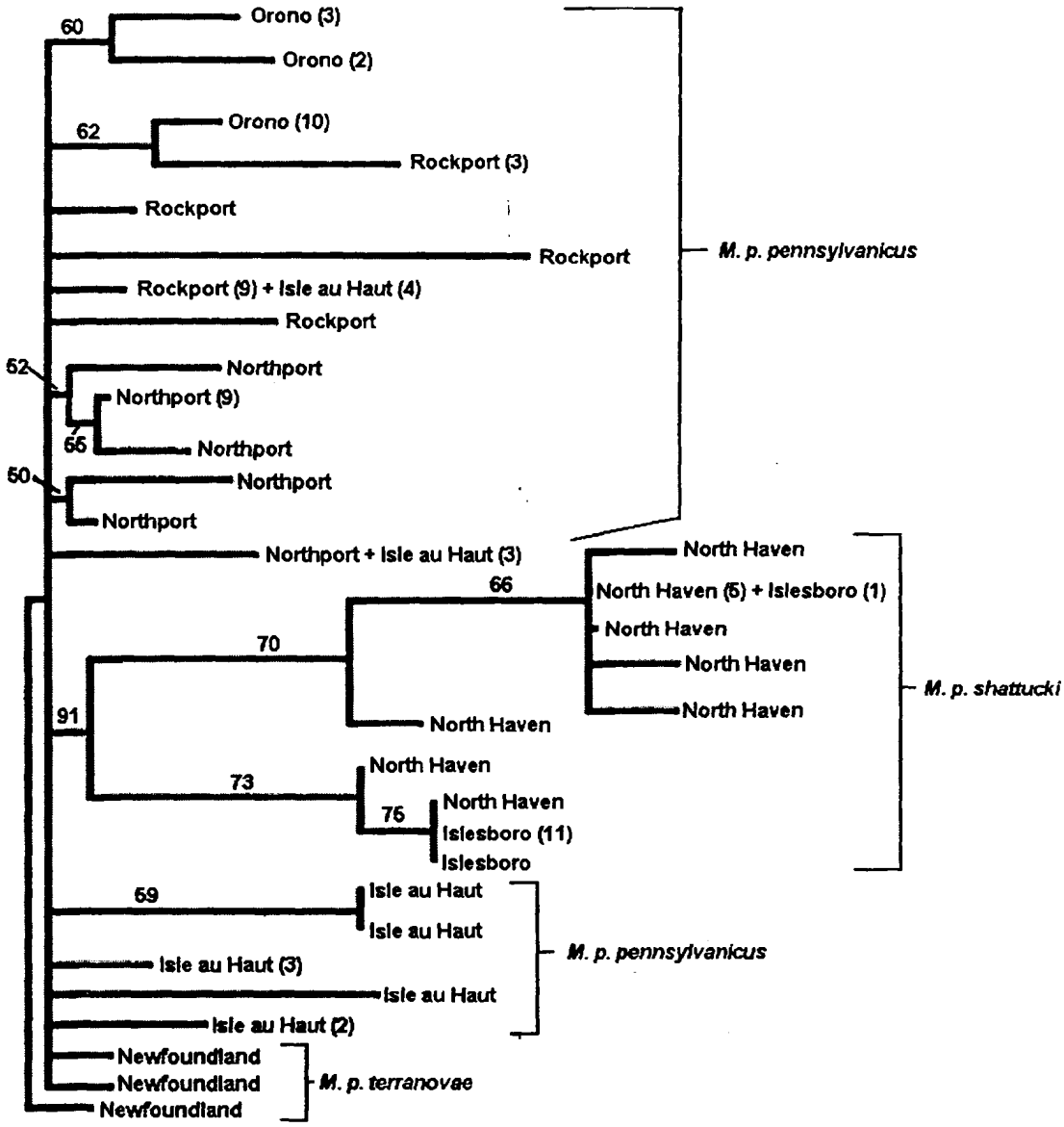

Figure 2.3. Neighbor-Joining tree using Tamura-Nei genetic distances in analysis of mtDNA control region of *M. p. pennsylvanicus* (Orono, Northport, Rockport, and Isle au Haut), two populations of *M. p. shattucki* (North Haven and Islesboro) and *M. p. terranovae* from Newfoundland. Numbers in parenthesis indicate number of individuals represented by the same haplotype, and numbers on branch lengths represent bootstrap score out of 100 replications.

within groups. This last comparison indicated that a significant amount of variation was due to the subspecies designation.

Pair-wise population comparisons were made using the genetic distance measure F_{ST}. All F_{ST} values were found to be significantly different from zero ($p<0.05$) (Table 2.5b). The most similar populations were *M. p. pennsylvanicus* from Isle au Haut and Rockport ($F_{ST}= 0.09$), with a corresponding estimate of gene flow of 5 females per generation.. When compared to all other populations, North Haven and Islesboro, and thus *M. p. shattucki*, had very large genetic distances from other populations, ranging from 0.65 to 0.86 for Islesboro and 0.59 to 0.69 for North Haven. Lowest estimates of gene flow were between Islesboro and Northport and Islesboro and Newfoundland (1 female vole every 10 generations). Because it is highly unlikely that voles are traveling between Newfoundland and Islesboro, the restricted gene flow between these localities suggested that there has been sufficient time since separation of these island subspecies for substantial genetic distance to evolve.

Discussion

Both microsatellite and mitochondrial DNA analyses indicated that *M. p. shattucki* is genetically distinct from mainland populations. Phylogenetic analysis based on mtDNA sequence variation showed that *M. p. shattucki* formed a monophyletic lineage. There was substructure within this lineage but it did not correspond to a specific island - individuals from both islands were found in both lineages. All populations of *M. p. pennsylvanicus* from Maine, as well as individuals of the *M. p. terranovae* subspecies from Newfoundland were in an unresolved group. Microsatellite analysis bolstered these

mtDNA results with highest genetic distances between populations of *M. p. shattucki* and *M. p. pennsylvanicus*. Additionally, this analysis showed that all populations of voles had significant heterozygote deficiency (high F_{IS}) and that there were no populations in Hardy-Weinberg Equilibrium (HWE).

While the overall conclusions for the two types of genetic analysis were the same, there were large differences between the microsatellite and mtDNA results. A portion of this discrepancy could possibly be due to the differences in mutation rates between the control region of the mtDNA (10^{-6}) and microsatellites (10^{-4} to 10^{-5}). Hedrick (1999) showed that the use of microsatellites would lead to an extreme underestimation of F_{ST} and population genetic structure because F_{ST} does not consider possible overlap in sets of alleles between populations due to physical constraints on allele size within the genome and possible back mutations. In Hedrick's view, an allele shared by two populations may not necessarily indicate the populations are closely related, because one population may have evolved that allele size through back mutation, or because it is the maximum size allowed. Therefore, the discrepancy between the population structure results of the two types of data was not an obstruction to my conclusion but supported it more strongly: North Haven and Islesboro are somewhat distinct based on the underestimated F_{ST}'s, leading me to believe that true differentiation is strongly supported.

