

Genetic relationships of meadow vole (*Microtus pennsylvanicus*) populations in central Appalachian wetlands

K.E. Franci, T.C. Glenn, S.B. Castleberry, and W.M. Ford

Abstract: We sequenced and compared variation within a 375-base-pair segment of the mitochondrial DNA control region of 323 meadow voles (*Microtus pennsylvanicus* (Ord, 1815)) among 14 populations to determine the influence of past and present landscape connectivity among isolated wetlands in the central Appalachian Mountains. To best explain observed differences among sites, we used genetic and landscape-level (GIS) data to test a null hypothesis (no genetic differences) and three alternate explanations of significant variation owing to founder effects, effective population size, or isolation by distance. Sequencing results revealed 16 distinct haplotypes (1–8 haplotypes/site), with two present in samples from most wetlands, and half of the remaining haplotypes concentrated in specific geographic clusters. Our findings best support the explanation that founder effects have influenced current genetic patterns among sites. These founder effects are likely due to historical land-use activities such as exploitative logging (ca. 1880–1920; creating early successional habitats for voles) and subsequent forest regeneration over the past half century; they were also likely influenced by postglacial colonization patterns. Therefore, current genetic diversity in these populations seems to largely reflect the number and source of voles that successfully colonized these isolated wetlands during the window of opportunity immediately following extensive logging.

Résumé : Nous avons séquencé un segment de 375 paires de bases de la région de contrôle de l'ADN mitochondrial chez 323 campagnols de Pennsylvanie (*Microtus pennsylvanicus* (Ord, 1815)) appartenant à 14 populations des terres humides isolées du centre des Appalaches et nous avons comparé la variation afin de déterminer l'influence des connectivités actuelles et passées des paysages. Afin de mieux expliquer les différences observées entre les sites, nous avons utilisé des données génétiques et des informations au niveau du paysage (GIS) pour tester une hypothèse nulle (aucune différence génétique) et trois explications de rechange de variations significatives, soit l'effet fondateur, la taille effective de la population et l'isolement par la distance. Le séquençage indique l'existence de 16 haplotypes distincts (1–8 haplotypes/site), dont deux sont présents dans les échantillons de la plupart des terres humides et la moitié des haplotypes restants concentrés dans des regroupements géographiques spécifiques. Nos résultats appuient principalement l'explication selon laquelle l'effet fondateur a influencé les patrons génétiques actuels au sein des sites. Cet effet fondateur est dû vraisemblablement aux utilisations des terres dans le passé, telles que la coupe commerciale de la forêt (vers 1880–1920, qui a créé des habitats de début de succession pour les campagnols) et la régénération subséquente au cours du dernier demi-siècle; il a aussi été influencé par les patrons de colonisation après les glaciations. La diversité génétique actuelle dans ces populations semble donc refléter en grande partie les nombres et les origines des campagnols qui ont colonisé avec succès ces terres humides isolées durant la période favorable qui s'est produite immédiatement après les importantes coupes de forêts.

[Traduit par la Rédaction]

Introduction

The pattern of wholesale forest habitat alteration followed by a gradual return toward pre-European settlement conditions and the recovery of many forest-obligate organisms is well documented for much of eastern North America. Conversely, the impacts of restoration upon species able to capitalize on earlier disturbance are less understood. Prior to the late-19th century, most of central Appalachia's higher eleva-

tions (>700 m) were densely forested and early successional habitats were uncommon (Stephenson 1993). Similarly, emergent wetlands also were uncommon; regionally most wetlands were heavily forested, dominated by red spruce (*Picea rubens* Sarg.; Clarkson 1993) with dense balsam fir (*Abies balsamea* (L.) P. Mill.) and rosebay rhododendron (*Rhododendron maximum* L.) thickets (Fortney 1993). However, extensive logging often followed by extreme fire events occurred in the central Appalachians from 1880 to

Received 22 February 2007. Accepted 17 December 2007. Published on the NRC Research Press Web site at cjz.nrc.ca on 28 March 2008.

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1920. In some areas, overstory tree removal created pockets of raised water tables, with sites revegetating to shrub-scrub or open-wetland systems. Currently, these open wetlands are locally abundant at high elevations in east-central and northern West Virginia and western Maryland west of the Allegheny Front in the Appalachian Plateau region of the central Appalachians. Although unglaciated, these high-elevation sites in our study once supported large areas of treeless alpine tundra (ca. 13 000 – 17 000 before common era) — clearly a result of glacial activity farther north in present-day Pennsylvania and Ohio (Delcourt and Delcourt 1998).

Over the past half-century, much of the surrounding upland landscape has reverted back to a mature second-growth mixed northern hardwood – montane conifer landscape. Therefore, in <125 years, this conifer-dominated forested landscape was clear-cut followed by burning and long-term grazing and then allowed to revert again to a mostly forested landscape (Stephenson 1993). Undoubtedly, these large-scale anthropogenic landscape changes and subsequent semi-restoration had profound effects, including the distribution of genetic variation within and among mammalian populations, particularly on those species favored by the temporary shift from forested to early-successional conditions (Lehman and Wayne 1991; Wayne et al. 1992; Tallmon et al. 2002).

As the most widely distributed microtine species in North America, meadow voles (*Microtus pennsylvanicus* (Ord, 1815)) are an early successional species that frequently inhabit pastures, old fields, and grassy rights-of-way, as well as the drier areas of open bogs, marshes, and glades (Reich 1981). Although meadow voles are common in the central Appalachians in patchily distributed, anthropogenically maintained grasslands and have occurred throughout the region since the last glacial maxima, we speculate that this species may have been largely absent from most pre-disturbance forested wetlands. This species generally is outcompeted by southern red-backed voles, *Myodes gapperi* (Vigors, 1830) (= *Clethrionomys gapperi* (Vigors, 1830)), in forested areas, and therefore are restricted to larger (>3 ha) open habitats (Grant 1969; Morris and Grant 1972; Kurta 1995; Franci et al. 2004). However, meadow voles have been successful in using relatively narrow open-habitat corridors, such as roads, day-lighted trails, and railroad grades as dispersal routes (Getz et al. 1978). Therefore, the vast clear-cut landscape with forest acreage at the historic minimum and open areas at its maximum at the turn of the 20th century probably provided large patches and connecting corridor habitat for dispersal and colonization by meadow voles. As forests regenerated throughout the region and pastures were abandoned, many of these wetlands remained the only open habitats that continued to serve as early-successional refugia for meadow voles.

