Naturally Occurring Sin Nombre Virus Genetic Reassortants

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Genetic reassortment has been shown to play an important role in the evolution of several segmented RNA viruses and in the epidemiology of associated diseases. Sin Nombre (SN) virus is the cause of hantavirus pulmonary syndrome throughout the western United States. Unlike other hantaviruses, it possesses a genome consisting of three negative-sense RNA segments, S, M, and L. Recent analysis has demonstrated the presence of at least three different hantaviruses in Nevada and eastern California, including SN, Prospect Hill-like, and El Moro Canyon-like viruses. In addition, two distinct lineages of SN virus can be found in Peromyscus maniculatus rodents (sometimes in close proximity) trapped at study sites in this region. Data obtained by phylogenetic analysis of sequence differences detected among the S, M, and L genome segments of these SN viruses are consistent with reassortment having taken place between SN virus genetic variants. The results suggest that M (and to a lesser extent S or L) genome segment flow occurs within SN virus populations in P. maniculatus in this region.

No reassortment was detected between SN virus and other hantavirus types present in the area. This finding suggests that as genetic distance increases, the frequency of formation of viable reassortants decreases, or that hantaviruses which are primarily maintained in different rodent hosts rarely have the opportunity to genetically interact.

INTRODUCTION

Genetic reassortment has been shown to occur with many segmented RNA viruses and can (as in case of the influenza viruses) profoundly influence the pathogenicity of viruses and the epidemiology of their associated diseases (Murphy and Webster, 1990). Within the family Bunyaviridae, reassortment has been experimentally shown in the Bunyavirus genus, including individual members of the California serogroup (e.g., La Crosse, snowshoe hare, Tahyna, trivittatus, Lumbo, and California encephalitis viruses), the Bunyamwera serogroup (e.g., Bunyamwera, Maguari, and Batai viruses), and the group C viruses (Pringle et al., 1984; Beaty et al., 1985; Bishop, 1990; Urquidi and Bishop, 1992). In addition, reassortment has been seen in the Phlebovirus genus among different strains of Rift Valley Fever virus (Turell et al., 1990). Within the Bunyaviridae family, no reassortment has been seen between viruses of different genera or viruses of different serogroups within the same genus.

Neutralization, hemagglutination inhibition (HI), and complement fixation (CF) tests are common serologic tests used to develop the taxonomic groupings of members of the Bunyaviridae family. Neutralizing and HI antibodies are thought to react predominantly with the surface glycoproteins (encoded by the virus M segment), whereas the CF test largely detects antibodies directed toward the virus nucleocapsid (N) protein (encoded by the S segment). Based on these different tests, several examples of relationship contradictions are seen among naturally occurring Bunyaviridae family members, including viruses of the Bunyavirus genus (group C and Gambboa and Patois groups) and the Phlebovirus genus. These data have been summarized previously and have been suggested to indicate that reassortment of these viruses may occur in nature (Fields et al., 1969; Ushijima et al., 1981; Peters and LeDuc, 1991).

Sin Nombre (SN) virus (also previously referred to as Four Corners virus or Muerto Canyon virus) is a negative-strand RNA virus belonging to the Hantavirus genus of the Bunyaviridae family (Nichol et al., 1993; Hjelle et al., 1994b; Spiropoulou et al., 1994; Li et al., 1995). Like other members of this genus, SN virus (strain NM H10) has a single-strand segmented genome, which consists of a 2059-nucleotide small (S) segment, a 3696-nucleotide medium (M) segment, and a 6562-nucleotide large (L) segment (Spiropoulou et al., 1994; Chizhikov et al., 1995). The S segment encodes a 428-amino-acid nucleocapsid protein; the M segment encodes a 1132-amino-acid poly-peptide that is presumably subsequently cleaved into two surface glycoproteins, G1 (652 amino acids) and G2 (488 amino acids); and the L segment encodes an RNA polymerase.
SN virus was identified as the etiologic agent for hantavirus pulmonary syndrome (HPS) which resulted in the 1993 epidemic in New Mexico, Arizona, and Colorado. An approximately 50% fatality is associated with HPS (CDC, unpublished). As of August 1995, 117 cases of HPS have been confirmed in 23 states across the United States (CDC, unpublished).