Average gene diversity (heterozygosity) within the microsatellite analysis was not largely different between any given population; however, it was significantly lower than expected, indicating departure from HWE. This departure may be an indication that mating is not random due to a social structure in which males and females both hold territories during the mating season, and each female chooses her mate from those males

that hold overlapping territories (Tamarin 1985). Alternatively, the departure from HWE may indicate presence of null alleles, which are alleles that do not amplify because of mutations in primer sequences (Pemberton 1995) and result in true heterozygotes being analyzed as homozygotes. Inbreeding coefficients were high in all populations sampled, however, these values were all within levels of a previous study, in which inbreeding in *M. pennsylvanicus* populations was estimated as 0.36 using allozymes (Pugh and Tamarin 1988). Behavioral experiments have found that *M. pennsylvanicus* have no reproductive inhibition between littermates such as the congenic *M. orhrogastor* and *M. californicus* have (Batzli et al. 1977). Pugh and Tamarin (1988) concluded that the costs of inbreeding are lower than the costs of dispersal to new territories. It is also possible that the chance characteristics of the seven loci used and over-interpretation of the genotyping data could have influenced the high inbreeding coefficient as well as the departure from HWE.

The two islands on which *M. p. shattucki* exist, Islesboro and North Haven, showed different results in estimates of inbreeding, number of private alleles, and genetic distance. These incongruent results may be due to differences between the two islands, the most important being isolation, as measured by geographic distance between the island and mainland, and human impact, which could be estimated by number of daily ferry trips to the island from the mainland. North Haven is more isolated from the mainland by distance (10.5 km) and human impact (6 ferry trips per day, on average), which influences the amount of gene flow between the island and the mainland. On the other hand, Islesboro is only 3.06 km from the closest mainland point, which is within a vole's dispersal distance over ice (6 km, Lomolino1989), and there are an average of 12

ferry trips per day between the island and mainland. North Haven voles had higher estimates of inbreeding, substantially more private alleles, and higher genetic distance values when compared to each other sampled population. Additionally, the largest genetic distance value overall was between voles on North Haven and Islesboro, which may at first, lead one to believe that Islesboro voles are not *M. p. shattucki*. However, the mtDNA results confirm that both populations are the same subspecies.

Using microsatellites as an indication of genetic differentiation has both problems and benefits. As previously mentioned, an enormous amount of variation was found per locus. Compared to the number of alleles per locus in the studies that originally developed the loci used, meadow voles had an increase of 140 to 500% more alleles per locus. While initially, more variation would seem to create noise in the data set to confound results, F_{ST} values have actually been found to be more precise (less variance) with larger number of alleles per locus (Ruzzante 1998). On the other hand, the lack of a reliable statistic to estimate population differentiation is a complex problem concerning microsatellite analysis. Current models oversimplify the multifaceted mutation dynamics of microsatellite loci, which are confounded by factors such as constraint of allele size and differential mutation rate between loci (Paetkau et al. 1997, see Estoup and Cornuet 1999 for complete overview of mutation models). Using the F_{ST} statistic allowed me to explore variation as typically explored and gave a baseline of differentiation of *M. p. shattucki*. Because of the uncertainty of the statistics for microsatellite loci, the mtDNA and morphological analyses were additionally employed, and I believe that, despite all the underlying issues, F_{ST}'s provided concrete support to the mtDNA results.

Morphological analysis (Chapter 1) concurs with the genetic analysis presented here. As with the morphological data, the genetic evidence presented here disagreed with Crowell's (1973) conclusion that meadow vole populations undergo frequent colonizations and extinctions. Voles on North Haven and Islesboro shared no mtDNA haplotypes with mainland populations, which would have been the case if voles had recently recolonized the islands. Additionally, the higher level of differentiation of *M. p. shattucki* voles would not have occurred in the microsatellite analysis.

The mtDNA analysis showed insight into the genetic history of North Haven, Islesboro, and Isle au Haut islands. I hypothesize the following history. Meadow voles first colonized Islesboro, which is only 3.06 km from the mainland, and Isle au Haut, which can be reached by a scattering of islands, by either crossing the ice when Penobscot Bay froze over or by accidental human introduction. North Haven which had the largest number of unique haplotypes and which is farther away from the mainland than an average vole's dispersal distance, was probably initially colonized by voles through human introduction. Subsequent immigration would also have to occur via human impact. Every icing incident after primary colonization as well as any additional human traffic could possibly bring individuals to Islesboro and Isle au Haut from the mainland, as would any possible human traffic. Islesboro voles only had two haplotypes, and shared one with North Haven, indicating that these voles either had not the time for mutation of the d-loop, or that gene flow was greater from the mainland. Alternatively, Isle au Haut shared haplotypes with Rockport and Northport, and was therefore not differentiated from others at the level of North Haven and Islesboro, leading me to

believe that Isle au Haut meadow voles are not *M. p. shattucki*. The morphological data also agreed with this conclusion (Chapter 1)

The genetic variation between *M. p. shattucki* and the populations of *M. p. pennsylvanicus* in this study warrant further exploration of the life history and possible reproductive isolation of these island voles. It is possible that the meadow voles on North Haven and Islesboro have expanded their habitat use and therefore, may have evolved different adaptations to an island environment (Williamson 1981). Additionally, further quantification of speciation would dictate a study of the hybrids of mainland and island voles to explore if reproductive isolation exists. Furthermore, other islands in Penobscot Bay (in addition to Isle au Haut) should be surveyed for presence of *M. p. shattucki* to investigate the range of this subspecies.

Some caveats should be noted. In the morphological analysis, significant characters in the discriminant function model should be diagnostic for subspecies designations. Historical analysis with museum specimens clearly separates *M. p. shattucki* from all other populations, using greatest length of skull, length of lower tooth row, and least interorbital breadth of which least interorbital breadth was found significantly larger in Youngman's (1967) analysis. Analysis of extant populations suggest that *M. p. shattucki* has diverged in multivariate space from mainland and Isle au Haut voles in Maine, but important morphological variables driving this analysis – tail length and length of upper tooth row – are different than those in the historical analysis. Tail length and length of upper tooth row were not included in Youngman's (1967) analysis. Howe's (1901) original description found that tail length of *M. p. shattucki* specimens was longer than in *M. p. pennsylvanicus*, however length of upper tooth row

was not studied. Because of these differences, it would be difficult to identify a Penobscot meadow vole without doing very detailed morphometrics. Analysis of another subset of voles could perhaps indicate that other variables are driving the analysis.

As for the genetic analysis, microsatellite data do not support subspecies status per se, as every population is significantly different from every other population. These results are unlike many recent population studies using microsatellites (Van de Zande 2000) However, loci in this study were so variable that population substructure may be masked by the variability. Additionally, the mtDNA analysis supported M. p. shattucki as a monophyletic lineage. These results are surprising, considering there was no divergence found between the Newfoundland subspecies (*M. p. terranovae*) and *M. p. pennsylvanicus* populations, although they are separated by great distances. However, more sampling of Islesboro and North Haven to increase sample size needs to be done to ensure that these populations are all significantly divergent.