We investigated genetic variation among meadow voles from 15 topographically isolated, high-elevation wetlands not connected by any obvious wetland or upland open habitat in West Virginia and western Maryland. We used variation of mitochondrial DNA (mtDNA) to determine colonization patterns and connectivity for vole populations. To detect differences at this regional scale and time frame, we sequenced a portion of the noncoding control region of

mtDNA. Mitochondrial DNA sequences provide great detail from a small region of the genome (Parker et al. 1998), can detect differences among populations at small spatial scales (Plante et al. 1989; Ishibashi et al. 1997; Aars et al. 1998), and are particularly sensitive to the effects of bottlenecks (Glenn et al. 1999). Relatively large amounts of genetic variation have been discovered in mtDNA of this or similar species in habitats that have been subjected to relatively recent glaciation (Plante et al. 1989; Aars et al. 1998; Heckel et al. 2005).

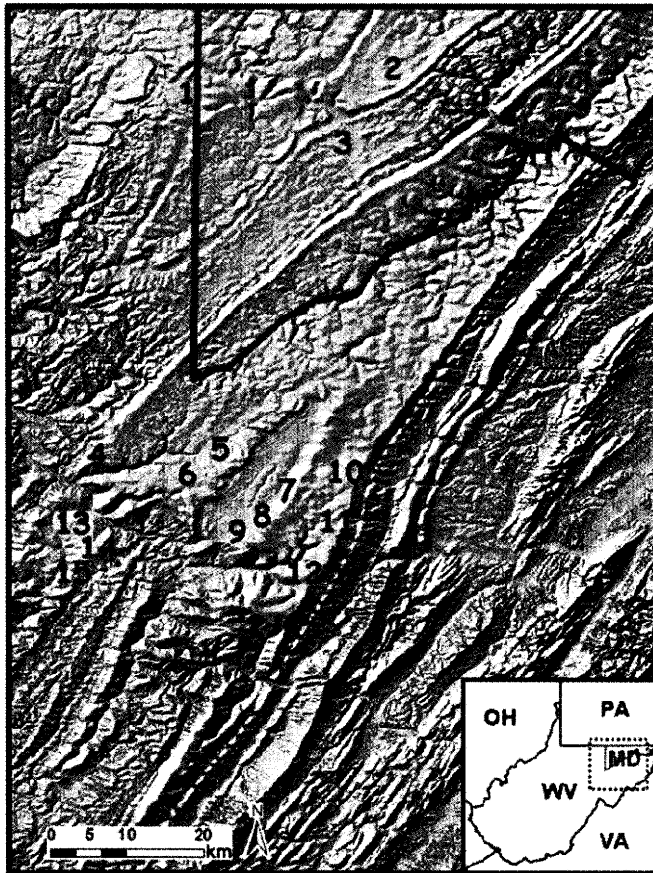
We analyzed the mtDNA data, sometimes coupled with landscape analyses, to test multiple hypotheses in specific scenarios for colonization. We considered differences that could have arisen as a result of post-glacial and (or) post-deforestation colonization. We began with the null hypothesis of no genetic differences among populations of meadow voles. Failure to reject this hypothesis would indicate homogenization of mtDNA haplotypes among vole populations following glaciation or forest removal and that no differences have yet emerged since the wetlands became more isolated. Three nonexclusive alternative explanations were tested. Our first alternative predicts that differences among populations are due to variation in founders, but that all founders came from a common gene pool. Evidence consistent with this explanation includes significant variation among populations, regardless of spatial distance to each other, and no clear spatial pattern among haplotypes. Our second alternative is that genetic variation has been affected by effective population size; that is, all populations began with similar levels of genetic diversity and differences now apparent are due to population size changes and genetic drift. A positive correlation of population size and genetic variation would support this alternative, as would departures from the expectations of a population in equilibrium assayed with neutral genetic markers. Our third alternative predicts that differences among populations are primarily due to isolation by distance. A positive correlation of genetic differentiation and landscape distance would support this hypothesis. Different tests used in this alternative could also indicate the relative effects of colonization following post-glacial maxima versus recent anthropogenic activities (i.e., there were multiple sources for the populations sampled). We secondarily tested whether habitat connectivity and migration significantly affects genetic variation. In this case, isolation-by-distance models would hold for metapopulations (i.e., groups of sites among which dispersal is likely to occur) but not among populations overall. Additionally, populations connected by more likely dispersal routes (e.g., along mountain ridges rather than between parallel ridges) will have fewer differences than populations separated by less likely dispersal routes of similar distance. The limited number of populations sampled per metapopulation restricts statistical inference from connectivity analyses, thus these results should be viewed as a guide for future hypothesis testing rather than tests of specific hypotheses within this study.

Materials and methods

Specimen collection

We collected meadow voles from May to August 2001

Fig. 1. High-elevation wetlands in the Allegheny Mountains of Preston, Tucker, and Randolph counties, West Virginia, and Garrett County, Maryland, at which meadow vole (*Microtus pennsylvanicus*) genetic samples were collected in 2001–2002. Background digital elevation model shows how topographical differences aided in grouping sites. Site numbers referenced in Tables 2, 3, and 5. For GPS coordinates of specific locations see Francel et al. (2004).



and 2002 in 15 high-elevation wetlands in Randolph and Tucker counties, West Virginia, and Garrett County, Maryland, using a combination of Sherman traps, Museum Special snap-traps, and pitfall traps (Fig. 1; background digital elevation model available from <http://esri.com/data/resources/geographic-data.html> [accessed 17 March 2008]; for GPS coordinates and thorough description of capture techniques see Francel et al. 2004). Ear clips of ca. 10 mm² were taken from live animals at point of capture, whereas entire ears were obtained from deceased animals. All tissue samples were immediately preserved in 95% ethanol. Because only one sample was obtained from Yellow Creek (YELL; site 14 in Fig. 1), we included it in descriptive haplotype results but excluded it from population comparison analyses. We estimated relative population size (log-transformed to ensure normality) for each site by multiplying the continuous area of suitable habitat for voles by vole capture success (no. of captures per trap-night).

