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ANALYSES OF GENE FLOW AMONG POPULATIONS OF DEER MICE (*PEROMYSCUS MANICULATUS*) AT SITES NEAR HANTAVIRUS PULMONARY SYNDROME CASE-PATIENT RESIDENCES

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ABSTRACT: Gene flow and potential for Sin Nombre virus (SNV) trafficking of the deer mouse (*Peromyscus maniculatus*) was studied in Delta and Mesa counties of western Colorado (USA). The study areas included Grand Mesa and surrounding grazing and agricultural areas. This area has several natural potential barriers to rodent gene flow, including rivers, cliffs, and mountains. Ten study sites were utilized in a spatially nested design ranging from 0.65–81 km apart; four of these sites were at or near human hantavirus pulmonary syndrome (HPS) case-patient residences. One HPS case occurred on the north side of Grand Mesa in 1993; the other three (two confirmed, one presumptive) occurred on the south side of Grand Mesa between 1999–2000. Blood and tissue samples were collected from each of 221 deer mice captured from 1999–2000. Blood samples were tested for IgG antibody to SNV. At least one deer mouse had antibody to SNV at nine of 10 sites. Genomic DNA was isolated from tissue samples and alleles at six microsatellite loci were amplified by polymerase chain reaction (PCR). Polymorphisms were resolved on denaturing polyacrylamide gels and visualized by silver staining. Traditional population genetic analyses of this study indicated moderate population subdivision among the populations surveyed, slight evidence of isolation by distance, and that the Gunnison River system may hinder gene flow in this area. Application of assignment tests indicated that approximately 73–85% of mice were assigned to their population of capture. Many of the misclassifications occurred among sites less than 1 km apart; however, some long-distance misclassifications were noted. Additionally, some misclassifications were noted among study sites on different sides of the Gunnison River system, indicating that the riparian corridor of this system may facilitate some gene flow. Overall, these data indicate that SNV trafficking is more likely at the local level, but some long-distance trafficking may be possible, especially where select habitat variables favor long-distance movements.

Key words: Deer mouse, hantavirus pulmonary syndrome, microsatellite, *Peromyscus maniculatus*, population genetics, Sin Nombre virus.

INTRODUCTION

In 1993, several hantavirus pulmonary syndrome (HPS; Butler and Peters, 1994; Elliott et al., 1994) cases were reported in the Four Corners region of the southwestern United States. These HPS cases were caused by Sin Nombre virus (SNV; family *Bunyaviridae*, genus *Hantavirus*). Deer mice (*Peromyscus maniculatus*) are the primary hosts of SNV (Childs et al., 1994). Several of these cases occurred in Colorado, one of which occurred in 1993 in Mesa County on the north side of Grand Mesa (Fig. 1). According to the Colorado Department of Health and Environment, from 1993 to October 2000, 27 HPS cases occurred in Colorado (http://www.cdphe.state.co.us/dc/Zoonosis/zoonosis.asp). Notably, of the 12 HPS cases that occurred in Colorado from January 1999 to October 2000, three (two confirmed, one presumptive) occurred in Delta County (on the south side of Grand Mesa), yielding one of the highest human HPS infection rates per county in Colorado during this time period.