Conservation Implications

The evolutionary significant unit (ESU) is associated with the distinct population segments that are protected under the US Endangered Species Act. The concept of what constitutes an ESU has changed over the last two decades. Initally, ESU was described as a population unit that has evolved significant adaptive variation based on concordance between different types of data (Ryder 1986). Waples (1991) later extended this definition to reproductively isolated populations. Moritz (1994) focused this definition on the evolutionary past and applied genetic methods by defining an ESU as reciprocal monophyly in mtDNA data and significant divergence of allele frequencies at nuclear loci. Crandall et al. (2000) suggested that both genetic and ecological information should

be used in delineating ESUs by determining distinctiveness of populations in terms of exchangeability, or whether an individual from one population can be placed in the second population and thrive in the same niche as the individuals in its new population. In other words, are individuals from one population essentially exchangeable with those of another, or are they unique? Ecological factors affecting exchangeability are those that limit the spread of variants through genetic drift and natural selection (e.g.. morphology, life history traits, demography) while genetic factors deal with gene flow estimates from genetic data (i.e. microsatellite and mtDNA estimates of *Nm*). Additionally, historic and recent indications of distinctiveness are considered in both ecological and genetic categories.

In my study, microsatellite analysis represented recent genetic divergence, and indicated support for rejecting exchangeability, while the mtDNA results correspond to historic genetic divergence and also indicated support for rejecting exchangeability. The morphological data (Chapter 1), rejected exchangeability in the historic data, as measured by the museum specimens. Recent exchangeability was not as well defined, but *M. p. shattucki* was still defined as an identifiable separate entity in morphological analyses of extant populations (80% correct classification). While this evidence is suggestive of *M. p. shattucki* as an ESU, additional study of *M. p. shattucki* is warranted before this conclusion can be made. The naming of a population as an ESU has possible political ramifications that need to be considered in conjunction with the biological data.

REFERENCES

Anderson, M.H. 1965. Anatomical Preparations. University of California Press, Los Angeles, CA.

Angerbjorn, A. 1986. Gigantism in island populations of wood mice (*Apodemus*) in Europe. Oikos 47: 47-56.

Batzli, G.O., L.L. Getz, and S.Hurley. 1977. Suppression of growth and reproduction of microtine rodents by social factors. Journal of Mammalogy 58: 583-591.

Berry, R.J. 1998. Evolution of small mammals. Pp. 114-127 in: Evolution on Islands, Oxford University Press, Inc., NY

Case, T.J. 1978. A general explanation for insular body size trends in terrestrial vertebrates. Ecology 59: 1-18.

Castro-Campillo, A., H.R. Roberts, D.J. Schmidly, and R.D. Bradley. 1999. Systematic status of *Peromyscus boylii ambiguus* based on morphologic and molecular data. Journal of Mammalogy 80: 1214-1231.

Chamberlain, J.L. 1954. The Block Island meadow mouse, *Microtus provectus*. Journal of Mammalogy 35: 587-589.

Cole, S. 1993. Terrestrial mammal survey of Isle au Haut. Maine Naturalist 1:161-168.

Conkling, P.W. 1999. Islands in Time: A natural and cultural history of the islands of the Gulf of Maine. Down East Books, Camden, ME.

Crandall, K.A., O.R.P. Bininda-Emonds, G.M. Mace, and R.K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15: 290-295.

Crowell, K.L. 1973. Experimental Zoogeography: Introductions of mice to small islands. American Naturalist 107: 535-558.

Estoup, A. and J. Cornuet. 1999. Microsatellite evolution: inferences from population data. Pp 49-64 in Microsatellites: evolution and applications (D.B. Goldstein and C. Schlotterer, eds). Oxford University Press, Oxford.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17:368-376.

Fivush, B., R. Parker, and R.H. Tamarin. 1975. Karyotype of the beach vole, *Microtus breweri*, an endemic island species. Journal of Mammalogy 56: 272-273.

Foster, J. B. 1964. Evolution of mammals on islands. Nature 202: 234-235.

Gallardo, M.H. and D. Reise. 1992. Systematics of *Aconaemys* (Rodentia, Octodontidae). Journal of Mammalogy 73: 779-788.

Galliari, C.A. and F.J. Pardinas. 2000. Taxonomy and distribution of the sigmodontine rodents of genus *Necromys* in central Argentina and Uruguay. Acta Theriologica 45: 211-232.

Graur, D. and W. Li. 2000. Fundamentals of molecular evolution: 2nd edition. Sinauer Associates, Inc., Sunderland, MA.

Gu, X. and J. Zhang. 1997. A simple method for estimating the parameter of substitution rate variation among sites. Molecular Biology and Evolution 14: 1106-1113.

Hartl, D.L. and A.G. Clark. 1997. Principles of population genetics, 3rd edition. Sinauer Associates, Inc., Sunderland, MA.

Hasegawa, M., H Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA sequences. Journal of Molecular Evolution 22: 160-174.

Heaney, L.R. 1978. Island area and body size of insular mammals: evidence from the tri-colored squirrel (*Calloscurius prevosti*) of southeast Asia. Evolution 32: 29-44.

Hedrick, P.W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313-318.

Howe, R.H. 1901. A new race of *Microtus pennsylvanicus*. Proceedings of the Portland Society of Natural History, Volume II. 201-202.

Ishibashi, Y., T. Saitoh, S. Abe, and M.C. Yoshida,. 1995. Polymorphic microsatellite DNA markers in the grey red-backed vole *Clethrionomys refocanus bedfordiae*. Molecular Ecology 4:127-128.

Jaenike, J.R. 1973. A steady state model of genetic polymorphism on islands. American Naturalist 107: 903-905.

Kohn, P.H. and R.H. Tamarin. 1976. Selection at electrophoretic loci for reproductive parameters in island and mainland voles. Evolution 32: 15-28.

Lomolino, M.V. 1989. Bioenergetics of cross-ice movements by Microtus pennsylvanicus, *Peromyscus leucopus*, and *Blarina brevicauda*. Holarctic Ecology 12: 213-218.

MacArthur, R.H. and E.O. Wilson. 1963. An equilibrium theory of insular zoogeography. Evolution 17: 373-387.

MacArthur, R.H. and E.O.Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey:

The Maine Atlas and Gazetteer. 1999. DeLorme. Yarmouth, ME.

Mayr, E. 1954. Change of genetic environment and evolution. Pp. 157-180 in Evolution as a process (J. Huxley, A.C. Hardy and E.B. Ford, eds). Allen and Unwin, London.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, MA.

Modi, W.S. 1986. Karotypic differentiation among two sibling species pairs of new world microtine rodents. Journal of Mammalogy 67: 159-165.

Moncrief, N.D., N.E. Cockett, A.D. Neff, W.L. Thomas, and R.D. Dueser. 1997. Polymorphic microsatellites in the meadow vole (*Microtus pennsylvanicus)*: conservation of loci across species of rodents. Molecular Ecology 6:299-301.

Moritz, C. 1994. Defining evolutionary significant units for conservation. Trends in Ecology and Evolution **9**:373-375.

Moyer, C.A., G.H. Adler, and R.H. Tamarin. 1988. Systematics of New England *Microtus*, with emphasis on *Microtus breweri*. Journal of Mammalogy 69: 782-794.

Paetkau, D., L.P. Waits, P.L. Clarkson, L. Craighead, and C. Strobeck. 1997. An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. Genetics 147: 1943-1957.

