

Phylogeography of the deer mouse (*Peromyscus maniculatus*) provides a predictive framework for research on hantaviruses

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Phylogeographical partitioning of Sin Nombre and Monongahela viruses (hantaviruses) may reflect that of their primary rodent host, the deer mouse (*Peromyscus maniculatus*). Lack of a comprehensive assessment of phylogeographical variation of the host has precluded the possibility of predicting spatial limits of existing strains of these viruses or geographical regions where novel viral strains might emerge. The complete cytochrome *b* gene was sequenced for 206 deer mice collected from sites throughout North America to provide a foundation for future studies of spatial structure and evolution of this ubiquitous host. Bayesian analyses of these sequences partitioned deer mice into six largely allopatric lineages, some of which may represent unrecognized species. The geographical distributions of these lineages were probably shaped by Quaternary climatic events. Populations of mice were apparently restricted to refugia during glacial advances, where they experienced genetic divergence. Expansion of these populations, following climatic amelioration, brought genetically distinctive forms into contact. Occurrence of parallel changes in virus strains can now be explored in appropriate regions. In New Mexico, for example, near the location where *Sin Nombre virus* was first discovered, there are three genetically distinctive lineages of deer mice whose geographical ranges need to be delineated precisely. The phylogeography of *P. maniculatus* provides a framework for interpreting geographical variability, not only in hosts, but also in associated viral variants and disease transmission, and an opportunity to predict the potential geographical distribution of newly emerging viral strains.

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INTRODUCTION

Strong interest in the ecology and population dynamics of deer mice (*Peromyscus maniculatus*) was generated with recognition of its role as the primary host of *Sin Nombre virus* (SNV) transmitted to humans (Childs *et al.*, 1994). SNV, first discovered in 1993 (Nichol *et al.*, 1993), has been responsible for 416 instances of hantavirus cardiopulmonary syndrome (HCPS) in humans in the USA until January 2006 (CDC, 2005a), of which about 36% were fatal. The majority of HCPS cases have occurred in western North America, but cases throughout the range of deer mice have been documented.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are DQ385624–DQ385827.

A table showing specimens of *Peromyscus* examined and the Nexus file used to generate Fig. 1 are available as supplementary material in JGV Online.

Hantaviruses (family *Bunyaviridae*) are RNA viruses that are suspected to have co-evolved with their murid rodent hosts. Pathogens causing persistent infections are more likely to co-evolve with their host through vicariance (co-speciation) than through dispersal (Holmes, 2004). Chronic infection by these viruses may contribute to the observed pattern of tight co-evolution with murid rodents, although further tests are needed to explore temporal and spatial aspects of this phenomenon more rigorously (Holmes, 2004). Analyses of the RNA sequence of SNV indicated that this virus was not introduced from the Old World recently, but instead has a long evolutionary history in North America (Yates *et al.*, 2002).

Several investigators (e.g. Plyusnin & Morzunov, 2001) found substantial concordance between phylogenies of New World hantaviruses and their murid reservoirs, whereas others reported substantial geographical variation within a

particular hantavirus that apparently reflects the evolutionary and biogeographical history of the respective host (Asikainen *et al.*, 2000). Instances of host switching, although demonstrated (Morzunov *et al.*, 1998; Vapalahti *et al.*, 1999), are not common. Recognition of this co-evolutionary process stimulated the rapid identification of other suspected hosts of novel hantaviruses. Delineation of viral and rodent phylogenies at the interspecific and intra-specific levels provides a framework for predicting the discovery of novel viruses (Yates *et al.*, 2002) and hosts (Salazar-Bravo *et al.*, 2002). For example, knowledge of the phylogenetic relationships of these rodent hosts focused survey efforts in the Americas subsequent to the 1993 discovery of SNV, and this knowledge, enabled by samples archived in museum collections, led quickly to the discovery of the first hantaviruses known from Central (Hjelle *et al.*, 1995) and South (Hjelle *et al.*, 1996) America. In the past dozen years, 28 new hantaviruses have been described from the western hemisphere; at least 16 are pathogenic to humans (Jentes & Mills, 2003). New hantaviruses continue to be discovered (Rosa *et al.*, 2005) and significant advances in our understanding of viral occurrence, evolution, epidemiology and transmission are on the horizon.