Genetic analyses

We extracted DNA from 3 mm² ear tissue samples with a DNeasy protocol for animal tissues (Qiagen Inc. 2006) or with a modified Chelex protocol (Glenn 2000). We ampli-

fied 420-base-pair (bp) fragments of the mitochondrial control region by polymerase chain reaction (PCR) with primers L-Pro+ (5'-ACCATCAGCACCCAAAGC-3') and H-424 (5'-CCCGTACCATCGAGATGT-3') designed from an alignment of common vole (*Microtus arvalis* (Pallas, 1778)) sequences (Haring et al. 2000; alignment available from T.C. Glenn). Sequences from both strands were determined directly from PCR products using Big-Dye version 3.0 terminator chemistry and were sequenced with an ABI Prism 377-96 or 3700 sequencer (Applied Biosystems, Foster City, California). We edited chromatograms in Sequencher version 4.0.5 (Gene Codes Corporation, Ann Arbor, Michigan). Following editing, 375 bp were available for all individuals. We resequenced and analyzed samples from 10 individuals as replicates to estimate error rate and ensure correct sample tracking throughout analyses.

We analyzed sequences in PAUP* version 4.0b10 (Swoford 2002) and Arlequin version 2.001 (Schneider et al. 2000). Duplicate haplotypes were identified in PAUP* and removed prior to analysis with Modeltest. We used Modeltest version 3.06 (Posada and Crandall 1998) with $\alpha = 0.01$ to determine the most appropriate model of molecular evolution. We determined the Hasegawa–Kishino–Yano (HKY85; Hasegawa et al. 1985) model with among nucleotide site rate variation (using a four-category discrete approximation of the γ distribution with a shape parameter = 0.0149) to be the most appropriate model. At the population level, we used Arlequin to construct a matrix of Tamura and Nei's genetic distances (TN93; Tamura and Nei 1993; $\gamma = 0.0149$; transition/transversion (Ti/Tv) ratio = 3.93), the model most similar to HKY85 available in Arlequin. We also calculated population pairwise F_{ST} values. An analysis of molecular variance (AMOVA) partitioned the variation among sites into hierarchical groupings using both the frequency only (F_{ST}), which is most sensitive to recent population differentiation, and genetic distance with frequency and nucleotide differences (Φ_{ST}), which better reflects long-term population differentiation. We based statistical significance of AMOVA diversity estimates on probabilities derived from 1023 permutations. Haplotype networks were constructed and visualized using Network version 4.2 (available from <http://fluxus-engineering.com/sharenet.htm> [accessed 20 December 2007]).

We used DnaSP version 4.10.4 from Rozas et al. (2003) to calculate Tajima's D (Tajima 1989a), Fu and Li's D^* (Fu and Li 1993), haplotype diversity, and R_2 of Ramos-Onsins and Rozas (2002), as well as to estimate significance of D , D^* , and R_2 . Values of D , D^* , and R_2 were tested for deviation from the expectations under neutrality, which may indicate natural selection on the mutations or departures from other assumptions such as stable population size (Tajima 1989b; Ramos-Onsins and Rozas 2002).

The frequency of haplotypes within populations was used to estimate haplotype diversity. We correlated the number of meadow voles of a haplotype with the mean physical distance between two voles of the same type to determine if less common haplotypes were restricted in space or a function of different sample sizes (Aars et al. 1998).

Landscape analyses

We obtained GIS data layers of landscape features sur-

rounding our sites, including roads, trails, and wetlands, using the National Wetlands Inventory (NWI; West Virginia GIS Technical Center, available from <http://wvgis.wvu.edu/> [accessed 11 January 2006]). Land use – land cover (LULC) data layers (WebGIS, available from <http://WebGIS.com> [accessed 11 January 2006]) and digital orthophoto quarter quadrangle (DOQQ) maps (Maryland Department of Natural Resources, available from <http://MSGIC.state.md.us> [accessed 11 January 2006]; West Virginia Department of Environmental Protection, available from <http://gis.wvdep.org> [accessed 11 January 2006]) also were obtained. All layers were in the UTM NAD83, Zone 17N coordinate system.

To optimize recognition of wetlands, we overlaid the smaller scale NWI layer onto the LULC layer so that NWI designations took precedence over the broader LULC wetland categories. If specific NWI wetland types (i.e., emergent or shrub–scrub wetlands) were not designated as LULC categories, we created them in the combined LULC and NWI table. In ArcView version 3.2 (Environmental Systems Research Institute, Inc., Redlands, California), we joined separate layers containing roads, trails, and LULC–NWI. Because all trail and some road layers were not available for sites outside of the Monongahela National Forest, DOQQ maps of the areas were overlaid and we recorded all visible roads and trail paths.

We determined distances among the 14 sites using three models of movement. The first model was the straight-line distance between sites (CROW model). In the OPTIMAL model, we measured distance between sites using routes that were optimal for meadow voles: roads (Getz et al. 1978) or agricultural, wetland, or transportation (power-line and gas-line right-of-ways) land-use categories (e.g., Reich 1981; Linzey 1984; Linzey and Cranford 1984). We considered forested trails and forested land-use categories as impassable. The SUBOPTIMAL model was similar to the OPTIMAL model, except that it allowed for additional movement corridors along trails.

Genetic data analyses

We used Mantel tests in NTSYS-pc version 2.02i (Applied Biostatistics, Inc., Port Jefferson, New York) to separately compare the three distance models to matrices of TN93 genetic distances and F_{ST} values among sites. $\text{Log}(x + 1)$ transformations were used on physical distances, whereas arcsine transformations were used to correct for restrictions on the 0–1 intervals of genetic distances and F_{ST} values. Our spatial analyses of molecular variance (SAMOVAs) were run in SAMOVA version 1.0, utilizing both geographic and genetic distances among sites to maximally differentiate single sites or groups of sites (Dupanloup et al. 2002). We input the geographic coordinates of the 14 sites and the distribution and sequences of haplotypes across these sites, and we ran separate SAMOVAs for grouping the sites into two to five groups.