Pemberton, J.M., J. Slate, D.R. Bancroft, and J.A. Barrett. 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. Molecular Ecology 4: 249-252.

Pugh, S.R. and R.H. Tamarin. 1987. Inbreeding in a population of meadow vole, *Microtus pennsylvanicus*. Canadian Journal of Zoology 66: 1831-1834.

Rencher, A.C. 1995. Methods of Multivariate Analysis. John Wiley and Sons, Inc. NY.

Ruzzante, D.E. 1998. A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. Canadian Journal of Fisheries and Aquatic Sciences 55: 1-14.

Ryder, O.A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution **1**: 9-10.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392: 491-494.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Sambrook, J., E.F. Fritch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Scheinder, S., J. Kueffer, D. Roessli, and L. Excoffier. 2000. Arlequin, Version 2.0; a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Smith, G.W. 1985. Chronology of Late Wisconsinan deglaciation of coastal Maine. Pp 11-25 in: Late Pleistocene history of northeastern New England and adjacent Québec, Borns, H., LaSalle, P., and W.B. Thompson.

Stewart, D.T. and A.J. Baker. 1997. A phylogeny of some taxa of masked shrews (*Sorex cinerus*) based on mitochondrial DNA d-loop sequences. Journal of Mammalogy 78: 361-376.

Stewart, W.A., B. Piertney, and J.F. Dallas. 1998. Isolation and characterization on highly polymorphic microsatellites in the water vole, *Arvicola terrestris*. Molecular Ecology 9: 1258-1259.

Sutton, D.A. and B.D. Patterson. 2000. Geographic variation of the western chipmunk *Tamias senex* and *T. siskoyou*, with two new subspecies from California. Journal of Mammalogy 81: 299-316.

Tamarin, R.H. 1985. Biology of New World Microtus. American Society of Mammalogy, Shippensburg, PA.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512-526.

United States Fish and Wildlife Service (U.S.F.W.S.) Federal Register. November 15, 1994. Endangered and threatened wildlife and plants, animal candid.

Van de Zande, L., R.C. van Apeldoorn, A.F. Blijdenstein, D. de Jong, W. van Delden, and R. Bijlsma. 2000. Microsatellite analysis of population structure and genetic differentiation within and between populations of the root vole, *Microtus oeconomus* in the Netherlands. Molecular Ecology 9: 1651-1656.

Waples, R.S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of 'species' under the endangered species act. Marine Fisheries Review: **53**:11-12.

Williamson, M.H. 1981. Island populations. Oxford University Press, Oxford.

Whittaker, R.J. 1998. Island biogeography: ecology, evolution, and conservation. Oxford University Press, NY.

Wilson, G.M. and J.R. Choate. 1997. Taxonomic status and biogeography of the southern bog lemming, *Synaptomys cooperi*, on the central great plains. Journal of Mammalogy 78: 444-458.

Wyman, L.C. 1953 The Validity of the Penobscot Field Mouse. Journal of Mammalogy 34:162-166.

Youngman, P.M. 1967. Insular populations of the meadow vole, *Microtus pennsylvanicus*, from northeastern North America, with descriptions of two new subspecies. Journal of Mammalogy 48: 579-588.

APPENDIX A: MEANS (± SD) OF EACH MORPHOLOGICAL VARIABLE OF MUSEUM SPECIMENS

Table A1. Mean(+ SD) skull and external measurements for meadow vole specimens from museum collections. [a] M= males, F= females, U= unknown gender, T= all specimens.

			New England			Maritime Canada		
			M. p. provectus	*M. p. breweri*	*M. p. shattucki*	*M. p. copelandi*	*M. p. magdalenensis*	*M.p.acadicus*
			9 M[a], 13 F[a]	12 M, 16 F	10 M, 12 F	17 M, 17 F	26 M, 16 F, 3 U	48 M, 40 F, 4 U
	Variable		**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**
Skull	Greatest Length of Skull	T[a]	26.90 (1.251)	29.10 (1.606)	28.40 (1.354)	27.44 (1.929)	28.17 (1.993)	25.94 (1.217)
		M	27.17 (0.689)	29.17 (1.729)	28.62 (1.799)	27.18 (2.337)	27.97 (0.622)	26.00 (1.229)
		F	26.72 (1.527)	29.06 (1.56)	28.22 (0.877)	27.69 (1.441)	28.45 (1.776)	25.96 (1.250)
		U					27.95 (2.408)	25.30 (0.722)
	Condylozygomatic Length	T	11.23 (0.777)	11.61 (0.838)	10.94 (0.626)	11.25 (0.662)	11.74 (0.745)	11.14 (1.629)
		M	11.37 (0.680)	11.59 (0.889)	10.95 (0.759)	11.26 (0.620)	11.82 (0.269)	11.19 (0.724)
		F	11.13 (0.850)	11.63 (0.827)	10.93 (0.526)	11.24 (0.720)	11.82 (0.757)	11.20 (0.767)
		U					11.65 (0.777)	10.58 (0.397)
	Zygomatic Breadth	T	14.38 (0.713)	14.56 (1.103)	14.12 (0.571)	13.73 (0.558)	14.51 (1.115)	13.60 (0.658)
		M	14.57 (0.498)	14.78 (1.275)	14.13 (0.636)	13.61 (0.577)	15.18 (0.325)	13.69 (0.700)
		F	14.25 (0.824)	14.39 (0.964)	14.11 (0.539)	13.85 (0.526)	14.65 (1.212)	13.55 (0.618)
		U					14.24 (0.989)	13.14 (0.350)
	Cranial Length	T	12.00(0.836)	11.98 (0.887)	11.29 (0.649)	11.17 (0.739)	12.16 (0.869)	11.23 (0.774)
		M	11.75 (0.559)	12.23 (0.973)	11.46 (0.798)	11.09 (0.703)	11.79 (1.556)	11.28 (0.885)
		F	12.17 (0.968)	11.79 (0.795)	11.15 (0.483)	11.25 (0.786)	12.35 (0.805)	11.19 (0.665)
		U					11.95 (0.900)	11.10 (0.482)
	Cranial Breadth at Squamosals	T	10.05 (0.459)	10.09 (0.502)	10.35 (0.337)	10.07 (0.694)	10.34 (1.004)	9.87 (0.781)
		M	10.11 (0.443)	10.10 (0.481)	10.28 (0.336)	9.90 (0.667)	9.98 (0.226)	9.93 (0.928)
		F	10.00 (0.482)	10.08 (0.533)	10.41 (0.341)	10.25 (0.694)	10.36 (0.928)	9.83 (0.582)
		U					10.38 (1.243)	9.62 (0.729)

Table A1. (continued)