Intraspecific variation of either host or virus, however, has received less attention, but phylogeographical studies could be as informative as phylogenetic studies in understanding viral evolution. *P. maniculatus* is a widespread species with considerable documented geographical variation (Carleton, 1989). Substantial geographical variation within a viral host is probably reflected in substantial genetic structure within the presumably co-adapting virus, especially given the high rates of molecular evolution characteristic of viruses. *P. maniculatus* harbours at least two hantaviruses: SNV, primarily in the western United States, and Monongahela virus (MONV) in the eastern United States and Canada, suggesting a phylogeographical split in both host and virus. Herein, we use molecular phylogeography to elucidate population structure among deer mice and to develop a predictive framework for discovering novel SNV variants.

Previous efforts to document geographical variation in deer mice demonstrated marked phylogeographical structure and raised awareness of the possibility that this nominal species may represent a complex of closely related species (Awise *et al.*, 1983; Hogan *et al.*, 1993, 1997; Lansman *et al.*, 1983). We extended earlier studies by sequencing the entire mitochondrial cytochrome *b* gene across a more extensive geographical sample of deer mice and by including as outgroups closely related species to provide a context for identifying significant geographical variation in *P. maniculatus*. Additionally, we used more advanced methods for phylogeographical and population analyses than were available in previous studies. Bayesian analyses, for example, suggest significant phylogeographical structure and population expansion among phylogeographical lineages of the host, thereby providing clues to the uneven prevalence and transmissibility of viruses such as SNV. Expanding

populations might create identifiable contact zones of interest for discovering novel strains of hantaviruses.

METHODS

Specimens. We analysed a total of 204 tissue samples (plus two GenBank mtDNA sequences, AF155398 and AF119261) representing specimens from localities across the range of *P. maniculatus*, and other nominal species (*Peromyscus melanotis*, *Peromyscus keeni* and *Peromyscus gossypinus*). These samples represent populations from approximately 20 nominal subspecies (Hall, 1981) ranging from Labrador, Canada, to Baja California, Mexico. Within the USA, populations from Washington to Tennessee and from southern California to Maine were sampled (see Supplementary Table S1, available in JGV Online, for specimens examined). Tissue samples were archived at the MSB, University of New Mexico, Albuquerque, USA, the Carnegie Museum of Natural History, Pittsburgh, USA, and Oswego State University, Oswego, New York, USA.

DNA sequences. Total genomic DNA was extracted from frozen tissues or tissues stored in 70% ethanol by using a Qiagen DNeasy tissue kit. Double-stranded symmetrical amplification and sequencing of the complete mitochondrial cytochrome *b* gene were performed via PCR with the primers L14724 and H15915 (Irwin *et al.*, 1991). Cleaned PCR products were sequenced by using BigDye Terminator Cycle Sequencing Ready Reaction mix v. 1.1 (Applied Biosystems). PCR products were run on an ABI 3100 automated DNA sequencer in the Molecular Biology Facility, Biology Department, University of New Mexico, Albuquerque, USA.

Analyses. Sequences were analysed by PAUP* (Swofford, 2002) using the MrModeltest block (24 evolutionary models) and model scores were submitted to MrModeltest v. 2.2 (Nylander, 2004) to determine the best model to use for Bayesian reconstruction. MrBayes (v. 3.1.1; Huelsenbeck & Ronquist, 2001) was used to conduct analyses using a GTR+ Γ +I model determined from MrModeltest to ascertain phylogenetic relationships. Four consecutive Markov chain Monte Carlo Metropolis coupling (MCMCMC) computations were run for 1 million generations, with tree sampling every 100 generations. A burn-in period of 250 000 generations was discarded for each run prior to calculating consensus trees. Trees were rooted by using *P. gossypinus*, a member of the *Peromyscus leucopus* species group (Musser & Carleton, 2005), as the outgroup. Only unique cytochrome *b* sequences within a population were included in phylogenetic analyses, but all samples were used for neutrality and population-expansion analyses.