We focused some of our analyses on smaller spatial scales where dispersal among populations seemed likely. We defined the following metapopulations based on known geologic divisions (which corresponded to elevational (watershed) boundaries) and distance: Maryland (sites 1–3), Big Run (site 4), Canaan (sites 5–9), Dolly Sods (sites 10–12), and Otter Creek (sites 13–15; Fig. 1).

Results

Sequences of 375 bp from 323 individuals revealed 19 variable nucleotide sites (14 transitions, 5 transversions) among 16 distinct haplotypes (1–8 haplotypes/site; GenBank accession Nos. AY369455–AY369777; Tables 1, 2). We discovered no haplotype that had more than seven nucleotide site differences (1.9%) from haplotype B, the most common haplotype, and the maximum difference among all haplotypes was nine nucleotides (2.4%; Table 1, Fig. 2). All replicate DNA sequences were identical to the initial sequences obtained, indicating high consistency of results obtained. For comparison, nucleotide differences calculated between meadow voles and outgroup microtine species, southern bog lemmings (*Synaptomys cooperi* Baird, 1858) and southern red-backed voles, were markedly higher (>22% and >18%, respectively) at these sites. Therefore, we found all sequences to be consistent with expectations of correct species identification of meadow voles and correct sample tracking. Additionally, the high Ti/Tv ratio strongly suggests that the DNA fragments we obtained were mitochondrial and not integrated nuclear copies (Zhang and Hewitt 1996).

Null hypothesis

We discovered that two haplotypes, B (164 individuals, 14 sites) and A (46 individuals, 10 sites), were common across sites, whereas several other haplotypes were site- or region-specific (Table 2; Figs. 2, 3). Five sites (Condon Run (COND), Cranesville Swamp (CRSW), The Glades (GLAD), Hammel Glade (HAMM), and Yellow Creek (YELL)) exhibited unique haplotypes. Our AMOVA examining all 14 sites revealed that 29%–35% of variations were explained by differences among populations ($F_{ST} = 0.354$, $P < 0.001$; $\Phi_{ST} = 0.293$, $P < 0.001$).

Alternative 1

From our constructed matrix of TN93 genetic distances for all 14 sites, pairwise distances among sites averaged 12.16 (range 2.28–31.41). F_{ST} values averaged 0.322 (range 0.081–0.796) among paired sites (Table 3). Based on pairwise F_{ST} significance values, we found four sites that were significantly different from all others (COND, CRSW, HAMM, and Moore Run (MOOR)) and one site (Main Bog (MAIN)) that was significantly different from all but one other site (Herz Tract (HERZ)).

Our SAMOVA results indicated that two of the three Maryland sites were most differentiated from all others. When two groups were specified, CRSW (Fig. 1) was separated from all other sites ($F_{CT} = 0.359$, $P = 0.061$; Table 4). For three groups, CRSW and HAMM were separated from one another and from all remaining sites ($F_{CT} = 0.362$, $P = 0.008$). When four groups were designated, CRSW and HAMM were again placed in separate groups, whereas the third group consisted of the two southernmost sites, MOOR and COND, and the fourth group contained all remaining sites ($F_{CT} = 0.375$, $P < 0.001$). The northernmost site, GLAD, remained grouped with the central sites in all of our SAMOVA analyses.

Some haplotypes were restricted to specific populations (Table 2), but there was little geographic structure to the relationships among haplotypes discovered (i.e., haplotypes

Table 1. Nineteen variable bases in the mitochondrial DNA sequences of the 16 haplotypes discovered among 323 meadow voles (*Microtus pennsylvanicus*) sampled from 15 high-elevation wetlands in the Allegheny Mountains of West Virginia and Maryland, 2001–2002.

Haplotype	Variable nucleotide sites																		
	2	4	4	6	7	8	8	9	9	0	1	1	1	1	1	1	2	2	3
Consensus	A	T	A	A	T	A	A	C	T	C	T	C	C	C	T	C	C	T	T
A	G	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	T	A	.
B	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.
D	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.
E	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
F	.	.	.	.	C	.	G	.	.	.	.	.	.	.	.	.	.	.	.
G	.	.	.	.	C	.	G	.	.	.	.	.	.	T	.	.	.	.	.
H	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C
I	.	.	.	T	.	G	.	.	.	A	.	.	.	.	A	.	T	A	.
J	.	.	.	.	.	G	.	.	C	.	.	T	.	T	.	.	.	.	.
K	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	A	.	A	C
L	.	.	.	.	.	.	.	.	.	.	C	T	.	T	.	.	.	.	.
M	.	C	G	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.
N	.	C	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.
O	.	C	.	.	.	.	.	T	.	.	.	T	T	.	.	.	.	.	.
P	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.

Note: Base positions are relative to an alignment of all sequences, which begin at position 7 of the control region relative to the common vole (*Microtus arvalis*) (Haring et al. 2000). Bases that match the consensus are represented by a period.

more similar phylogenetically were not restricted in space; see Figs. 2 and 3). Despite the site-specific haplotypes, the correlation between the number of meadow voles with a haplotype and the mean physical distance between two voles with the haplotype was not significant ($r = 0.316$, $P = 0.233$), indicating that less common haplotypes are not restricted in space and their presence could be correlated to different sample sizes across sites (Aars et al. 1998).

Alternative 2

Observed haplotype diversity averaged 0.465 across the 14 sites (range 0.165–0.757; Table 2). The highest levels of haplotype and nucleotide diversity were found in Maryland (GLAD, HAMM) and the Canaan Valley (BEAL, HERZ, MAIN) areas, whereas the lowest levels were in Otter Creek (COND, MOOR) and Dolly Sods (FISH) areas. Nucleotide diversity averaged 6.41×10^{-3} (range 1.37×10^{-3} – 10.50×10^{-3} ; Table 2). Gene and nucleotide diversity were correlated ($r = 0.726$, $P = 0.003$). Correlations between relative population size and haplotype diversity revealed a

nearly significant positive relationship ($r = 0.516$, $P = 0.059$; Table 5). However, we observed no significant correlation between relative population size and nucleotide diversity ($r = 0.378$, $P = 0.182$). No population deviated significantly from neutrality using Tajima's D ($P > 0.10$ for all values except MAIN, which had a $P > 0.05$) or Ramos-Onsins and Rozas' R_2 , but five populations deviated significantly ($P < 0.05$) when using Fu and Li's D^* (Table 2). Haplotype diversity differed from the expectations of equilibrium and neutrality in all but five populations (Big Run Bog (BGR), COND, Fisher Springs Run (FISH), High Ridge (HIGH), and MOOR), always yielding a positive D^* .