			New England			Maritime Canada		
			M. p. provectus	*M. p. breweri*	*M. p. shattucki*	*M. p. copelandi*	*M. p. magdalenensis*	*M. p. terranovae*
			9 M[a], 13 F[a]	12 M, 16 F	10 M, 12 F	17 M, 17 F	26 M, 16 F, 3 U	23 M, 29 F
	Variable		**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**
Skull (cont.)	**Least Interorbital Breath**	T	4.07 (0.197)	3.62 (0.154)	3.96 (0.144)	3.98 (0.211)	3.93 (0.289)	3.67 (0.255)
		M	4.14 (0.216)	3.59 (0.148)	3.97 (0.181)	3.97 (0.252)	3.85 (0.141)	3.66 (0.287)
		F	4.01 (0.169	3.65 (0.157)	3.94 (0.112)	3.99 (0.167)	3.93 (0.280)	3.65 (0.223)
		U						
	Palatine Foramina Length	T	2.57 (0.510)	2.21 (0.491)	1.94 (0.196)	1.95 (0.272)	2.03 (0.336)	2.03 (0.336)
		M	2.71 (0.476)	2.13 (0.493)	1.97 (0.235)	1.92 (0.293)	2.12 (0.177)	2.12 (0.177)
		F	2.48 (0.531)	2.26 (0.498)	1.92 (0.163)	1.98 (0.255)	2.11 (0.356)	2.11 (0.356)
		U						1.83 (0.322)
	Palatine Foramina Greatest Breadth	T	2.94 (0.268)	3.42 (0.325)	3.19 (0.472)	2.98 (0.313)	2.99 (0.462)	2.87 (0.283)
		M	3.06 (0.262)	3.43 (0.357)	3.06 (0.589)	3.00 (0.347)	3.43 (0.481)	2.82 (0.325)
		F	2.86 (0.249)	3.41 (0.310)	3.29 (0.323)	2.96 (0.286)	3.03 (0.482)	2.90 (0.246)
		U					2.84 (0.413)	
	Palatine Foramina Least Breadth	T	1.98 (0.218)	2.36 (0.371)	2.24 (0.297)	2.09 (0.359)	2.13 (0.395)	2.04 (0.255)
		M	1.96 (0.292)	2.37 (0.322)	2.22 (0.338)	2.15 (0.338)	2.17 (0.042)	2.01 (0.249)
		F	1.99 (0.161)	2.35 (0.414)	2.25 (0.273)	2.04 (0.382)	2.12 (0.423)	2.07 (0.260)
		U					2.19 (0.346)	
	Nasal Length	T	7.36 (0.558)	8.10 (1.285)	8.14 (0.779)	7.66 (1.165)	7.67 (0.974)	7.25 (0.726)
		M	7.49 (0.431)	8.38 (0.890)	8.13 (0.819)	7.61 (1.307)	7.96 (0.141)	7.21 (0.867)
		F	7.27 (0.633)	8.07 (0.997)	8.14 (0.780)	7.71 (1.041)	7.85 (0.852)	7.28 (0.604)
		U					7.37 (1.178)	
	Nasal Breadth	T	2.95 (0.317)	2.84 (0.440)	2.35 (0.228)	2.72 (0.391)	2.64 (0.376)	2.48 (0.324)
		M	3.01 (0.352)	2.92 (0.514)	2.36 (0.242)	2.61 (0.313)	3.05 (0.651)	2.54 (0.314)
		F	2.90 (0.296)	2.78 (0.382)	2.35 (0.226)	2.83 (0.442)	2.61 (0.315)	2.44 (0.330)
		U					2.60 (0.436)	

Table A1. (continued)

			New England			Maritime Canada		
			M. p. provectus	*M. p. breweri*	*M. p. shattucki*	*M. p. copelandi*	*M. p. magdalenensis*	*M. p. terranovae*
			9 M[a], 13 F[a]	12 M, 16 F	10 M, 12 F	17 M, 17 F	26 M, 16 F, 3 U	23 M, 29 F
	Variable		**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**
Skull (cont.)	Cranial Breadth at Zygomatic Arch	T	9.86 (0.426)	9.84 (0.567)	10.43 (0.298)	10.13 (0.524)	10.25 (0.862)	9.68 (0.585)
		M	9.89 (0.436)	10.09 (0.388)	10.49 (0.328)	10.00 (0.561)	10.20 (0.233)	9.71 (0.607)
		F	9.83 (0.435)	9.65 (0.617)	10.38 (0.273)	9.74 (2.130)	10.23 (0.567)	9.67 (0.577)
		U					10.35 (1.267)	
	Length of Upper Tooth Row	T	6.52 (0.687)	7.07 (0.543)	7.20 (0.346)	6.74 (0.432)	6.84 (0.495)	6.50 (0.329)
		M	6.47 (0.737)	7.14 (0.628)	7.11 (0.307)	6.71 (0.374)	6.61 (0.431)	6.45 (0.399)
		F	6.55 (0.679)	7.02 (0.485)	7.27 (0.370)	6.77 (0.492)	6.85 (0.445)	6.55 (0.261)
		U					6.90 (0.552)	
	Length of Lower Tooth Row	T	6.53 (0.453)	7.15 (0.413)	6.97 (0.427)	6.71 (0.324)	7.08 (0.554)	6.57 (0.545)
		M	6.71 (0.244)	7.22 (0.507)	7.08 (0.580)	6.64 (0.345)	7.16 (0.354)	6.48 (0.673)
		F	6.40 (0.526)	7.10 (0.333)	6.88 (0.229)	6.79 (0.293)	7.07 (0.539)	6.63 (0.416)
		U					7.12 (0.629)	
External	Tail Length	T	44.82 (5.338)	49.43 (5.495)	44.86 (4.529)	45.07 (5.826)	48.61 (7.188)	45.87 (5.736)
		M	46.06 (4.876)	50.79 (6.747)	42.30 (4.596)	43.91 (7.285)	46.67 (2.121)	44.30 (7.582)
		F	43.96 (5.662)	48.41 (4.286)	47.00 (3.330)	46.24 (3.750)	50.38 (5.601)	47.10 (3.342)
		U					47.75 (6.856)	
	Hind Foot Length	T	22.00 (0.900)	22.46 (0.871)	22.77 (0.612)	22.70 (1.757)	23.15 (3.305)	22.31 (2.616)
		M	22.22 (0.870)	22.50 (0.977)	22.90 (0.876)	29.20 (7.285)	21.67 (0.707)	23.04 (3.548)
		F	21.85 (0.922)	22.44 (0.814)	22.67 (0.246)	23.05 (0.879)	22.94 (0.898)	21.72 (1.334)
		U					22.53 (1.176)	
	Total Body Length	T	168.11 (12.158)	174.89 (17.653)	182.59 (10.027)	169.56 (23.276)	172.37 (20.551)	164.87 (16.397)
		M	170.50 (9.975)	180.58 (16.373)	184.90 (3.573)	165.03 (29.202)	190.67 (7.071)	163.74 (17.014)
		F	166.46 (13.605)	170.63 (17.861)	180.67 (13.138)	174.09 (14.866)	177.08 (13.316)	165.76 (16.137)
		U					164.94 (22.988)	

Table A2. Mean(+ SD) skull and external measurements for male, female and both meadow vole specimens from museum [a] M= males, F= females, U= unknown gender, T= all specimens.