Hantaviruses are distributed worldwide. Different serotypes of Hantavirus can be found in different geographic areas. Before the 1993 HPS outbreak, at least six distinct serotypes of hantavirus had been clearly defined: Hantaan (HTN), Seoul (SEO), Thailand, Puumala (PUU), Prospect Hill (PH), and Thottapalayam viruses (McKee et al., 1991). These serotypes differ from each other in their geographic distribution, primary rodent reservoirs, and the associated human disease symptoms (McKee et al., 1991). PH virus, the first hantavirus shown to be associated with a rodent indigenous to North America, was identified in Microtus pennsylvanicus (meadow vole) in Maryland (Lee et al., 1985). Peromyscus maniculatus (deer mouse) was identified as the principal rodent host for SN virus (Childs et al., 1994). SN virus, like other hantaviruses, is thought to be transmitted to humans via aerosol from infected rodent excreta. The main clinical manifestations of SN virus infection are different from those of infections associated with other serotypes; SN virus causes HPS while viruses of other hantavirus serotypes, HTN, SEO, and PUU, cause hemorrhagic fever with renal syndrome of varying degrees of severity. To date, PH virus, which is closely related to SN virus (Nichol et al., 1993), has not been found to be associated with any human disease. The genetic basis for the apparent differences in the disease potential of these viruses remains unclear.

All of the 17 HPS cases confirmed in Nevada and California have been caused by SN virus. However, analysis of the geographic distribution and genetic variation of hantaviruses in Nevada and neighboring areas of eastern California has shown that at least three distinct hantaviruses exist in this region: SN virus in P. maniculatus, PH-like viruses in Microtus montanus (mountain vole), and El Moro Canyon (ELMC) virus (Hjelle et al., 1994a) in Reithrodontomys megalotis (western harvest mouse) (Rowe et al., 1995). To add further complexity to the picture, two genetically distinct SN virus lineages were shown to be present in P. maniculatus in this region, in some cases coexisting within the same study site (Rowe et al., 1995). This prompted an investigation into whether genetic reassortment occurs among the various hantaviruses in this region, and if it correlates in any way with virus pathogenicity or disease epidemiology.

**MATERIALS AND METHODS**

Specimens collected from rodents and humans

Forty-three rodent samples were collected from several counties in Nevada and eastern California: Washoe (NV WA-), Eureka (NV EU-), Lyon (NV LY-), Nye (NV NY-), and Clark (NV CL-) Counties, and the capitol territory, Carson City (NV CC-), in Nevada, and Butte (CA BU-), Mono (CA MO-), and Nevada (CA NV-) Counties in California. The animals were trapped using Sherman (H. B. Sherman Traps, Inc., Tallahassee, FL) live-animal traps. Animals were anesthetized with Metofane (Pitman-Moore) and then released after being bled from the retro-orbital sinus. Some of the trapped rodents were sacrificed to be used in tissue analysis for virus sequences later. Blood and tissue samples were placed on dry ice for transport to a biosafety level 3 (BSL3) laboratory. Human autopsy tissues were obtained from patients when cause of death was suspected to be HPS. Blood was also obtained from patients in the convalescent phase of disease.

Total RNA extraction

Total RNA extraction was done in a laminar flow hood inside a BSL3 facility to avoid cross-contamination and possible infection. RNA was extracted from human/rodent tissues or blood using a RNaid PLUS kit (BIO 101, Inc., La Jolla, CA) as described previously (Nichol et al., 1993). Briefly, 300 µl of cell lysis solutions with guanidine thiocyanate was added to approximately 100 mg of tissue or 50 µl of blood. RNA was then extracted using phenol-chloroform extraction, followed by purification with RNA matrix beads. RNA was eluted from the matrix beads in RNase-free water and stored at −80°C. Great care was taken to avoid possible cross-contamination of RNA samples as described previously (Childs et al., 1994).

Nested reverse transcriptase – polymerase chain reaction amplification and sequence analysis of virus RNA

Reverse transcription of hantavirus RNA into cDNA and DNA amplification by nested reverse transcriptase–polymerase chain reaction (RT-PCR) was carried out as described previously (Nichol et al., 1993; Spiropoulou et al., 1994), using the primers outlined in Table 1. The second-round PCR products were analyzed by electrophoresis in 1% agarose gel (Fisher Scientific) and DNA bands of the correct predicted size were excised from the gel and purified using a GeneClean kit (BIO 101) according to the manufacturer’s instructions. Dideoxynucleotide sequence analysis of the virus-specific PCR products was carried out by either manual or automated methods as described previously (Nichol et al., 1993; Applied Biosystems, Foster City, CA; Winship, 1989), using the primers listed in Table 1. This procedure resulted in sequence fragments of 169, 139, and 282 nucleotides in length for the S, M, and L segments, respectively.