Hantaviruses are thought to be contracted by their rodent hosts through passage

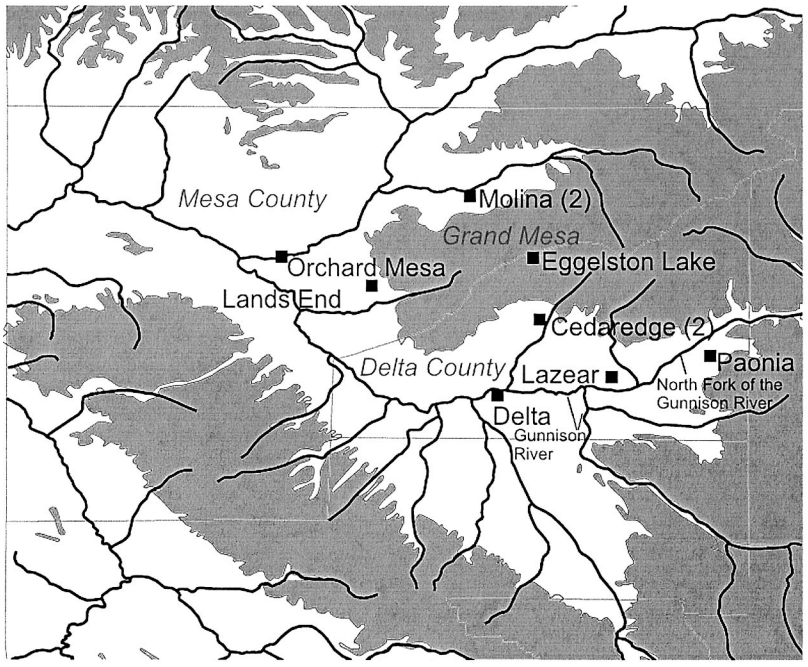

FIGURE 1. Locations of study sites on and near Grand Mesa, Colorado. The Delta, Lazear, Paonia, and Molina study sites were associated with residences of human HPS case-patients (See Table 2). Replicate populations were sampled at the Cedaredge and Molina study sites.

of urine or saliva via bite wounds during fighting (Glass et al., 1988) or possibly by direct contact with virus-infected rodents, contact with their virus-contaminated nest material, or contact with aerosolized virus in closed spaces (Gavrilovskaya et al., 1990). Unfortunately, little is known about the determinants of intraspecific transmission, maintenance, persistence, or trafficking of hantaviruses (Mills et al., 1999a; Shope, 1999).

Several genetic analyses have been conducted on members of the genus *Peromyscus.* Methods for these include, but have not been limited to, microsatellites (Schmidt, 1999; Wooten, 1999), randomly amplified polymorphic DNA (RAPD; Landry and Lapointe, 1999), and restriction fragment length polymorphisms (RFLP; Walpole et al., 1997). Use of microsatellites to study aspects of the biology of small mammals has gained popularity over recent years, especially for *Spermophilus* sp. (May et al., 1997; Stevens et al., 1997), *Clethrionomys* sp. (Reese et al., 2001), and *Peromyscus* sp. (Schmidt, 1999; Wooten et al., 1999; Chirhart et al., 2000). Within the genus *Peromyscus, P. maniculatus* has received the most attention in terms of systematic biology and evolutionary genetics (Chirhart et al., 2000); however, surprisingly few microsatellite studies have addressed *P. maniculatus.*

Several recent reports outlined the endemicity of SNV and SNV-like viruses throughout much of the United States (Douglass et al., 1996; Mills et al., 1997; Abbott et al., 1999; Calisher et al., 1999; Kuenzi et al., 1999). However, the spatial and temporal prevalence of SNV in deer mouse populations varies dramatically (Mills et al., 1997), which could be conditioned by the genetic composition of the populations or by trafficking of infected mice. The objective of this study was to use microsatellite markers developed for *P. maniculatus* to assess the gene flow and the SNV trafficking potential of *P. maniculatus* to local and distant sites. The use of assignment tests, which utilize individ-

TABLE 1. Study site locations of *Peromyscus maniculatus* collections in west central Colorado.

County	Study site	Abbreviation	Locality description	Site description	Elevation (m)	GPS coordinates
Delta	Cedaredge A	CA	Near Cedaredge	Livestock pasture	1,981	38°55′24.2″N 107°56′00.1″W
Delta	Cedaredge B	CB	Near Cedaredge	Livestock pasture	1,981	38°55′47.3″N 107°55′57.6″W
Delta	Delta	DE	Near Delta	Peri-domestic site	1,554	38°44′54.6″N 108°01′15.9″W
Delta	Eggelston Lake	EL	Grand Mesa	Coniferous forest	3,139	39°02′49.5″N 107°56′30.8″W
Delta	Lazear	LA	Between Hotchkiss and Lazear	Agricultural	1,737	38°47′50.8″N 107°47′18.7″W
Delta	Paonia	PA	Near Paonia	Pasture/juniper	1,890	38°50′1.8″N 107°33′58.3″W
Mesa	Lands End	LE	Base of Lands End Road	Piñon pine/juniper	1,829	38°59′18.9″N 108°17′13.3″W
Mesa	Molina A	MA	Near Molina	Sage/juniper	1,951	39°09′45.8″N 108°03′18.4″W
Mesa	Molina B	MB	Near Molina	Sage/juniper	1,951	39°10′05.9″N 108°03′26.8″W
Mesa	Orchard Mesa	OM	Near Clifton	Agricultural	1,463	39°02′34.9″N 108°28′21.6″W

uals as experimental units, rather than populations, could have application for this objective. Indeed, the use of assignment tests when using highly variable loci (i.e., microsatellite) to determine population membership of specific individuals appears to be a powerful approach and may even be used to identify recent migrants or descendants of recent migrants (Waser and Strobeck, 1998; Hedrick, 1999).