Alternative 3

Our correlations to test isolation by distance (third alternative hypothesis) revealed no apparent patterns between physical distance ($\log(x + 1)$ -transformed) and genetic distances, as measured by both TN93 measures ($r = 0.348$, $P = 0.969$) and population pairwise F_{ST} values (arcsine-transformed; $r = 0.330$, $P = 0.970$).

Table 2. Distribution of haplotypes discovered among meadow voles (*Microtus pennsylvanicus*) sampled from 15 high-elevation wetlands in the Allegheny Mountains of West Virginia and Maryland, 2001–2002.

Pop ID	Site	Haplotypes																Sum	Haplotype diversity (SD)	Nucleotide diversity (SD)	Fu and Li's D*
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P				
1	CRSW		1		2			13	2	1	1							20	0.579 (0.124)	7.26 (1.99)	-0.439
2	GLAD	8	21	5			5	3				2	2	1				47	0.757 (0.052)	10.50 (0.99)	1.536
3	HAMM	3	2	3				2					21		1			32	0.561 (0.098)	9.47 (1.80)	1.499
4	BIGR		6			12	2											20	0.568 (0.086)	2.36 (0.64)	1.001
5	NORT	2	8															10	0.356 (0.159)	6.64 (2.97)	1.382
6	MAIN	14	11						1									26	0.551 (0.048)	9.91 (0.77)	0.253
7	ABER	2	19	1	4													26	0.452 (0.109)	4.63 (1.53)	0.253
8	HERZ	2	8						2									12	0.546 (0.144)	10.18 (2.41)	1.410
9	BEAL	2	5	4														11	0.691 (0.086)	9.99 (1.98)	1.421
10	ALDR	8	26															34	0.371 (0.079)	6.92 (1.47)	1.265
11	FISH	3	42			1												46	0.165 (0.072)	2.43 (1.18)	1.247
12	HIGH	2	12															14	0.264 (0.136)	4.92 (2.54)	1.331
13	MOOR		1		1		13											15	0.257 (0.142)	1.37 (0.76)	-1.081
14	YELL																1	1	—	—	—
15	COND		2					7					2	23	1	1		9	0.389 (0.164)	3.11 (1.32)	1.188
	Sum	46	164	13	7	13	20	7	18	5	1	1	2	23	1	1		323			

Note: Listed is the number of individuals with each haplotype, total number of individuals per site, total number of each haplotype across sites, gene diversity, nucleotide diversity $\times 10^3$, and Fu and Li's *D** (values with $P < 0.05$ are in boldface type). Sites listed according to region: Pop ID corresponds to the geographic numbering system in Fig. 1.

Fig. 2. Haplotype network indicating relationships and relative abundance of 16 haplotypes, discovered among 323 meadow voles (*Microtus pennsylvanicus*) sampled at 15 high-elevation wetlands in the Allegheny Mountains of West Virginia and Maryland, 2001–2002. Letters indicate distinct haplotypes; circle area indicates relative abundance of specific haplotypes. Numbers along lines indicate specific base-pair changes among haplotypes (Table 1); see text for details.

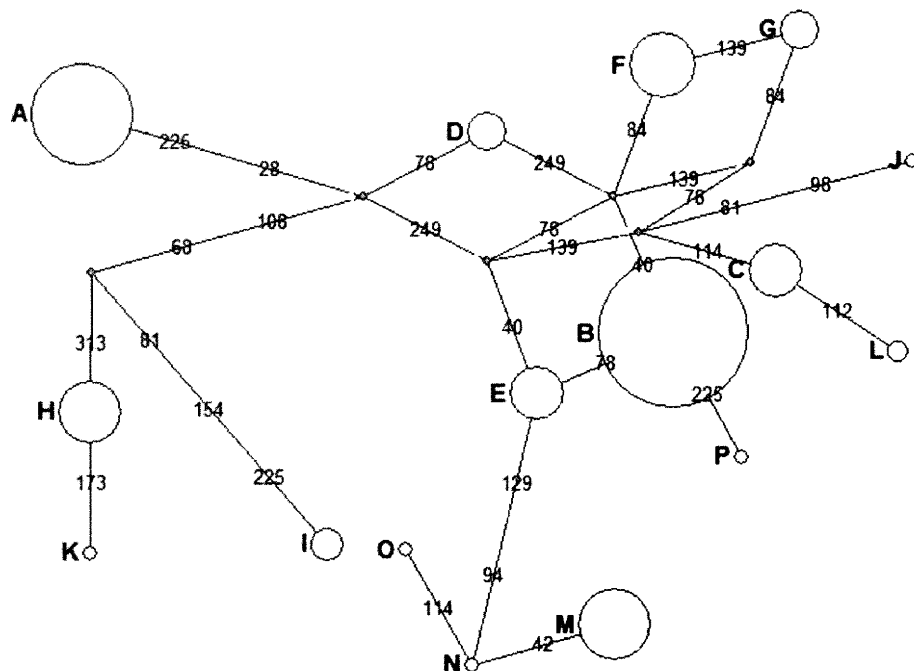


Fig. 3. Haplotype distributions from 323 meadow voles (*Microtus pennsylvanicus*) sampled at 15 high-elevation wetlands in West Virginia and Maryland, 2001–2002. Haplotype diagrams reveal the presence and relative frequency of each haplotype found among groups of geographically clustered sites. For locality names and referenced numbers see Tables 2, 3, and 5.