Phylogenetic analysis

Phylogenetic analysis of nucleotide sequence differences between the virus-specific PCR products was car-
TABLE 1
Oligonucleotide Primers

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<th>Segment</th>
<th>1st round primers</th>
<th>2nd round primers</th>
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<td>5’ AAAGATGCAAGAAGAGCGGTGGA3’</td>
<td>5’ GTGACCC’GATGAGTGTAACAA3’</td>
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<tr>
<td></td>
<td>5’ TCACGTTTGGCACCAG3’</td>
<td>5’ ACATCAAGGGACACTT’CCATA3’</td>
</tr>
<tr>
<td></td>
<td>5’ AATATTGAGTCCGTG’ATGGG3’</td>
<td>OR 5’ GGGTTTTAGTTTTTTGTGAAA’CCAG3’</td>
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<tr>
<td></td>
<td>5’ GCCATGG’TTTTCTCATATCGT’G3’</td>
<td>5’ TCTGG’GTCACATGCAA’ACCA3’</td>
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<tr>
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<td>5’ TCA/GATAGATTGTGATGCA3’</td>
<td>5’ CATGGTTATCTCATAGG’TC3’</td>
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<tr>
<td>M</td>
<td>5’ TGTGTGTGTGGACCATGCTG3’</td>
<td>5’ ATGCAAAAC’AGTGGATG3’</td>
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<td></td>
<td>5’ TC’/ATAGATTTGTGATGCA3’</td>
<td>5’ CATGGTATCTCATAGG’TC3’</td>
</tr>
<tr>
<td>L</td>
<td>5’ TCAACCATACATCAATGGGCC3’</td>
<td>5’ TCCAGGCTGATATATTAGTC3’</td>
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<td>5’ CTCCATATCTTTTATAGCGATC3’</td>
<td>5’ TGGTCAGAAGCTAATAGTTG3’</td>
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Additional primers for dideoxynucleotide sequencing (nucleotide sequence)

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<th>Segment</th>
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<tr>
<td>L</td>
<td>5’ TAATACACCCATTATACCC’</td>
<td>5’ CATGACATTATGCCTGTAAC3’</td>
</tr>
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</table>

ried out by the maximum parsimony method, using PAUP software version 3.1.1 (Swofford, 1993) run on a Macintosh Quadra 800 (Apple Computers). Analysis was carried out by a heuristic search, using a 4:1 weighing of transversions over transitions. This weighing was based on prior MacClade software analysis (Maddison and Maddison, 1992) of trees generated from nucleotide sequence differences detected among numerous SN virus variants which estimated transition:transversion ratios of 4.6:1 (Spiropoulou et al., 1994; Nichol, unpublished). Such weighing is predicted to improve the effectiveness of the maximum parsimony method for estimation of the correct phylogeny (Hillis et al., 1994). Bootstrap confidence limits were calculated by 500 heuristic search repetitions. A bootstrap limit of 70% or higher at a particular node or branch point has been shown to correspond to a probability of 95% or greater that the node (and corresponding clade) is real (Hillis and Bull, 1993).

The following previously published hantavirus sequences were included in the analysis: S segment—HTN strain 76-118 (Schmaljohn et al., 1986), GenBank Accession No. M14626, SEO strain SR-11 (Schmaljohn et al., 1987), M34881, PUU strain CG18-20 (Stohwasser et al., 1990), M32750, PH strain PHV1 (Parrington and Kang, 1990), M34011, SN strain CA H19 (Spiropoulou et al., 1994), L25784, and SN strain CC107 (Li et al., 1995), L33683; M segment—HTN strain 76-118 (Schmaljohn et al., 1987; Yoo and Kang, 1987), M14627 and X61034, SEO strain SR-11 (Arikawa et al., 1990), M34882, PUU strain CG18-20 (Giebel et al., 1989), M29979, PH strain PHV-1 (Parrington et al., 1991), X55129, and SN strain CA H19 (Spiropoulou et al., 1994); and L segment—HTN strain 76-118 (Schmaljohn, 1990), X55901, SEO strain 80-90 (Antic et al., 1991), X56492, PUU strain CG18-20 (Stohwasser et al., 1991), M63194, SN strain NMH10 (Spiropoulou et al., 1994), L25782, and PH strain PHV1 (Spiropoulou et al., 1994), L27796. In total, 46 SN virus samples were analyzed, and information on all three segments (S, M, and L) was available for 35 of these.