MATERIALS AND METHODS

Study sites

Study sites were established at or near Cedaredge (two sites, CA and CB), Delta (DE), Eggelston Lake (EL), Lazear (LA), and Paonia (PA) in Delta County and the base of Lands End Road (LE) and Orchard Mesa (OM) in Mesa County in 2000 (Fig. 1; Table 1). Additional study sites were located near Molina (two sites, MA and MB) as part of a longitudinal study of SNV and small mammals in Mesa County (Calisher et al., 1999). Four of these study sites (PA, DE, LA, and Molina sites) were near or at residences of human HPS case-patients (the DE case is presumptive; Table 2). The study sites ranged from 0.65–81 km apart. The study sites within close proximity (CA and CB, MA and MB, both pairs <1 km apart) were nested samples, and were strategically chosen at similar elevations (Table 1) on the southern (CA and CB) and northern foothills (MA and MB) of Grand Mesa (Fig. 1).

The Delta County study sites included grazing lands (CA and CB), a disturbed peri-domestic site (DE), a high elevation coniferous forest (EL), an experimental orchard (LA), and a cattle ranch (PA) dominated by grazing pastures surrounded by junipers (*Juniperus* sp.). The Mesa County study sites consisted of a piñon pine (*Pinus edulis*)-juniper woodland (LE), uplands dominated by sage (*Artemisia* sp.) and juniper (MA and MB), and an experimental orchard (OM; Table 1).

Field sampling

Deer mice were intensively sampled at short-term sites in July and August 2000 (CA, CB, DE, EL, LA, LE, and PA) or in September 2000 (OM) using Sherman non-folding aluminum live-traps (Table 2). The two longitudinal study sites at Molina (MA and MB) were sampled a total of six times from October 1999 to October 2000 as part of a longitudinal monitoring program of hantaviruses and their rodent hosts (see Calisher et al., 1999; Mills et al., 1999b). Small mammal processing was conducted according to protocols for trapping and sampling small mammals for virologic testing (Mills et al., 1995a, b). Briefly, we identified the

TABLE 2. Field study sites, trapping effort, number of captures, number of infected individuals, and prevalence of infection with Sin Nombre virus in *Peromyscus maniculatus* from west-central Colorado. Associations of study sites with human hantavirus pulmonary syndrome case-patient residences are also noted. Point prevalence estimates of Sin Nombre virus were greatest at the Cedaredge B and Delta study sites.

Study site	Trap-nights	*n* (captures)	*n* (infected)	Prevalence (%)	Hantavirus pulmonary syndrome (HPS) association
Cedaredge A	575	16	2	13	None
Cedaredge B	500	10	3	30	None
Delta	400	13	4	31	Near presumptive HPS case residence
Eggelston Lake	450	21	1	5	None
Lazear	450	37	2	5	Near HPS case residence
Paonia	530	17	3	18	HPS case residence
Lands End	450	14	1	7	None
Molina A	2,610	44	2	5	Near HPS case residence
Molina B	2,610	41	5	12	Near HPS case residence
Orchard Mesa	450	8	0	0	None

species, recorded presence of scars and wounds, collected blood samples, and excised a small piece of ear tissue from each deer mouse captured. Ear tissue samples were stored in 70% ethanol in cryovials on ice in the field and at −20 C in the laboratory until genomic DNA was extracted. At longitudinal sites (MA and MB), individuals were marked with individually numbered ear tags. At the remaining sites (CA, CB, DE, EL, LA, LE, OM, and PA), individuals were batch-marked with permanent ink. All deer mice were released where they were captured.

Genomic DNA extractions and antibody assays

Genomic DNA was isolated from ear tissue using the CTAB (hexadecyltrimethlyammonium bromide) method (Black and DuTeau, 1997). The tissue was blotted with a paper towel and then macerated by freezing it to −70 C and smashing it between two metal blocks (in sealed plastic bags) with a 1.4 kg sledgehammer. Following maceration, genomic DNA was isolated following the procedures described by Black and DuTeau (1997). Genomic DNA was resuspended in 100 μl of 0.05 M Tris-EDTA buffer (pH 8.0). A small (approximately 30 μl) aliquot was stored in a refrigerator and the remaining genomic DNA was stored at −70 C in cryovials. Blood samples were assayed for IgG antibody to SNV (Feldmann et al., 1993).