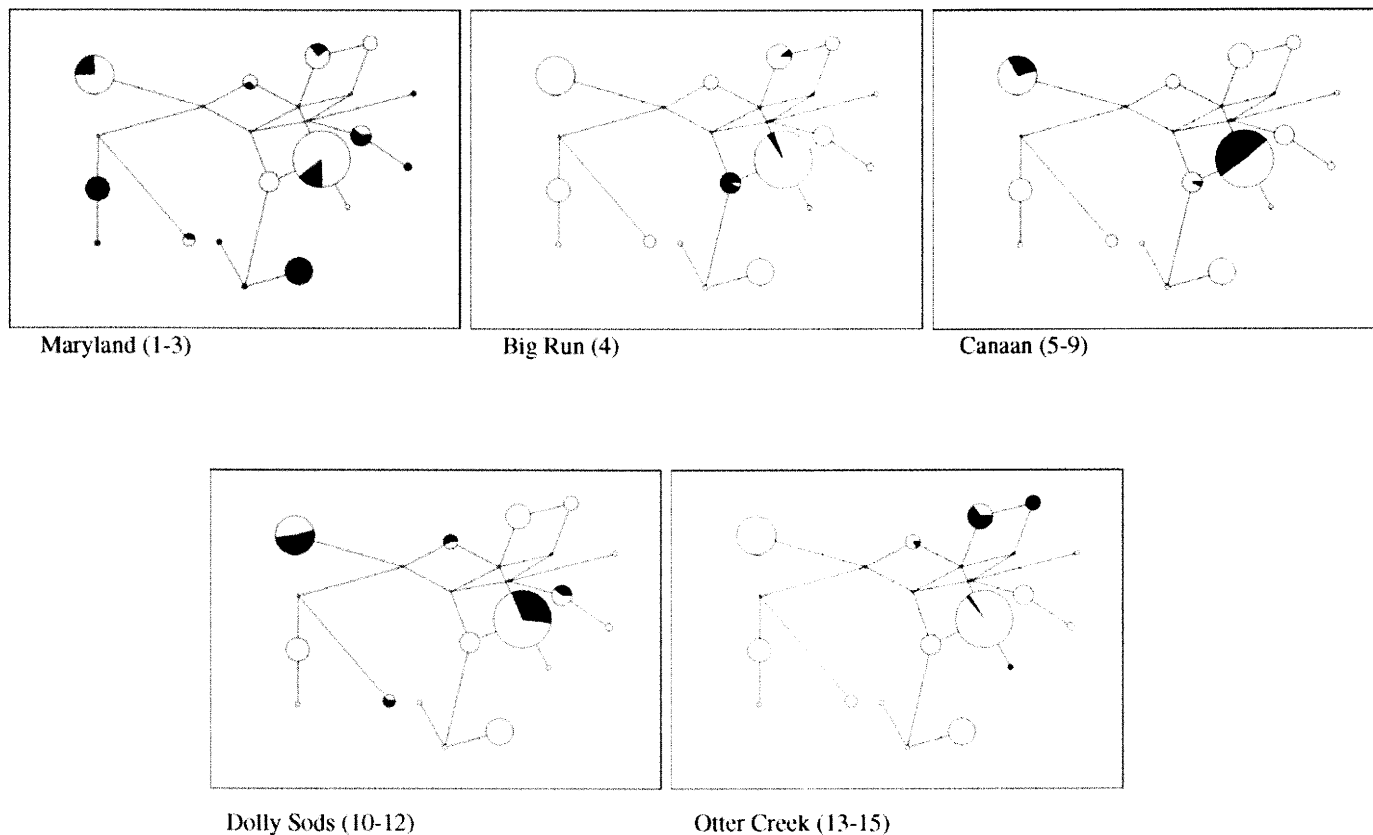


Table 3. Pairwise genetic differences (Tamura and Nei 1993 with $\gamma = 0.0149$ and Ti/Tv = 3.93; above the diagonal) among 322 meadow voles (*Microtus pennsylvanicus*) sampled from 14 high-elevation wetlands in the Allegheny Mountains of West Virginia and Maryland, 2001–2002.

Pop ID	CRSW	GLAD	HAMM	BIGR	NORT	MAIN	ABER	HERZ	BEAL	ALDR	FISH	HIGH	MOOR	COND
1 CRSW	—	17.60	31.41	14.06	16.66	13.56	16.21	15.69	16.89	16.37	17.64	17.14	15.7	21.29
2 GLAD	0.32	—	22.37	7.75	11.51	15.87	10.19	14.20	13.89	11.89	9.99	10.89	10.25	12.78
3 HAMM	0.58	0.29	—	10.24	17.56	27.64	16.18	22.73	23.09	18.47	13.98	16.09	21.75	30.37
4 BIGR	0.66	-0.05	0.06	—	4.61	11.33	3.42	7.86	8.10	5.22	2.28	3.62	4.26	6.66
5 NORT	0.51	-0.01	0.24	0.01	—	12.56	6.07	10.21	12.11	7.67	5.37	6.42	7.59	10.04
6 MAIN	0.24	0.15	0.47	0.37	0.18	—	12.41	13.51	15.37	12.44	12.92	12.74	16.51	20.78
7 ABER	0.61	0.03	0.30	0.06	-0.05	0.32	—	9.27	10.72	6.64	3.90	5.15	5.12	6.81
8 HERZ	0.34	0.02	0.32	0.12	-0.04	0.07	0.06	—	14.37	10.50	9.05	9.73	11.95	15.19
9 BEAL	0.37	-0.03	0.31	0.11	0.10	0.16	0.18	0.06	—	12.39	10.95	11.66	10.13	10.98
10 ALDR	0.49	0.03	0.31	0.04	-0.07	0.18	0.00	-0.01	0.13	—	6.05	6.99	8.39	11.02
11 FISH	0.73	0.12	0.32	0.04	0.04	0.47	0.02	0.22	0.38	0.09	—	4.26	4.56	6.41
12 HIGH	0.58	0.03	0.24	-0.01	-0.08	0.27	-0.05	0.03	0.17	-0.03	-0.01	—	6.28	8.47
13 MOOR	0.69	0.16	0.51	0.72	0.47	0.55	0.39	0.45	0.32	0.37	0.53	0.47	—	2.17
15 COND	0.71	0.23	0.59	0.74	0.48	0.58	0.43	0.46	0.24	0.43	0.58	0.49	0.32	—

Note: F_{ST} values are listed below the diagonal (those significantly different from 0 are in boldface type, $P < 0.05$). Pop ID corresponds to the geographic numbering system in Fig. 1.