Possible historical changes in population size or selective regimes were examined in clades with sample sizes >10. The null assumptions of neutrality and constant population size were tested with Tajima's *D* test of selective neutrality (Tajima, 1989) and Fu's *F_s* test of neutrality (Fu, 1997). *F_s* *P* values for clades were estimated from 1000 replicates with ARLEQUIN (v. 2.000; Schneider *et al.*, 2000) and DnaSP (Rozas *et al.*, 2003).

RESULTS

Six well-supported major lineages were identified within the sampled range of *P. maniculatus* (Fig. 1). These results indicate that the nominal species *P. maniculatus*, as currently recognized by Musser & Carleton (2005), is paraphyletic with respect to *P. keeni* and *P. melanotis* and may represent at least four distinct species. One lineage (clade 1, Fig. 1) includes samples from Rocky Mountain states, including northern and central New Mexico, and closely

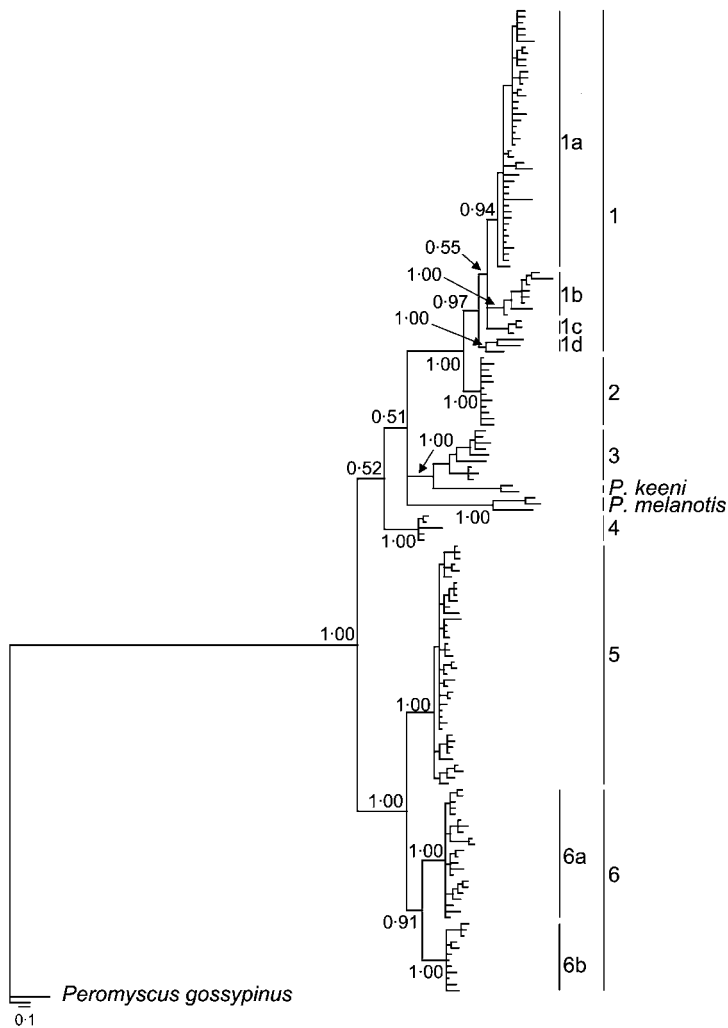


Fig. 1. Bayesian estimate (GTR+ Γ +I) of the phylogeographical relationship of populations of *Peromyscus maniculatus*. *P. gossypinus* was used as an outgroup. Consensus tree of four runs of four MCMCMC chains of 750 000 (after burn in of 250 000) generations. Numbers with branches are the posterior probabilities associated with each clade. Numbers and lines to the right of the tree identify the major and minor clades. Clades discussed in text: 1, Rocky Mountain states; 2, Plains; 3, Pacific Coast (including *P. keenii*); 4, south-western New Mexico; 5, north-eastern United States and eastern Canada; 6, north-eastern and north-central United States and south-central Canada. See supplementary tree file (perosGTRcontree.nex) with samples used to generate this tree, available in JGV Online.