Genetic analyses

Six microsatellite loci were evaluated in this study (Table 3). Microsatellite loci have high

TABLE 3. Characterization of microsatellite loci for *Peromyscus maniculatus*. The approximate length of the PCR product was determined from the original microsatellite sequences for primers with PMs prefixes. Primers with the Pml prefix are from Chirhart et al. (2000).

Locus[a]	Primer sequence 5′–3′	Annealing temperature (C)	Approximate product size (bp)
PMs13	F: GAGATTCCAAGGAGACATGGAG	53	249
PMs13	R: TCATATGCCTGCACCTGTAGAG		
PMs19	F: ATACAACACCAATAACGACCCA	56	233
PMs19	R: AGTCTACCTCAAATGGCAAGCT		
PMs21	F: GTGAAGCCTGAACAGTTACTTA	51	196
PMs21	R: GAGATAGAGGTTTGCTAAAAGA		
PMs26	F: ATGCAGTCTCCACATATCCTGT	56	242
PMs26	R: ATTGTCCTTGGTCTGACCACTA		
PMs29	F: GCTGAGTTCTTGCCCAGTTATA	54	198
PMs29	R: TTGAGTTCTGCTACCCCTCTAA		
Pm101	See Chirhart et al. (2000)		

[a] Full details of primer development are available on request.

mutation rates (i.e., 10^{-2}–10^{-6} mutations/generation) with DNA replication slippage being the predominant mechanism of mutation in microsatellite DNA (Schlötterer, 2000); thus, their abundance and high degree of polymorphism make microsatellite alleles useful markers for evaluating genetic differences among populations (Reese et al., 2001).

Polymerase chain reaction (PCR) amplification of microsatellite DNA was conducted in 50-μl reaction volumes using 2 μl of *P. maniculatus* template DNA in 96-well plates. Amplification was conducted in a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA) using one of each of the six primer pairs (Table 3). Polymerase chain reaction conditions consisted of heating the plates to 95 C for 5 min and then cooling the plates to 80 C prior to the addition of one unit of *Taq* DNA polymerase. The remainder of the program consisted of 30 cycles of 1 min at 95 C, 1 min at primer-specific annealing temperatures (Table 3), and 2 min at 72 C. A final extension step was carried out for 1 hr at 72 C and the samples were then cooled indefinitely at 4 C. Each 96-well plate of PCR product was tested for contamination using a negative control (no template DNA included). Amplified products were size-fractioned by electrophoresis using large (38×50 cm) denaturing polyacrylamide gels (6%, 19:1 cross-linking, BioRad Laboratories, Hercules, California, USA). Gel mixtures included 15 ml 40% acrylamide, 20 ml 5x TBE, and 42 g urea brought to a 100 ml volume with ddH_2O. Seven μl of PCR product were mixed with 3 μl of loading buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol), heated to 95 C, and loaded onto the gel using sharktooth combs. Electrophoresis was conducted at 12 constant watts for approximately 15 hr at room temperature. Gels were silver stained to visualize DNA fragments, which were viewed for scoring using a light box (Black and DuTeau, 1997).

Variation in microsatellite allele frequencies within and among populations was examined using Arlequin 2.0 (Schneider et al., 2000) to compute F_{st} values and pairwise Slatkin's linearized F_{st} values (Slatkin, 1993). Additionally, Cavalli-Sforza and Edwards' (1967) chord distances were calculated with POPDIST (Guldbrandtsen et al., 2000).

Geographic distances were obtained with a field GPS unit and pairwise geographic distances were calculated using geographic information systems (ARC-INFO software, Environmental Systems Research Institute, Inc., Redlands, California). The pairwise Slatkin's linearized F_{st} values ($F_{st}/1- F_{st}$) were regressed on pairwise geographic distances (and the natural logarithm transformation of these distances) among populations to determine whether geographic distance among populations serves as a barrier to gene flow (Slatkin, 1993). Regression analyses and Mantel tests (Mantel, 1967) were conducted using program MANTEL (available from slozano@colostate.edu).

Matrices of pairwise Slatkin's linearized F_{st} values (Slatkin, 1993) and Cavalli-Sforza and Edwards' (1967) chord distances were used to construct dendrograms among all collections using the unweighted pair-group method with arithmetic averaging cluster analysis (UPGMA) in the NEIGHBOR procedure in PHYLIP3.6 (see Felsenstein 1993).