RESULTS

The phylogenetic analysis of nucleotide sequence differences among 139-bp fragments of the G2 protein-encoding region of virus M segments (Rowe et al., 1995) has demonstrated previously that two distinct SN virus lineages coexist in various regions of Nevada and eastern California. Analysis of a larger series of samples confirms and extends this finding (Fig. 1, M tree). As before, all the samples from HPS cases or hantavirus-infected P. maniculatus clearly fell into the SN virus genetic group separate from other related hantaviruses. Of the 46 samples analyzed, 19 fell into one lineage (referred to as M1) and 27 into the other (referred to as M2). The nodes separating these lineages were highly supported by bootstrap analysis (88 and 72%, respectively). The M1 and M2 groups showed a minimum divergence of 11.5% at the nucleotide level. These two SN virus groups are distinct from those present in the Four Corners region (New Mexico, Arizona, and Colorado) of the southwestern United States (Spiropoulou et al., 1994; Rowe et al., 1995).

Analysis of the S and L trees shows that they, too, can be divided into at least two main groups, here labeled S1, S2 and L1, L2 (Fig. 1, S and L trees). It can be seen that placement of viruses into group 1 or 2 does not
Fig. 1. Phylogenetic relationship of each of the S, M, and L genome segments of SN virus. Phylogenetic analysis was performed by the maximum parsimony method, using the PAUP software. Sequence fragment lengths were 169 bp (S), 139 bp (M) and 282 bp (L). Bootstrap confidence limits are indicated at the branch points. Analysis of each segment included the available sequences for HTN, SEO, PUU, and PH viruses in addition to SN viruses (see Materials and Methods). Identical overall tree topologies were seen in each case, and only the portion of each tree including the PH and SN virus samples is shown for simplicity. The horizontal lengths on each branch represent the number of nucleotide step differences. The vertical lines are used for graphic representation only. Samples are designated with the following nomenclature: state (NV or CA), followed by county (e.g., WA, LY; see text for abbreviations); rodent (R) or human (H) sample; for example, NV WA-R199 is rodent sample 199 obtained from Washoe County, Nevada.
remain constant between each of the three genome segments analyzed, suggesting that reassortment has occurred between viruses. The groupings were analyzed in a manner consistent with the concept of maximum parsimony (i.e., minimum evolution), so as to minimize the number of hypothesized reassortants. This approach suggested that the groupings be viewed predominantly relative to those obtained from the L segment analysis (Fig. 1, group 1 shown in lightface, group 2 in boldface). Taking the most parsimonious view, it appears that the L and S segment groupings correlate well with only three exceptions, samples NV WA-199, NV LY-R758, and NV CL-R313. However, many exceptions were seen in the M segment tree groupings relative to the L and S segment groupings. The possibility of RNA or PCR product cross-contamination could be ruled out as a cause of the group switching observed. Apart from the very extensive precautions taken to avoid cross-contamination (see Materials and Methods and Childs et al., 1994), several of the samples with identical nucleotide sequences were collected independently and at different sites. In addition, many were independently processed in laboratories approximately 2500 miles apart (i.e., Atlanta and Reno). Further, the switching of group assignment depending on the segment analyzed is not due to instability in the phylogenetic trees, since the important nodes (branch points) are well supported in bootstrap analysis. However, the data set for the S segment contained less nucleotide sequence variation than those for the M and L segments, as the original S sequence fragment (169 bp) analyzed corresponds to a relatively conserved region of the N protein coding region. Although the bootstrap figures are reasonable at the important nodes on the S segment tree, only a minimum nucleotide sequence divergence of 2.7% separates viruses belonging to groups 1 and 2. To ensure that the correct virus grouping had been obtained, we repeated the analysis of many of the samples, using primers which resulted in the amplification of a 164-bp fragment of a more highly variable region of the S RNA segment (Fig. 2). The same overall grouping of virus sequences was obtained, but was more clearly seen due to the greater sequence differences between samples.