related forms from Washington, northern California and Michigan (Isle Royale). A second lineage (clade 2, Fig. 1) is represented by individuals from the Plains states. A third (clade 3, Fig. 1) includes populations along the Pacific Coast region from Washington to Baja California; this lineage also consists of mice recognized as *P. keenii*. A fourth (clade 4, Fig. 1) comprises mice from southern New Mexico and Mexico. A fifth lineage (clade 5, Fig. 1) is represented by mice from north-eastern USA and eastern Canada. The sixth lineage (clade 6, Fig. 1) occurs in north-eastern and north-central USA and south-central Canada (Fig. 2).

Analyses of population expansion and neutrality were only performed on four lineages (1a, Rocky Mountains; 2, Plains; 5, north-eastern; 6, north-east central; Fig. 1) with larger sample sizes and multiple geographical locations. Three smaller lineages within the Rocky Mountain clade (clades 1b, 1c and 1d), consisting of mice from Washington, Michigan and northern California, were not analysed due to small sample sizes. Two eastern lineages (clade 5 and 6) were analysed separately, as were the lineages found in the Plains states and the Rocky Mountains. Three of the major lineages

analysed showed signs of demographic expansion and/or departures from strict neutrality (Table 1). Tajima's D and Fu's F_s test values for the lineages representing the Rocky Mountains (clade 1, Fig. 1), Plains states (clade 2, Fig. 1) and north-eastern USA and Canada (clade 6, Fig. 1) populations are both negative and significant and the mismatch distributions are unimodal (Fig. 3). However, the north-east central lineage (clade 5, Fig. 1) shows a negative and significant F_s value, but D is not significant and the mismatch distribution is bimodal.

DISCUSSION

Our analyses support the following main conclusions. First, *P. maniculatus*, as currently defined (Musser & Carleton, 2005), is a complex of several deeply divergent phylogeographical lineages. Some of these lineages may represent distinct species. Second, the deepest genetic divergence occurs between the eastern clades (5 and 6; Fig. 1) and the western clades (1–4; Fig. 1), corresponding roughly to the geographical ranges of MONV and SNV. Drobot *et al.* (2001) provided similar results from Canada and suggested

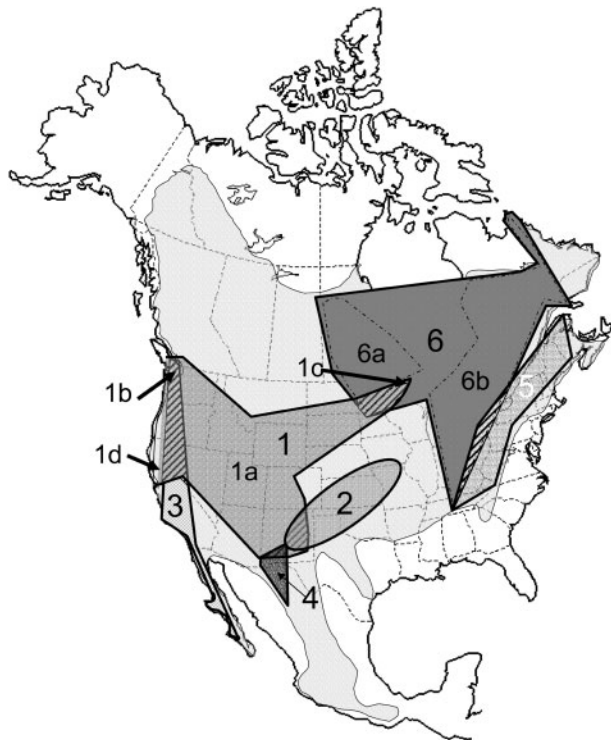


Fig. 2. Distribution map showing sampled populations of *Peromyscus maniculatus* [based on Hall (1981)]. Solid polygons around samples represent major clades and numbers refer to clades shown in Fig. 1. Hatched areas represent potential contact zones between lineages.