The computer program GeneClass (Cornuet et al., 1999; available from http://www.ensam.inra.fr/URLB/geneclass/geneclass.html) was used to perform assignment tests to estimate the population of origin of all individuals sampled. The computation of some of the methods (i.e., likelihood based) of program GeneClass relies on two explicit assumptions: loci should be at Hardy-Weinberg equilibrium and at linkage equilibrium (Cornuet et al., 1999). However, these assumptions are relaxed for distance-based methods (Cornuet et al., 1999). Several of the loci examined in this study were not at Hardy-Weinberg equilibrium; therefore, we present results using two distance methods (Cornuet et al., 1999). Individuals were tested using the D_{AS} (shared allele distance) and Cavalli-Sforza and Edwards' (1967) chord distance method with the "leave one out" option (i.e., they were individually excluded from their population when performing their assignment [Cornuet et al., 1999]). Additional tests were conducted using the simulation option (i.e., exclusion analyses).

RESULTS

In 9,025 trap-nights during October 1999 to October 2000, we captured 221 individual deer mice at our study sites (Table 2). Aside from our LA sample, *P. maniculatus* populations were low in this area from 1999–2000 (unpubl. data). Sample sizes ranged from 44 (MA) to eight (OM). Due to consistent template DNA problems, only 208 individuals were included in genetic analyses. The percent of deer mice with IgG antibody to SNV at the 10 locations on and around Grand Mesa was highly variable, ranging from 0 (OM)–31%

(DE). No geographic pattern of antibody prevalence was noted (Table 2).

All loci yielded multiple alleles in all populations. The average total number of alleles per locus/population was 8.8. The average total number of alleles per locus was 28.5. Average expected heterozygosities (H_e) were high (mean=0.86) in all instances, ranging from 0.81 (DE) to 0.91 (CB). Multiple populations and loci yielded significant deviations from expected heterozygosities. The vast majority of these were lower than expected, possibly a problem with "null alleles" (see discussion), but a few (i.e., PA at PMs19 and DE at PMs21) were greater than expected. Average observed heterozygosities (H_o) ranged from 0.47 (CB) to 0.69 (LE), and had an overall average of 0.59. To reduce the likelihood of type I errors, a Bonferroni correction was applied to these data, setting the (Bonferroni-corrected) probability level to $P=0.0083$ (0.05/6). Nonetheless, many populations still yielded significant deviations from expected heterozygosities.

The overall estimate of F_{st} across all populations was 0.10, indicating moderate genetic subdivision. Pairwise F_{st} values were smallest at the local level among replicated sites (i.e., CA and CB, pairwise $F_{st}=0.031$; MA and MB, pairwise $F_{st}=0.043$) indicating little differentiation among these local sites (Fig. 1). F_{st} values were greatest for pairwise comparisons of all populations with PA, DE, EL, and MB. Notably, the PA and DE sites were located south of the Gunnison River system (the North Fork of the Gunnison River (PA) and the Gunnison River (DE) of the Gunnison River system; Fig. 1).

A slight positive relationship was observed between pairwise geographic distances and pairwise Slatkin's linearized F_{st} values. The relationship was noted for comparisons of untransformed geographic distances versus Slatkin's linearized F_{st} ($r^2=0.15$, Mantel probability<0.05) and for natural logarithm transformed geographic distance data versus Slatkin's linearized F_{st} ($r^2=0.22$, Mantel probability<0.01). However, when the PA site was removed from this comparison, the relationship was insignificant.

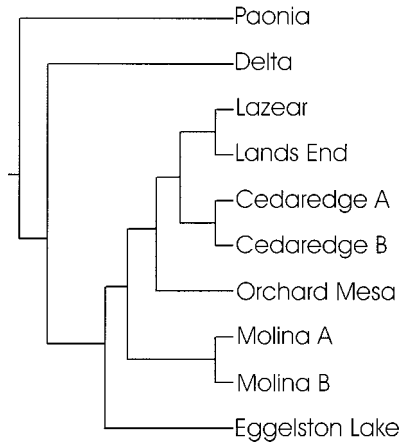

FIGURE 2. Unweighted pair-group method with arithmetic averaging cluster analysis of pairwise Slatkin's linearized F_{st} relationship between collections of *Peromyscus maniculatus* in Colorado. Analysis was done using microsatellite DNA markers. The Paonia and Delta sites do not cluster with the remaining sites.