Mantel tests revealed that none of the movement models were significantly correlated to matrices of F_{ST} or TN93 genetic distances. Matrix correlation values (r) for the CROW, OPTIMAL, and SUBOPTIMAL models were 0.33, 0.34, and 0.32, respectively, for F_{ST} and 0.43, 0.39, and 0.39, respectively, for TN93 genetic distances ($P > 0.05$ for all correlations). Similar results were discovered with distance models and the F_{ST} matrix (CROW, OPTIMAL, and SUBOPTIMAL models were 0.33, 0.34, and 0.32, respectively; $P > 0.05$). However, when we examined metapopulations separately with TN93 values, movement among the three Maryland sites best fit the OPTIMAL and SUBOPTIMAL models ($r = 0.91$ for both), and was negatively correlated to the CROW model ($r = -0.65$). We found no significant correlations for the Dolly Sods ($r \leq 0.11$ for all three models) or the Canaan ($r \leq 0.40$ for all three models) clusters.

Discussion

The amount of mtDNA diversity we discovered in meadow voles was relatively low compared with other microtine rodents at similar (Aars et al. 1998; Heckel et al. 2005; Piernney et al. 2005; Blois and Arbogast 2006; Miller et al. 2006) or smaller (Ishibashi et al. 1997; Meeks et al. 2007) spatial scales. For example, Aars et al. (1998) found 26 haplotypes in 120 bank voles (*Clethrionomys glareolus* (Schreber, 1780)) in Norway, separated by a maximum distance of 9 km, and Ishibashi et al. (1997) discovered 13 haplotypes with 14 variable sites among 280 bases of the control region among grey red-backed voles (*Clethrionomys rufocanus* (Sundevall, 1846)) in a 1 ha trapping grid in Japan. In the only study in which similar levels of genetic variation have been described across samples at a similar spatial scale, Meeks et al. (2007) sampled 468 bank voles from 14 Ukrainian populations, discovering 15 haplotypes, 11 variable sites among 265 bases of control region mtDNA, with average gene and nucleotide diversities of 0.660 and 4.1×10^{-3} , respectively. Obviously, differences in population history affect genetic diversity significantly, which limit such interspecific comparisons, and all comparisons of mtDNA reflect only maternal histories.

Microtus species recently have been shown to have high rates of molecular evolution in mtDNA (Triant and De-Woody 2006). All other studies of vole control region sequences, however, do show an even higher bias for transitions. Although it would be unlikely that mutation rates are limiting variation in the ancestral (source) population(s), the mutations we observed might suggest a somewhat different set of mutational processes in this species. Alternately, we may have sampled variants much older than most other intraspecific studies of microtine rodents. Overall, however, the amount of variation in the mtDNA surveyed is sufficient to address the hypotheses that we sought to test.

Null hypothesis

AMOVA, pairwise F_{ST} estimates, and SAMOVA indicated marked genetic heterogeneity among meadow vole populations. We therefore rejected the null hypothesis of no genetic differences among populations. Not surprisingly, the level of differentiation that we observed is substantially

Table 4. Results of SAMOVA analyses of meadow vole (*Microtus pennsylvanicus*) haplotypes for two, three, and four groups of 14 high-elevation wetlands in the Allegheny Mountains of West Virginia and Maryland, 2001–2002.

No. of groups	Source of variation	Total variation	Percentage of total	P
2	Among groups	0.946	35.9	0.061
	Among sites within groups	0.445	16.9	<0.001
	Within sites	1.242	47.2	<0.001
3	Among groups	0.873	36.2	0.008
	Among sites within groups	0.295	12.2	<0.001
	Within sites	1.242	51.6	<0.001
4	Among groups	0.860	37.5	<0.001
	Among sites within groups	0.191	8.3	<0.001
	Within sites	1.242	54.2	<0.001

Note: Refer to text for sites included in each group.

Table 5. Capture success and approximate wetland area used to estimate relative population size of meadow voles (*Microtus pennsylvanicus*) among 14 high-elevation wetlands (YELL, set in italic type, was not included in further analysis) in the Allegheny Mountains of West Virginia and Maryland, 2001–2002.

Pop ID	Site	Approximate wetland area (ha)	Capture success (no. of individuals/trap-night)	Relative population size
1	CRSW	225.0	0.0133	2.993
2	GLAD	240.0	0.0393	9.432
3	HAMM	20.0	0.0520	1.040
4	BIGR	15.0	0.0187	0.281
5	NORT	15.0	0.0060	0.090
6	MAIN	30.0	0.0136	0.408
7	ABER	3.0	0.0387	0.116
8	HERZ	10.0	0.0074	0.074
9	BEAL	1.2	0.0141	0.017
10	ALDR	5.0	0.0537	0.269
11	FISH	25.0	0.0356	0.890
12	HIGH	28.0	0.0111	0.311
13	MOOR	2.0	0.0307	0.061
14	<i>YELL</i>	2.5	0.0008	0.002
15	COND	3.0	0.0069	0.021

Note: Pop ID corresponds to geographic numbering system in Fig. 1.

smaller than that noted by Heckel et al. (2005), Blois and Arbogast (2006), and Miller et al. (2006), all of whom surveyed much larger geographic areas.

Alternative 1 (founder effects)

The presence of site- or region-specific haplotypes suggests some degree of genetic isolation, but other geographic patterns among the haplotypes were not evident in the West Virginia localities. Several lines of evidence support the first alternative hypothesis. First, two haplotypes were dominant and nearly ubiquitous, indicating that these haplotypes are common at the broadest landscape scale sampled and that both pre-date the current populations. Second, some sites, especially those within the Dolly Sods cluster, exhibited relatively low haplotype diversity (all but one individual was haplotype A or B). Low diversity can result when one or a few related females colonize an unoccupied site (Aars et al. 1998). We recorded the lowest diversity measures in this study at isolated wetlands, surrounded by forested lands that

have low habitat suitability for meadow voles. Our results suggest that a few individuals successfully colonized these sites and their common haplotypes have persisted since the time when the locally suitable habitat was created. Third, similar haplotypes are not grouped geographically (Fig. 3).