that *P. maniculatus* was separated into two distinct haplotypes corresponding to genetic divergence seen in SN-like viruses. Finally, post-Pleistocene population expansion has created zones of potential contact between members of divergent host clades, potentially stimulating recombination of closely related viruses.

Phylogeographical implications

Data provided here suggest that *P. maniculatus* is paraphyletic with respect to other recognized species. The type

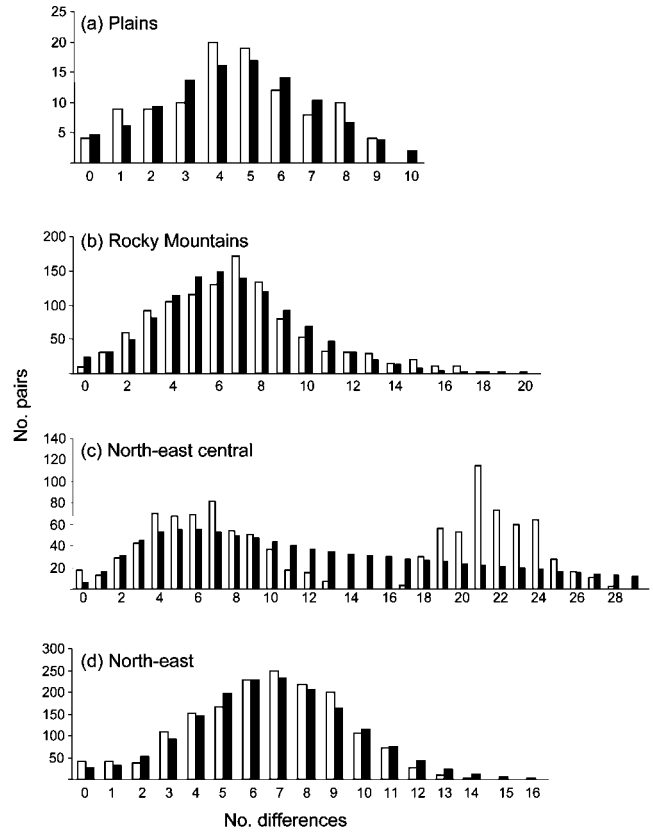


Fig. 3. Mismatch distributions depicting four of the major, reciprocally monophyletic clades of *Peromyscus maniculatus*. (a) Plains states; (b) Rocky Mountains; (c) north-east and central; (d) north-east. Empty bars, observed; filled bars, simulated.

locality for *P. maniculatus* is the Moravian settlements of Labrador, Canada (Musser & Carleton, 2005); this taxon herein is restricted to mice from the north-eastern and central clades (clades 5 and 6; Fig. 1). This mouse harbours MONV, a virus related closely to the New York virus carried by *P. leucopus* (Song *et al.*, 1996). MONV has been found in *P. maniculatus* from Canada and throughout the eastern USA (Monroe *et al.*, 1999). Our data restrict the distribution of *P. maniculatus* to Canada, east of approximately the

Table 1. Sample size (*n*), Tajima's *D* and Fu's *F_s* values with *P* values for test of expansion of major clades of *Peromyscus maniculatus*

Clade	<i>n</i>	Distinct haplotypes	<i>S</i> *	π †	Θ (<i>W</i>)‡	<i>D</i>	<i>P</i>	<i>F_s</i>	<i>P</i>
Plains	15	12	29	4.533	8.919	-2.065	0.006	-5.283	0.006
Rocky Mountains	48	41	77	6.840	17.350	-2.145	0.006	-34.194	0.001
North-east central	47	33	76	13.515	17.207	-0.762	0.239	-9.471	0.010
North-east	58	36	64	6.559	13.826	-1.803	0.024	-21.328	0.001

*Total no. segregating (polymorphic) sites.