The UPGMA cluster analysis of pairwise Slatkin's linearized F_{st} among collections indicated that the PA and DE collections appear by themselves and all the remaining populations form the remaining cluster (Fig. 2). Notably, the former two populations failed to cluster with the remaining populations even though they were closer (in geographic distance) to some of the populations that formed the cluster than were some populations within the cluster. A similar observation was made for the PA and DE collections when Cavalli-Sforza and Edwards' (1967) chord distances were used in a UPGMA cluster analysis (Fig. 3). However, some of the remaining populations clustered differently (Fig. 3).

The assignment test of program GeneClass yielded an overall 73.6% classification of individual *P. maniculatus* to their population of origin (i.e., population of capture; Table 4) using the D_{AS} method (non simulated). Many of the misclassifications in this test were at the local level

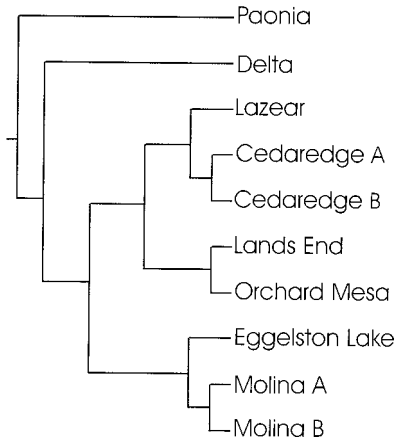

FIGURE 3. Unweighted pair-group method with arithmetic averaging cluster analysis of pairwise Cavalli-Sforza and Edwards' (1967) chord distance relationship between collections of *Peromyscus maniculatus* in Colorado. Analysis was done using microsatellite DNA markers. Similarly to Figure 2, the Paonia and Delta sites do not cluster with the remaining sites.

among replicated sites but several others were noted (Table 4). When these data were simulated (i.e., exclusion analysis), however, only 47% were assigned only to their population of capture, 40% were assigned to multiple populations or a single different population, and 13% were not assigned to any population. When Cavalli-Sforza and Edwards' (1967) chord distance method (non simulated) was employed, 85.0% of individual *P. maniculatus* were classified to their population of origin (Table 4). Similarly, many of the misclassifications in this test were at the local level among replicated sites but several others were noted (Table 4). When these data were simulated (i.e., exclusion analysis), only 53% were assigned only to their population of capture, 16% were assigned to multiple populations or a single different population, and 31% were not assigned to any population.

DISCUSSION

Population structure within a habitat mosaic could play a significant role in the spatial distribution of infection with a hantavirus in local populations of reservoir species (Glass et al., 1988). If this were the case, increasing population densities would result in increased rodent-to-rodent contact, a greater number of potential virus-transmission events, and a higher overall incidence and cumulative prevalence of infection within host populations (Mills et al., 1999a). This clearly is not always the case (see Douglass et al., 1996; Mills et al., 1997; Boone et al., 1998) because population trends are not always clearly associated with periods of virus transmission (Childs et al., 1987). Although estimates of *P. maniculatus* population density were not objectives of this study, we accumulated no evidence that our point prevalence estimates were associated with relative population size (Table 2). However, considering that eight of 10 sites we surveyed were merely point prevalence estimates, this observation should be interpreted with caution. Mills et al. (1997) determined that the lowest overall prevalence of infection was found at altitudinal and climatic extremes (i.e., desert and alpine tundra), but no discernible pattern in the geographic distribution of infected animals was observed. Similarly, we observed no geographic pattern in the distribution of infected animals and no clear habitat association of infected animals.

Many of the loci and populations examined in this study deviated from expected Hardy-Weinberg equilibrium values. It is common for microsatellites to have nonamplifying alleles (Pemberton et al., 1995). On occasion, mutations at the priming site of one allele will prevent primer annealing, resulting in only a single product for that individual (Reese et al., 2001). This may have occurred with PMs 13 as it consistently yielded very low observed heterozygosity values. Further, nonamplification of one allele can be difficult to discriminate from amplification of two identical, comigrating alleles (Reese et al., 2001). Although recent technologies appear to help alleviate this concern, the aforementioned situation was not easily teased apart using the electrophoresis and

TABLE 4. Numbers of individual *Peromyscus maniculatus* assigned to each population (study site) using assignment tests. The D_{AS} (top values in each row) and Cavalli-Sforza and Edwards' (1967) chord distance (bottom value in each row) methods were employed in program GeneClass (Cornuet et al., 1999). Numbers of individuals on the diagonal represent individuals assigned to their population of capture. The percent of individuals correctly assigned is noted in parentheses. Numbers of individuals below the diagonal represent the number of individuals misclassified among population pairs. Numbers above the diagonal (separated by a comma) represent the number of individuals misclassified from each population for each population pair. These numbers are listed in the same order that the populations are presented. Note that two of the greatest numbers of misclassifications occur among closely spaced replicate populations (CA and CB; MA and MB) for both methods.