			Maritime Canada (continued)		*M. p. pennsylvanicus*			
			M. p. terranovae	*M. p. enixus*	Island Maine	Mainland Maine	New Brunswick	Gaspé Pennisula
			23 M, 29 F	29 M, 30 F, 7 U	12 M, 10 F	25 M, 16 F	23 M, 7 F	27 M, 29 F, 5 U
	Variable		**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**
Skull	Greatest Length of Skull	T[a]	26.69 (1.236)	26.40 (1.472)	26.39 (1.012)	26.54 (1.270)	25.68 (1.373)	25.50 (1.597)
		M	26.45 (1.414)	26.79 (1.525)	26.74 (1.049)	26.43 (1.421)	25.52 (1.470)	25.35 (1.735)
		F	26.88 (1.060)	26.22 (1.363)	25.97 (0.825)	26.73 (0.984)	26.22 (0.868)	25.46 (1.549)
		U		25.57 (1.396)				26.51 (0.696)
	Condylozygomatic Length	T	11.68 (0.512)	11.49 (0.802)	11.71 (0.596)	11.58 (0.678)	10.88 (0.699)	11.04 (0.762)
		M	11.62 (0.359)	11.52 (0.747)	11.69 (0.655)	11.68 (0.664)	10.83 (0.759)	10.87 (0.817)
		F	11.74 (0.609)	11.51 (0.732)	11.72 (0.553)	11.43 (0.694)	11.06 (0.449)	11.08 (0.694)
		U		11.25 (1.304)				11.83 (0.085)
	Zygomatic Breadth	T	13.83 (0.615)	13.98 (0.737)	13.98 (0.636)	14.02 (0.578)	13.33 (0.528)	13.29 (0.922)
		M	13.84 (0.608)	14.21 (0.770)	14.08 (0.813)	14.04 (0.545)	13.29 (0.506)	13.21 (0.906)
		F	13.81 (0.630)	13.79 (0.614)	13.87 (0.331)	13.99 (0.647)	13.47 (0.617)	13.27 (0.997)
		U		13.80 (0.907)				13.85 (0.163)
	Cranial Length	T	11.58 (0.753)	11.56 (0.697)	11.98 (0.721)	11.58 (0.600)	11.18 (0.900)	11.06 (0.969)
		M	11.62 (0.699)	11.61 (0.723)	12.08 (0.795)	11.73 (0.669)	11.03 (0.777)	10.93 (0.940)
		F	11.55 (0.805)	11.51 (0.699)	11.86 (0.642)	11.32 (0.346)	11.66 (1.161)	11.13 (1.068)
		U		11.59 (0.664)				11.30 (0.369)
	Cranial Breadth at Squamosals	T	9.71 (0.643)	10.06 (0.578)	10.21 (0.474)	10.11 (0.552)	9.62 (0.484)	10.03 (0.646)
		M	9.62 (0.592)	10.03 (0.524)	10.17 (0.475)	10.10 (0.565)	9.62 (0.501)	9.98 (0.434)
		F	9.78 (0.683)	10.16 (0.604)	10.25 (0.493)	10.12 (0.547)	9.59 (0.463)	9.97 (0.804)
		U		9.77 (0.647)				10.64 (0.043)

Table A2. (continued)

			Maritime Canada (continued)		*M. p. pennylvanicus*			
			M.p.acadicus	*M. p. enixus*	Island Maine	Mainland Maine	New Brunswick	Gaspé Pennisula
			48 M, 40 F, 4 U	29 M, 30 F, 7 U	12 M, 10 F	25 M, 16 F	23 M, 7 F	27 M, 29 F, 5 U
	Variable		**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**
Skull (cont.)	Cranial Breadth at Zygomatic Arch	T	10.00 (0.686)	10.28 (1.519)	9.94 (0.466)	10.11 (0.551)	10.00 (0.437)	9.91 (0.483)
		M	10.04 (0.672)	10.62 (2.142)	9.95 (0.405)	9.95 (0.549)	9.98 (0.451)	9.82 (0.533)
		F	9.97 (0.710)	10.03 (0.678)	9.94 (0.553)	10.39 (0.449)	10.07 (0.412)	9.92 (0.434)
		U	9.89 (0.753)	9.92 (0.576)				10.34 (0.225)
	Length of Upper Tooth Row	T	6.35 (0.563)	6.55 (0.493)	6.24 (1.154)	6.70 (0.308)	6.32 (0.404)	6.32 (0.460)
		M	6.33 (0.624)	6.59 (0.512)	6.55 (0.427)	6.73 (0.321)	6.28 (0.422)	6.25 (0.433)
		F	6.43 (0.497)	6.54 (0.518)	6.48 (0.349)	6.64 (0.287)	6.43 (0.338)	6.33 (0.471)
		U	5.93 (0.159)	6.46 (0.309)				6.60 (0.520)
	Length of Lower Tooth Row	T	6.28 (0.546)	6.49 (0.529)	6.39 (0.321)	6.56 (0.354)	6.18 (0.360)	6.26 (0.527)
		M	6.21 (0.633)	6.51 (0.435)	6.34 (0.353)	6.62 (0.326)	6.18 (0.372)	6.08 (0.460)
		F	6.37 (0.441)	6.42 (0.513)	6.45 (0.284)	6.46 (0.387)	6.20 (0.344)	6.38 (0.562)
		U	6.21 (0.354)	6.26 (0.350)				6.52 (0.411)
External	Tail Length	T	42.71 (5.593)	42.21 (7.238)	51.20 (4.963)	45.91 (2.515)	43.52 (3.085)	42.75 (6.720)
		M	43.00 (5.745)	42.97 (4.709)	52.17 (6.147)	44.91 (6.265)	43.11 (6.678)	40.78 (6.072)
		F	43.50 (3.883)	44.07 (6.091)	50.05 (2.929)	47.57 (7.561)	44.86 (9.371)	43.93 (7.294)
		U	38.80 (6.760)	41.43 (3.867)				46.60 (3.209)
	Hind Foot Length	T	20.92 (2.898)	22.33 (3.669)	21.20 (1.186)	21.53 (1.357)	20.85 (1.845)	20.94 (0.949)
		M	20.53 (0.946)	21.95 (1.256)	21.75 (0.941)	21.53 (1.062)	20.83 (2.704)	20.96 (1.074)
		F	20.75 (0.906)	21.68 (1.235)	20.53 (1.139)	22.21 (2.507)	20.93 (4.382)	20.95 (0.827)
		U	20.40 (0.547)	20.71 (1.976)				20.80 (1.095)
	Total Body Length	T	156.69 (14.807)	159.98 (17.080)	170.41 (11.241)	166.41 (6.867)	153.97 (7.250)	153.28 (19.631)
		M	156.63 (13.183)	162.93 (18.283)	174.92 (9.539)	164.39 (13.995)	151.57 (16.673)	158.60 (20.852)
		F	155.85 (17.146)	158.77 (16.247)	165.01 (11.143)	169.78 (12.570)	161.86 (23.054)	154.37 (17.178)
		U	164.00 (7.000)	153.00 (14.776)				151.34 (21.900)

Table A2. (continued)