Evidence of reassortment between SN viral genomes could also be seen by pairwise comparison of sequence identities for the S, M, and L segments of viruses in the Lyon (Table 2) or Eureka County samples sets (Table 3). For example, the S fragment sequences of samples NV LY-R724 and NV LY-R758 were 100% homologous, while their M and L segments differed by 14.4 and 5.6%, respectively. As for Eureka County, the S fragment sequence of samples NV EU-R693 and NV EU-R576 were 98.8% homologous, but differed in their M and L segments by 12.3 and 2.2%. It was shown previously that differences in percentage sequence divergence seen between the M and S segments of any two hantaviruses were generally less than 2% (Li et al., 1995). Thus, the differences observed here (up to 14%) when comparing different segments of SN virus pairs are inconsistent with differential rates of accumulation of mutations.

Overall, the phylogenetic and sequence comparison data obtained for the three virus genome segments would be most simply explained by several of these viruses representing SN virus M segment reassortants and a few S or L segment reassortants. No evidence was seen of reassortment having occurred between different hantavirus types, such as the PH-like viruses or ELMC viruses which are also present in rodents in these same trap sites.

Analysis of the geographic distribution of reassortants shows that many are present throughout the Nevada and eastern California region (Fig. 3). With the exception of samples from Butte County, samples from all other count-
ies studied contained two to three different virus genome segment constellations. All samples from Butte County were of the S2M2L2 type. It is quite possible that only one variant group exists for this county because it is geographically isolated (by the Sierra Nevada mountain range) from the other counties studied (Rowe et al., 1995).

**DISCUSSION**

Pairwise nucleotide sequence comparisons and phylogenetic analysis of SN virus S, M, and L segments indicate that segment reassortment can occur between SN viruses belonging to distinct lineages. The data are consistent with most of these reassortment viruses representing M segment exchanges and a lesser number of S or L segment exchanges. This correlates well with earlier genetic analysis of two SN virus isolates (CC107 and CC74) which also suggested that RNA segment reassortment occurs (Li et al., 1995). These isolates were obtained from SN virus-infected rodents MO-CA119 and MO-CA161 described here. Sequence analysis of the entire S, M, and partial L segments of CC107 and CC74 showed that while their M segment sequences diverged by less than 1%, their S and L (partial) segments differed by 13 and 6.2%, respectively (Li et al., 1995). This result is in agreement with the S2M2L2 (MO CA-119, source of CC107) and S1M2L1 (MO CA-161, source of CC74) grouping determined here (Fig. 1). Thus, CC107 appears to be the parental S2M2L2 virus, whereas CC74 is an M segment reassortant with S1M1L1 virus. The fact that the same relationships are seen whether the entire segment or a sequence fragment is analyzed shows that the reassortment analysis based on PCR fragments is valid and also rules out genetic recombination (as opposed to segment reassortment) as a cause of virus samples flipping position on the S, M, and L phylogenetic trees.

It is interesting that these rodents were trapped as part of the investigation of case CA MO-H757. While genotypes S2M2L2 and S1M2L1 were found in the five P. maniculatus tested, the human case was infected with S2M1L2 virus. Presumably, a sampling of a greater number of rodents would have located P. maniculatus infected with S2M1L2 virus at the case sample site. Surprisingly, the first three HPS cases tested in California and Nevada all had the S2M1L2 genotype despite being from different geographic locations (Fig. 3). This may suggest that virus with this genetic makeup is more pathogenic for humans than SN viruses containing other RNA segment combinations. However, there is not a strict correlation of the virus M1 segment with human disease, since M2 segment-containing SN viruses have also recently been associated with HPS cases in the western United States (data not shown).

The appearance of reassortants implies that it must at least occasionally be possible for rodents to become infected simultaneously with two or more genotypes of  

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<th>LY-R786</th>
<th>LY-R792</th>
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**TABLE 2**

Pairwise Nucleotide Sequence Identities of S, M, and L Fragments of Virus Samples Found in Lyon County, Nevada
SN virus. The source of virus presumably would be two or more contact rodents singly infected with the different genotype viruses. Given the relatively high amino acid identity between viruses of the two SN lineages, it seems unlikely that different lineage viruses could superinfect rodents that were already SN virus seropositive; however, this observation needs to be experimentally confirmed.