Locations[a]	Locations[a]									
	PA	DE	LA	CA	CB	LE	EL	OM	MA	MB
PA	13 (81)	3, 1	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	14 (88)	2, 1	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
DE	4	12 (92)	0, 9	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	3	12 (92)	0, 2	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
LA	0	9	17 (47)	3, 0	1, 0	4, 0	2, 0	0, 0	0, 0	0, 0
	0	2	28 (78)	2, 0	1, 1	2, 0	1, 0	0, 0	0, 0	0, 0
CA	0	0	3	9 (56)	3, 5	1, 0	0, 0	3, 0	0, 0	0, 0
	0	0	2	13 (81)	3, 4	0, 0	0, 0	0, 0	0, 0	0, 0
CB	0	0	1	8	4 (40)	1, 1	0, 0	0, 0	0, 0	0, 0
	0	0	2	7	4 (40)	0, 1	0, 0	0, 0	0, 0	0, 0
LE	0	0	4	1	2	12 (86)	1, 0	0, 0	0, 1	0, 0
	0	0	2	0	1	11 (79)	1, 0	1, 0	0, 0	0, 0
EL	0	0	2	0	0	1	21 (100)	0, 0	0, 1	0, 0
	0	0	1	0	0	1	21 (100)	0, 0	0, 1	0, 0
OM	0	0	0	3	0	0	0	7 (88)	0, 1	1, 1
	0	0	0	0	0	1	0	7 (88)	0, 1	1, 0
MA	0	0	0	0	0	1	1	1	29 (69)	10, 2
	0	0	0	0	0	0	1	1	37 (88)	4, 2
MB	0	0	0	0	0	0	0	2	12	29 (91)
	0	0	0	0	0	0	0	1	6	30 (94)

[a] PA=Paonia, DE=Delta, LA=Lazear, CA=Cedaredge site A, CB=Cedaredge site B, LE=Lands End, EL=Eggelston Lake, OM=Orchard Mesa, MA=Molina site A, MB=Molina site B.

visualization procedures employed in this study. Therefore, heterozygous individuals with null alleles may be falsely identified as homozygotes, resulting in erroneous estimations of equilibrium parameters (Reese et al., 2001). However, the inequality between H_o and H_e we observed may not be unexpected considering the distinction between the large number of alleles per locus and the relatively small sample sizes surveyed per population (Chirhart et al., 2000).

The overall estimate of F_{st} (0.10) we observed was within the range of those reported by others for *Peromyscus* sp. Examples include the use of RAPDS for *P. maniculatus* (F_{st}=0.13; Landry and Lapointe, 1999), allozymes for *P. leucopus* (F_{st}=0.18; Loxterman et al., 1998; F_{st}=0.14; Schnake-Greene et al., 1990) and *P. attwateri* (F_{st}=0.06), and salivary amylases for *P. leucopus* (F_{st}=0.05; Merriam et al., 1989); thus, these workers report low to moderately high population differentiation for various species of *Peromyscus.* In general, one might expect moderately low population differentiation for a widespread habitat generalist such as the deer mouse.

If there is complete or nearly complete replacement of alleles among populations, the use of F_{st} may not be the most appropriate statistic to use in comparisons of populations (Wright, 1978). Recent works have supported this statement. Measures of differentiation between groups of highly variable loci, such as microsatellite loci, may be highly dependent on the level of

within-group variation (Hedrick, 1999). Further, because these loci often have very high within-population heterozygosity, the magnitude of differentiation measures may be quite small (Hedrick, 1999). As a result, measures that are variation independent are recommended for highly variable loci (Hedrick, 1999). Also, overlap in the sets of alleles in completely isolated populations can be observed (e.g., Paetkau et al., 1997), probably because of constraints on repeat number and back mutation (Nauta and Weissing, 1996; sensu Hedrick, 1999), which can be referred to as "size homoplasy." Unfortunately, as is the case with many statistics, F_{st} is sensitive to small sample sizes, which tends to inflate estimates of F_{st} and underestimate N_em (Apostol et al., 1996).