			Maritime Canada (continued)		*M. p. pennsylvanicus*			
			M.p.acadicus 48 M, 40 F, 4 U	*M. p. enixus* 29 M, 30 F, 7 U	Island Maine 12 M, 10 F	Mainland Maine 25 M, 16 F	New Brunswick 23 M, 7 F	Gaspé Pennisula 27 M, 29 F, 5 U
	Variable		**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**	**Mean (± SD)**
Skull (cont.)	Cranial Breadth at Zygomatic Arch	T	10.00 (0.686)	10.28 (1.519)	9.94 (0.466)	10.11 (0.551)	10.00 (0.437)	9.91 (0.483)
		M	10.04 (0.672)	10.62 (2.142)	9.95 (0.405)	9.95 (0.549)	9.98 (0.451)	9.82 (0.533)
		F	9.97 (0.710)	10.03 (0.678)	9.94 (0.553)	10.39 (0.449)	10.07 (0.412)	9.92 (0.434)
		U	9.89 (0.753)	9.92 (0.576)				10.34 (0.225)
	Length of Upper Tooth Row	T	6.35 (0.563)	6.55 (0.493)	6.24 (1.154)	6.70 (0.308)	6.32 (0.404)	6.32 (0.460)
		M	6.33 (0.624)	6.59 (0.512)	6.55 (0.427)	6.73 (0.321)	6.28 (0.422)	6.25 (0.433)
		F	6.43 (0.497)	6.54 (0.518)	6.48 (0.349)	6.64 (0.287)	6.43 (0.338)	6.33 (0.471)
		U	5.93 (0.159)	6.46 (0.309)				6.60 (0.520)
	Length of Lower Tooth Row	T	6.28 (0.546)	6.49 (0.529)	6.39 (0.321)	6.56 (0.354)	6.18 (0.360)	6.26 (0.527)
		M	6.21 (0.633)	6.51 (0.435)	6.34 (0.353)	6.62 (0.326)	6.18 (0.372)	6.08 (0.460)
		F	6.37 (0.441)	6.42 (0.513)	6.45 (0.284)	6.46 (0.387)	6.20 (0.344)	6.38 (0.562)
		U	6.21 (0.354)	6.26 (0.350)				6.52 (0.411)
External	Tail Length	T	42.71 (5.593)	42.21 (7.238)	51.20 (4.963)	45.91 (2.515)	43.52 (3.085)	42.75 (6.720)
		M	43.00 (5.745)	42.97 (4.709)	52.17 (6.147)	44.91 (6.265)	43.11 (6.678)	40.78 (6.072)
		F	43.50 (3.883)	44.07 (6.091)	50.05 (2.929)	47.57 (7.561)	44.86 (9.371)	43.93 (7.294)
		U	38.80 (6.760)	41.43 (3.867)				46.60 (3.209)
	Hind Foot Length	T	20.92 (2.898)	22.33 (3.669)	21.20 (1.186)	21.53 (1.357)	20.85 (1.845)	20.94 (0.949)
		M	20.53 (0.946)	21.95 (1.256)	21.75 (0.941)	21.53 (1.062)	20.83 (2.704)	20.96 (1.074)
		F	20.75 (0.906)	21.68 (1.235)	20.53 (1.139)	22.21 (2.507)	20.93 (4.382)	20.95 (0.827)
		U	20.40 (0.547)	20.71 (1.976)				20.80 (1.095)
	Total Body Length	T	156.69 (14.807)	159.98 (17.080)	170.41 (11.241)	166.41 (6.867)	153.97 (7.250)	153.28 (19.631)
		M	156.63 (13.183)	162.93 (18.283)	174.92 (9.539)	164.39 (13.995)	151.57 (16.673)	158.60 (20.852)
		F	155.85 (17.146)	158.77 (16.247)	165.01 (11.143)	169.78 (12.570)	161.86 (23.054)	154.37 (17.178)
		U	164.00 (7.000)	153.00 (14.776)				151.34 (21.900)

APPENDIX B: MEAN (+SD) OF MORPHOLOGICAL VARIABLES OF EXTANT SPECIMENS

Table B1. Mean (+ SD) for male, female, and total meadow vole specimens from extant populations. [a] M= males, F= females, T= all specimens.

			M. p. pennsylvanicus				*M. p. shattucki*	
			Mainland Maine	Coastal Maine		Penobscot Bay Island		
			Orono	Rockport	Northport	Isle au Haut	North Haven	Islesboro
			11 M[a], 13 F[a]	10 M, 8 F	7 M, 13 F	6 M, 4 F	6 M, 14 F	9 M, 11 F
	Variable		**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**
Skull	Greatest Length of Skull	T[a]	27.37 (1.089)	26.88 (1.475)	26.18 (1.227)	25.75 (1.624)	28.47 (1.367)	28.24 (2.356)
		M	27.12 (1.374)	26.57 (1.909)	26.6 (1.302)	25.58 (1.735)	28.14 (1.662)	28.25 (3.215)
		F	27.57 (0.774)	27.27 (0.529)	25.96 (1.175)	25.99 (1.663)	28.61 (1.265)	28.24 (1.509)
	Condylozygomatic Length	T	12.19 (0.701)	11.71 (1.073)	11.51 (0.644)	11.41 (0.905)	12.29 (1.038)	12.29 (1.043)
		M	12.45 (0.584)	11.71 (1.423)	11.63 (0.617)	11.15 (0.899)	12.03 (0.843)	12.42 (0.465)
		F	11.98 (0.740)	11.73 (0.441)	11.44 (0.673)	11.80 (0.880)	12.41 (1.120)	12.19 (1.366)
	Zygomatic Breadth	T	14.87 (0.622)	14.21 (0.552)	14.00 (0.714)	14.25 (1.069)	14.69 (1.340)	15.34 (0.485)
		M	15.02 (0.677)	14.09 (0.621)	14.44 (0.848)	13.93 (1.119)	15.02 (0.592)	15.5 (0.169)
		F	14.75 (0.571)	14.36 (0.443)	13.77 (0.524)	14.73 (0.914)	14.54 (1.554)	15.21 (0.618)
	Cranial Length	T	12.36 (1.131)	11.75 (0.467)	11.54 (0.759)	12.13 (0.695)	12.2 (0.727)	12.34 (1.222)
		M	12.4 (1.176)	11.55 (0.446)	11.84 (0.442)	12.06 (0.800)	12.47 (0.542)	13.02 (0.653)
		F	12.33 (1.137)	12.01 (0.369)	11.37 (0.854)	12.24 (0.595)	12.08 (0.783)	11.78 (1.315)
	Cranial Breadth at Squamosals	T	10.11 (0.572)	9.82 (0.577)	9.63 (0.580)	10.28 (0.631)	10.37 (1.073)	10.3 (0.513)
		M	10.20 (0.559)	9.94 (0.645)	9.84 (0.494)	10.21 (0.671)	10.14 (0.361)	10.23 (0.566)
		F	10.03 (0.593)	9.67 (0.476)	9.51 (0.608)	10.39 (0.643)	10.48 (1.264)	10.35 (0.487)
	Least Interorbital Breath	T	3.85 (0.130)	3.78 (0.349)	3.81 (0.117)	4.02 (0.238)	4.01 (0.156)	4.09 (0.15)
		M	3.82 (0.129)	3.83 (0.22)	3.81 (0.136)	4.03 (0.159)	4.11 (0.184)	4.17 (0.048)
		F	3.87 (0.133)	3.71 (0.472)	3.82 (0.112)	4.02 (0.358)	3.97 (0.126)	4.03 (0.175)
	Palatine Foramina Length	T	2.21 (0.210)	2.19 (0.370)	2.16 (0.207)	2.08 (0.351)	2.37 (0.431)	2.79 (0.457)
		M	2.21 (0.217)	2.30 (0.400)	2.17 (0.184)	2.08 (0.408)	2.19 (0.368)	3.05 (0.513)
		F	2.21 (0.214)	2.05 (0.301)	2.15 (0.225)	2.09 (0.304)	2.45 (0.445)	2.59 (0.286)