†Average no. pairwise differences between haplotypes.

‡Estimate of the population parameter based on *S*.

Manitoba–Saskatchewan border [but possibly as far west as the coastal region of British Columbia, as mapped by Hall (1981)] and southward through the eastern USA (Fig. 2).

Given the foregoing implications, our data suggest that populations in the western United States and Mexico may represent a previously described taxon, *Peromyscus sonoriensis* (Le Conte, 1853). The phylogeographical lineage represented by these mice is the host of SNV and would comprise the Rocky Mountain clade (clade 1a, Fig. 1), including north-western New Mexico, where SNV was first discovered. Three of the major lineages of deer mice meet in the south-western USA (clades 1a, 2 and 4, Figs 1 and 2). One lineage occurs in eastern New Mexico, extends into the Plains states (clade 2, Fig. 1) and may be conspecific (as a closely related sister clade) with mice from the Rocky Mountain clade (1a, Fig. 1). Mice from south-western New Mexico (clade 4) at the base of the western radiation may represent a different, formerly recognized species, *Peromyscus blandus* (Osgood, 1904). This taxon has been reported to occur from the southern to the eastern part of the state and into Mexico and the Trans-Pecos of Texas (Hall, 1981). The extent of sympatry and interbreeding, if any, among these lineages is unknown.

Cryptic species of *Peromyscus* have been detected in other regions within the range of *P. maniculatus*. Previous work hypothesized a connection between *P. keeni* of the Pacific North-West and *Peromyscus sejugis* (which we have not analysed), an island form from Baja California del Sur (Hogan *et al.*, 1997). Those two species are allied closely with *P. maniculatus coolidgei* from Baja California (Hogan *et al.*, 1997). Our data also place *P. keeni* and *P. maniculatus coolidgei* in the same clade and suggest that our Pacific Coast clade can be linked, along with *P. sejugis*, as a distinctive coastal form. Lucid & Cook (2004) suggested that *P. keeni* survived the ice age in coastal refugia in south-east Alaska and thus were in the Pacific North-West prior to the arrival of *P. maniculatus*. Populations of deer mice along the Pacific coast are more similar to *P. keeni/sejugis* than to *P. maniculatus* and may represent a distinct species. Avise *et al.* (1983) found *P. maniculatus* to be paraphyletic with respect to *Peromyscus polionotus*. *P. polionotus* may be sister species to *P. maniculatus gambeli* from southern California (Avise *et al.*, 1983). We analysed the 321 bp of cytochrome *b* sequence available from GenBank for *P. polionotus* (X89792) and the results suggest a close relationship with the Californian samples, represented by *P. maniculatus gambeli*, *P. maniculatus coolidgei* and *P. keeni*, from our study (results not shown). Avise *et al.* (1983) suggested that *P. polionotus* is a peripheral population of *P. maniculatus* that underwent speciation approximately 1.5 million years ago. This is approximately the time period suggested by other authors for *P. polionotus*, as well as *P. melanotis* and *P. sejugis*, based on morphology (Blair, 1950; Hooper, 1968), chromosomes and protein electrophoresis (Bowers *et al.*, 1973; Greenbaum *et al.*, 1978).

Population expansion

The pattern of phylogeographical structure found in *P. maniculatus* corresponds to hypothesized Pleistocene expansion in other mammalian taxa (Brant & Ortí, 2003; Hayes & Harrison, 1992; Runck & Cook, 2005). Those studies uncovered genetic signals of postglacial colonization among members of distinct clades found in eastern and western regions of the USA and Canada. These studies and others suggest that mammalian populations (and their respective viruses) were isolated in refugia during the Pleistocene glacial periods and later expanded to their current distributions. Three of the four lineages of *Peromyscus* that we analysed show genetic signatures of geographical expansion similar to that seen in other mammalian taxa (Lessa *et al.*, 2003). The lack of expansion signature in clade 6 (Fig. 1) is intriguing, although not completely surprising. Some clades cover fairly broad areas that include both recolonized and refugial areas. The prominent genetic breaks among lineages, as reported here for deer mice, suggest a history of allopatric divergence (Avise, 2000).