Many factors can influence the accumulation of reassortant viruses in natural rodent populations, making it difficult to gain insight into the frequency with which SN virus reassortment events are taking place based on the frequency of appearance of viable reassortant viruses in field samples. However, the frequent appearance of M segment reassortants might suggest that such reassortment events are more common than S or L reassortments. Similar findings were obtained with Bunyamwera serogroup viruses (Pringle et al., 1984). In tissue culture experiments involving mixed infections with Batai, Bunyamwera, or Maguari viruses, it was shown that reassortment did not occur at random, and only the M RNA segment appeared to segregate freely. However, unrestricted reassortment could be seen when recombinant viruses with nonhomologous subunit combinations were used as the parental viruses. It was suggested that this indicated that the restriction was mediated at the gene product level and that nonrandom reassortment was not due to incompatibility of genome RNA segments (Pringle et al., 1984). Along these lines, actual SN virus segment reassortment may be relatively random, but M segment reassortants may be more viable than other reassortants. Both the S and the L segments encode internal proteins (nucleocapsid and L polymerase) that must interact closely to result in high-efficiency replication. The M segment encodes the surface glycoproteins G1 and G2, which may allow more sequence flexibility or more opportunity for positive selection (e.g., by the host immune system or host cell receptors) for M segment reassortants.

Several other examples of nonrandom segment reassortment have been seen with other members of the Bunyaviridae family. S and M segment reassortants were experimentally produced by coinfection of mosquito or vertebrate hosts with different strains of Rift Valley fever virus (Turell et al., 1990). Dual infection of BHK-21 cells with the bunyaviruses La Crosse and snowshoe hare led to nonrandom reassortment, with a preference for homologous L-M and M-S associations (Urquidi and

<table>
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**Table 4**

Combinations of S, M, and L Segments in the Viral Genome of the 35 SN Virus Samples Studied

<table>
<thead>
<tr>
<th>Possible combinations/genotypes</th>
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<tr>
<td>M2</td>
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<td>L2</td>
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</table>

*MOR119* | LY-R786 | LY-R758 | MO-R159 | WA-R199 | CL-R313 | CL-R341 | EU-R451 | EU-R455 | EU-R576 | EU-R578 | EU-R309 | EU-R231 | EU-R845 | EU-R872
LY-R724 | LY-R792 | MO-R162 | MO-R170 | MO-R161 | NY-R301 | NY-R327 | NY-R309 | NY-R231 | NY-R845 | NY-R872
LY-R725 | WA-H791b | MO-R162 | MO-R170 | MO-R161 | NY-R301 | NY-R327 | NY-R309 | NY-R231 | NY-R845 | NY-R872
LY-R730 | MO-H757 | MO-R161 | NY-R301 | NY-R309 | NY-R231 | NY-R845 | NY-R872
LY-R726 | NY-H575 | MO-R161 | NY-R301 | NY-R309 | NY-R231 | NY-R845 | NY-R872
BU-R608 | NY-R309 | NY-R231 | NY-R845 | NY-R872
BU-R615 | NY-R309 | NY-R231 | NY-R845 | NY-R872
BU-R616 | NY-R309 | NY-R231 | NY-R845 | NY-R872
WA-R211 | NY-R309 | NY-R231 | NY-R845 | NY-R872

*a* Lightface characters represent rodent samples.

*b* Boldface characters represent human samples.
Bishop, 1992). Experiments are being initiated with the NMR11 and CC107 SN virus isolates to see if nonrandom reassortment occurs on coinfection of Vero E6 cells in culture.

In examining the geographic distribution of reassortant viruses, it appears that most regions (with the exception of Butte County, CA) contain reassortants with different segment constellations (Fig. 3). In several cases we failed to detect the “parental” type viruses. This may have been because of the small number of viruses typed at some locations (i.e., if we had looked at a greater number of samples we might have found the parental type viruses). Alternatively, our inability to detect these viruses may reflect the possibility that some of these reassortment events occurred some time ago, and the parental type virus no longer exists in that location. The broad geographic distribution of some specific reassortants (e.g., S1M2L1) would suggest that the latter is likely to be true, and the frequency with which viable reassortants form in nature may be relatively low, but when they do occur they can become fixed in the virus genome segment pool. If some particular combination of segments provides the reassortant with a higher rate of transmissibility than the parental viruses, this may provide another potential explanation of why parental viruses have not been detected in some geographic regions.

The ability of SN viruses to increase the complexity of their genetic pool via reassortment may have important implications for evolution of these viruses and for the epidemiology of HPS. Reassortment provides the potential for segmented RNA viruses to make rapid stepwise transitions across virus fitness landscapes which are difficult (or impossible) by accumulation of incremental mutational changes. This process can allow rapid exploration of the fitness potential of different virus genetic compositions in new ecological niches or in changing environments. More extensive studies will be necessary to fully evaluate the past or potential future role of hantavirus genome reassortment in the emergence of newly characterized hantaviruses or in the relative pathogenicity of these viruses for humans.

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