The use of assignment tests with highly variable loci to determine population membership of specific individuals appears to be a powerful approach and may even be used to identify recent migrants or descendants of recent migrants (Waser and Strobeck, 1998, Hedrick, 1999). Therefore, we present multiple population genetic statistics for comparison purposes, but acknowledge that the assignment tests are probably the most robust.

As noted previously, there are multiple geographic features on and around Grand Mesa that could hinder the gene flow among populations of *P. maniculatus.* These features include major river systems, cliffs, land areas with little vegetative structure, and high mountains. Although a slight relationship was observed for isolation by distance, any of these features might have disrupted an isolation by distance pattern. For example, all of the individuals at the high elevation EL site were correctly assigned to their population of capture (Fig. 1; Table 4). Additionally, several of our study sites were associated with the Gunnison River system. As an example of dispersal ability, *P. leucopus* are thought not to readily enter water or to possess adaptations for over-water dispersal (Carter and Merritt, 1981). However, earlier observations indicated that *P. leucopus* could swim considerable distances (Sheppe, 1965). Additionally, mountain rivers only represent weak to moderate barriers to common shrew (*Sorex araneus*) dispersal at some locations (Lugon-Moulin et al., 1999). If a river were indeed unnegotiable to individual mice, collections from each side of the river, even if directly across from each other (i.e., a short geographic distance), could yield a high level of genetic dissimilarity. However, collections from the same side of the river, even if sampled at relatively large distances from each other, could yield a high level of genetic similarity. Except for rare occasions, major river systems could be unnegotiable to a deer mouse. However, rafting on vegetation, portaging in a fisherman's boat, crossing on a bridge, or crossing during exceptionally low river levels could allow individuals to cross a river. We observed the North Fork of the Gunnison River at very low levels during the summer of 2000. Obviously, this could further compromise the "expected" effect of a river. Notably, assignment test misclassifications were observed between the Delta and Lazear study sites and between the Delta and Paonia study sites (Table 4). The LA site is located to the north of the Gunnison River system while the DE site is located south of the Gunnison River (Fig. 1). Similarly, the Gunnison River flows between the DE and PA sites. Rather than this river system being a major hindrance to gene flow, it may help facilitate some gene flow by providing a riparian corridor for long-distance movements of deer mice, perhaps only in select sections of this river system.

In contrast, the clustering in the dendrogram (Fig. 2) based on Slatkin's linearized F_{st} values indicates that the river systems associated with these study sites may be a slight hindrance to gene flow and that the other geographic features that we proposed as potential hindrances to gene flow pose little effect. All populations to the north of the Gunnison River system formed the single major cluster. The two

remaining populations, PA and DE, were located south of the Gunnison River system (i.e., the North Fork of the Gunnison River and the Gunnison River), but the Gunnison River bisects the land between these two populations (Fig. 1). Perhaps both rivers pose a slight hindrance to gene flow (at least at certain times of the year), but low river levels, river regulation, bridges, and riparian corridors facilitate some gene flow across these rivers. The second dendrogram (Fig. 3) based on Cavalli-Sforza and Edwards' (1967) chord distance yielded a similar overall pattern.

Long-term presence of SNV among rodent hosts is likely dependent on infected immigrants reestablishing the virus in populations of deer mice when the virus apparently goes extinct among these populations. We have observed the apparent temporal extinction of SNV at sites in Colorado on multiple occasions (Calisher et al., 1999). Results of this study indirectly support the idea of infected immigrants reestablishing SNV in populations where it has gone extinct; however, assignment tests indicate that immigration is more likely on a local scale than between distant sites (Table 4). Therefore, these data provide more support for SNV trafficking at the local level because intuitively, anywhere where there is gene flow among populations or sub-populations of deer mice, there is the potential for SNV transmission. The high misclassification rates at relatively short-distance sites indicates that SNV should readily be introduced among local populations, assuring re-introductions of this virus, and precluding any long-term extinctions within any particular local population as long as it is in contact with other populations over a wide area. On the other hand, a population that is more isolated (i.e., an island population) might run the risk of long-term extinctions of SNV.

In the future, we intend to use microsatellite markers to address questions pertaining to SNV transmission. More specifically, the application of several of these highly variable markers should allow us to detect if deer mice infected with SNV are more, equally, or less related to one another than they are to the uninfected portion of the population. If followed longitudinally, these results should lend some insight into the mysterious facets of SNV transmission.

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