Deer mice from the Rocky Mountain lineage (clade 1a, Fig. 1) probably represent an expanding population (Fig. 3). In general, genetic evidence of expanding populations would persist for about $1.5 \times N_{fe}$ generations (N_{fe} being the female effective population size), although the pattern is also consistent with a departure from strict neutrality. Sample sizes prevented testing for population expansion along the Pacific Coast. The north-east central clade (clade 6, Fig. 1) does not appear to have undergone historical population expansion, which could be a result of the minor phylogeographical break between clades 6a and 6b obscuring the detection of expansion, whereas the Rocky Mountain and Plains states clades do appear to have undergone population expansion (Table 1). Members of these clades intersect in Michigan (Fig. 2).

Potential contact zones

Zones of contact among divergent viral elements may increase the possibility of formation of recombinant variants. For example, Klempa *et al.* (2003) reported that Dobrava virus, which occurs in two species of *Apodemus* (*Apodemus agrarius* and *Apodemus flavicollis*), is separated into two distinct lineages. There is evidence of reassortment in a small area where the two viral lineages come into contact in Slovakia. Schmaljohn *et al.* (1995) reported new hantavirus variants from *P. maniculatus* of northern California in the zone that we identified as potentially being a region of sympatry among distinctive deer-mice lineages (Fig. 2). Genomic reassortment apparently distinguished these variants from SNV. These two lineages may contact in the Pacific North-West (Fig. 2) and additional sampling across Oregon and northern California would help to determine the geographical range of each taxon and reveal the extent of variation in associated hantaviruses.

Within the deer-mouse complex, distinct geographical populations of mice (and their associated viruses) have different evolutionary trajectories. The focus of new hantavirus discovery to date has been at the interspecific level, but new viral strains may emerge within currently recognized species that span broad geographical ranges and cross-ecological boundaries, such as we have identified in the large complex of deer mice. In addition, hantaviruses may undergo recombination when closely related viruses come into contact within the same individual host (Plyusnin, 2002). Thus, zones of sympatry should be productive areas to survey for recombinant viruses.

Our samples from Isle Royale (clade 1d, Fig. 1) clustered with the Rocky Mountain clade, whilst samples from central Michigan clustered in the north-east central clade. Lansman *et al.* (1983) reported specimens from northern Michigan clustering with mice from eastern states and mice from southern Michigan clustering more with the western mice. Thus, New Mexico, northern California and Michigan are areas where different lineages of deer mice are potentially in sympatry or at least in parapatry.

Many of the cases of HCPS in the eastern states were confirmed serologically, but actual viruses were not genotyped by RNA sequencing (CDC, 2005b). It is possible that these cases may actually represent exposure to MONV rather than SNV. The apparent presence of multiple, closely related species in the *P. maniculatus* complex merits more rigorous identification of geographical variation in hantaviruses. In addition to highlighting the significance of systematic and phylogeographical work for understanding the co-evolution of viruses and their hosts, this study points to several areas that require attention. For instance, distinctiveness of these phylogroups should be analysed more thoroughly with additional individuals and independent molecular markers (nuclear gene sequences, microsatellites or single-nucleotide polymorphisms) to characterize levels of gene flow among divergent populations of deer mice.

Additionally, co-evolutionary histories of viruses and their hosts should be examined in more detail, especially in areas of potential contact between hosts and associated viral strains. Evidence of population expansion of some of the host units raises the intriguing issue of a corresponding expansion of particular viral strains. Repeated cycles of range expansion and contraction (as during the Pleistocene glacial stages) might have made a substantial contribution to viral diversity. Phylogeographical and population-level analyses may provide key insight into situations that promote the emergence of novel viral elements.

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