Table B1. (continued)

	Variable		Mainland Maine Orono 11 M[a], 13 F[a] Mean (+ SD)	Coastal Maine Rockport 10 M, 8 F Mean (+ SD)	Coastal Maine Northport 7 M, 13 F Mean (+ SD)	Penobscot Bay Island Isle au Haut 6 M, 4 F Mean (+ SD)	*M. p. shattucki* North Haven 6 M, 14 F Mean (+ SD)	*M. p. shattucki* Islesboro 9 M, 11 F Mean (+ SD)
Skull (cont.)	Palatine Foramina Greatest Breadth	T	2.60 (0.243)	2.52 (0.324)	2.38 (0.365)	2.61 (0.287)	3.04 (0.549)	3.17 (0.452)
		M	2.69 (0.247)	2.56 (0.378)	2.59 (0.457)	2.61 (0.331)	2.63 (0.276)	3.14 (0.652)
		F	2.52 (0.218)	2.46 (0.255)	2.26 (0.258)	2.62 (0.254)	3.22 (0.547)	3.21 (0.215)
	Palatine Foramina Least Breadth	T	1.76 (0.194)	1.72 (0.259)	1.65 (0.296)	1.63 (0.256)	1.87 (0.206)	2.11 (0.367)
		M	1.76 (0.189)	1.81 (0.216)	1.68 (0.355)	1.73 (0.204)	1.76 (0.262)	2.19 (0.444)
		F	1.76 (0.206)	1.61 (0.276)	1.63 (0.273)	1.47 (0.265)	1.92 (0.162)	2.04 (0.297)
	Nasal Length	T	7.56 (0.653)	7.40 (0.838)	7.05 (0.78)	7.26 (0.703)	8.25 (0.56)	8.00 (0.585)
		M	7.44 (0.632)	7.18 (1.024)	7.08 (0.312)	7.49 (0.715)	8.07 (0.513)	8.25 (0.587)
		F	7.66 (0.679)	7.68 (0.451)	7.03 (0.244)	6.92 (0.611)	8.32 (0.58)	7.80 (0.527)
	Nasal Breadth	T	3.32 (0.233)	3.42 (0.095)	3.27 (0.351)	3.33 (0.517)	3.55 (0.407)	3.50 (0.236)
		M	3.38 (0.265)	3.46 (0.112)	3.3 (0.57)	3.61 (0.402)	3.62 (0.408)	3.52 (0.207)
		F	3.27 (0.199)	3.38 (0.049)	3.24 (0.386)	2.91 (0.376)	3.52 (0.418)	3.48 (0 266)
	Cranial Breadth at Zygomatic Arch	T	9.75 (0.585)	9.24 (0.530)	9.43 (0.632)	10.07 (0.538)	10.05 (0.443)	9.91 (0.414)
		M	9.73 (0.562)	9.41 (0.428)	9.64 (0.353)	10.12 (0.617)	10.33 (0.422)	9.94 (0.488)
		F	9.76 (0.627)	9.03 (0.596)	9.31 (0.249)	9.98 (0.468)	9.93 (0.409)	9.89 (0.366)
	Length of Upper Tooth Row	T	6.65 (0.383)	6.65 (0.418)	6.73 (0.398)	6.12 (0.416)	6.98 (0.353)	6.94 (0.416)
		M	6.68 (0.303)	6.61 (0.458)	6.82 (0.302)	6.13 (0.502)	6.87 (0.322)	6.84 (0.426)
		F	6.63 (0.451)	6.71 (0.383)	6.68 (0.284)	6.09 (0.311)	7.03 (0.365)	7.03 (0.408)
	Length of Lower Tooth Row	T	6.58 (0.357)	6.21 (0.581)	6.32 (0.268)	6.13 (0.22)	6.82 (0.542)	6.71 (0.45)
		M	6.62 (0.350)	6.19 (0.632)	6.51 (5.264)	6.07 (0.196)	6.55 (0.448)	6.82 (0.474)
		F	6.54 (0.372)	6.24 (0.552)	6.22 (5.707)	6.22 (0.255)	6.94 (0.551)	6.63 (0.431)

Table B1. (continued)

			Mainland Maine	Coastal Maine		Penobscot Bay Island	*M. p. shattucki*	
			Orono	Rockport	Northport	Isle au Haut	North Haven	Islesboro
			11 M[a], 13 F[a]	10 M, 8 F	7 M, 13 F	6 M, 4 F	6 M, 14 F	9 M, 11 F
	Variable		**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**	**Mean (+ SD)**
External	Tail Length	T	43.33 (4.546)	45.89 (6.85)	44.65 (5.019)	46.30 (4.572)	40.05 (4.571)	45.4 (4.43)
		M	42.73 (4.650)	45.4 (8.195)	46.29 (0.813)	45.83 (4.446)	40.33 (2.066)	45.44 (3.644)
		F	43.85 (4.580)	46.5 (5.182)	43.77 (0.378)	47.00 (5.354)	39.93 (5.37)	45.36 (5.163)
	Hind Foot Length	T	19.92 (1.018)	19.78 (1.166)	20.35 (0.641)	20.20 (1.033)	20.85 (0.988)	21.55 (1.234)
		M	20.00 (1.000)	19.6 (1.174)	21.14 (15.73)	20.33 (1.211)	21 (0.894)	21.78 (0.667)
		F	19.85 (1.068)	20 (1.195)	19.92 (15.424)	20.00 (0.816)	20.79 (1.051)	21.36 (1.567)
	Total Body Length	T	147.42 (11.594)	147.44 (12.055)	144.2 (16.178)	151.90 (14.302)	151.5 (14.877)	167.6 (15.716)
		M	145.45 (13.0948)	146.8 (11.98)	147.71 (15.424)	149.50 (15.909)	152.17 (15.549)	170.67 (10.344)
		F	149.08 (10.404)	148.25 (12.926)	142.31 (16.178)	155.50 (12.767)	151.21 (15.172)	165.09 (19.191)

BIOGRAPHY OF THE AUTHOR

Jennifer Lowry was born in Decatur, Georgia in 1976. She grew up in Simsbury, Connecticut where she graduated from Simsbury High School in 1994. She then traveled to Florida and earned her Bachelor's in Science majoring in Zoology in 1998. While at Florida, Jennifer started working in a molecular genetics lab under Dr. Michael Humphreys-Beher, which she continued for six months after graduation. She then worked as a fisheries biologist intern in Oregon before moving to Maine and starting graduate school in 1999. Jennifer is a candidate for the Master of Science degree in Wildlife Ecology in August, 2002.