The Maryland populations are the most distinct based on SAMOVA analyses. This cluster also contains the largest number of haplotypes, which span the entire network (Fig. 3). Therefore, it is possible that founding meadow voles generally invaded the wetlands sampled from the North. Future studies should investigate this, as it may be valuable from a conservation and ecological standpoint.

Alternative 2 (genetic drift)

Three of our observations support this alternative. First, there was a weak correlation of estimated population size and haplotype diversity. Second, most populations deviated from the expectations for haplotype diversity. Third, D^* deviated significantly from neutral expectations in five popula-

tions (note, however, that we were not able to correct for multiple comparisons because exact probability values were unknown, but only one value would be expected to be <0.05 based on the number of tests done). All five of the populations that deviated from neutrality had positive D^* values and 12 of 14 populations sampled had positive values. Additionally, observed pairwise mismatch distributions for these five populations (data not shown) deviated significantly from expected values, further indicating that these populations have experienced changes in population size (see Ramos-Onsins and Rozas 2002 and references therein).

It is not surprising that D^* detected significant departures from the expectations of neutrality when D did not, because D^* is known to be more powerful (Fu and Li 1993). We find it surprising, however, that D^* detected differences when R_2 did not, because R_2 should be more powerful given the parameters of our study (see Ramos-Onsins and Rozas 2002). However, none of the commonly used assays of departure from the expectations of equilibrium and neutrality have been tested using simulations similar to what we predicted for this species (colonization and rapid population growth followed by isolation and a slow decline in population size).

We suggest that some sites may have undergone genetic bottlenecks which have reduced differentiation. Rare haplotypes are the most likely to be lost when populations experience bottlenecks (Glenn et al. 1999 and references therein). Thus, bottlenecks can reduce differences among populations. This would be consistent, for example, with the patterns that we observed at the Dolly Sods and Canaan Valley sites which were dominated by haplotypes A and B.

Alternative 3 (isolation by distance)

Mantel tests for both $TN93$ and F_{ST} values across 14 sites failed to find any significant relationship of genetic and physical distance. This lack of significance did not differ whether we used a straight-line distance among sites or through estimating movement among sites through corridors of suitable habitat. However, some movement may have occurred through suitable corridors among the Maryland sites, likely detected because of the relatively high amount of haplotype variation within this cluster. We note that SAMOVA results indicated CRSW and HAMM were significantly different from all remaining sites. These populations were unique because of the high frequency of haplotypes H and M in these two populations, and the rarity of haplotypes H and M in other populations. However, the Maryland populations did not seem to simply be different because they were the farthest from the other populations. In fact, the population farthest from the West Virginia samples (GLAD) was grouped with the West Virginia populations by SAMOVA, whereas the populations from there and nearby HAMM were grouped separately. Therefore, the landscape features and habitat connectivity that we focused on probably were not the driving factors that explained meadow vole genetic separation at the broadest landscape scale.

We note, however, that large landscape barriers such as the alternating pattern of mountains and valleys were not taken into account in our study. These mountainous landforms combined with a matrix of unsuitable forested habitat may be more influential than distance alone. Similar geo-

graphic barriers, such as large rivers, have been noted to be more influential than distance alone for other vole species (e.g., Aars et al. 1998). As physical distances and genetic distances were not strongly related, we believe that other factors are operating in these populations. Additional studies, using more genetic markers such as microsatellites, are warranted (cf. Schweizer et al. 2007).

Connectivity and migration

When we examined genetic variation and potential meadow vole movement at a smaller scale (within metapopulations), landscape features were most influential and only significant within the Maryland metapopulation. Still, we observed that the Maryland populations had the highest number of (rare) haplotypes. Accordingly, our power to detect migration and associations was greatest among sites in this cluster. Conversely, the low haplotype diversity we observed at the Dolly Sods sites would prevent detection of most migration events. It is possible that landscape barriers existed prior to changes following exploitative logging and that these sites had been relatively isolated from one another and from West Virginia sites from the early to mid-Holocene until the late 1800s and early 1900s. If true, we suggest that clear-cutting simply increased opportunities for migration among the Maryland sites rather than stimulating colonization. However, as we note in the Introduction, these results should be considered tentative because of the low power of the analyses.

Conclusion

Our results illustrate the intrinsic relatedness of landscape and population genetic structures, providing insight into the impact of landscape changes of the last 125 years on small-mammal movement. The genetic patterns detected at the small scale support a model of local populations deriving from a single source, which is consistent with our assertion that many of the study sites were forested wetlands, largely devoid of meadow voles until the 1880s–1920s, when timber-harvesting created extensive early successional habitats across the region allowing the establishment of new populations. The current genetic diversity at the West Virginia sites likely reflects the number of voles that successfully invaded the site during the window of opportunity for dispersal and colonization following clear-cutting, followed by genetic drift within the wetlands once the forests regenerated. Given the evolutionarily short time since colonization and (or) mass migration, and pronounced effects of genetic drift on mtDNA, we were not surprised that haplotype diversity was relatively low across sites. Wider geographic sampling may allow determination of source populations or regions. Hypervariable nuclear markers may also better elucidate relationships among populations (cf. Heckel et al. 2005). Therefore, given the current physical isolation and limited dispersal among these wetlands, we suggest that these sites may be ideal systems to study founder effects using a larger array of populations and genetic makers.

Acknowledgements

This material is based upon work supported by the US Department of Energy, through Financial Assistance Award No. DE-FC09-07SR22506 to the University of Georgia Re-

search Foundation, USDA Forest Service Northeastern Research Station, Georgia Museum of Natural History, the D.B. Warnell School of Forestry and Natural Resources, and the Genome Analysis Facility at the Center for Applied Genetic Technologies at the University of Georgia. The Canaan Valley National Wildlife Refuge provided housing and field assistance. The Nature Conservancy allowed use of its lands for the study and the Maryland Branch (in coordination with Maryland Department of Natural Resources) provided DOQQ coverages and other GIS maps of Garrett County, Maryland. S.M. Dumpert, R.R. Kotecki, and H.M. Hadley assisted with fieldwork, and N.A. Schable and L.A. Dueck provided guidance with genetic laboratory work. J.L. Glenn provided helpful editorial comments on earlier drafts of the manuscript.

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