Western North American Naturalist 60(3), © 2000, pp. 281–290

ALLOZYME VARIABILITY, ISOLATION, AND DISPERSAL OF *EUSATTUS MURICATUS* (COLEOPTERA: TENEBRIONIDAE) WITHIN SILVER STATE DUNE COMPLEX, GREAT BASIN, WESTERN NORTH AMERICA

Tammy M. Epps[1], Hugh B. Britten[1,2], and Richard W. Rust[1,3]

ABSTRACT.—Genetics of *Eusattus muricatus* (Coleoptera: Tenebrionidae) populations within Silver State Dune Complex in the northern Great Basin of North America were assayed using allozyme electrophoresis. Hierarchical *F*-statistics showed no significant population subdivision among 3 valleys comprising Silver State Dune Complex. A regression of estimated pairwise gene flow (*Nm*) against pairwise geographic distance was not significant for populations within these valleys, but it was significant over larger distances. A neighbor-joining phenogram did not represent geographic relationships among populations in the 3 valleys but reflected the past geologic history of these dunes. Pairwise divergence times were low (<7000 yr) for populations within Silver State Dune Complex but were high (>18,000 yr) in outgroups.

Key words: allozymes, Tenebrionidae, Eusattus, *population genetics, biogeography.*

The following article is dedicated to Gary L. Vinyard. Gary was an aquatic biologist who loved the Great Basin. He was an excellent teacher, a dedicated researcher, a conservation biologist, and a friend of Great Basin fishes. His works will long be remembered and used.

The Great Basin in western North America (Morrison 1991) contains extensive sand dunes that are of Holocene origin (Born 1972, Benson et al. 1990). These dunes and their association with pluvial lakes and water courses have been well studied (Smith 1982, Benson et al. 1990, Eissmann 1990, Morrison 1991). *Eusattus muricatus* (Coleoptera: Tenebrionidae) is perhaps the most abundant of the 15–20 dune-obligate, flightless insects confined to these dunes (Bechtel et al. 1983, Doyen 1984, Rust 1986). Adults may be found foraging on the dune surface at dawn and dusk throughout the warmer months of the year (Doyen 1984, Rust 1986). Their ecology appears typical of other dune-obligate beetles, and therefore *E. muricatus* serves as a kind of "umbrella species" for this understudied fauna. The distribution of this insect on relatively isolated sand dunes makes it an excellent subject for examining population genetics, dispersal, and fragmentation events (Stock and Castrovillo 1981, Higby and Stock 1982, Larson et al. 1984, Slatkin 1985, Foster and Knowles 1990, Britten et al. 1994, 1995, Yamashita and Polis 1995, Porter and Rust 1996).

The relatively well-understood geologic histories of dunes in the Great Basin further aid in studying population genetic consequences of habitat isolation for these beetles. Earlier studies showed that populations of *E. muricatus* are isolated from gene flow in sand dunes within different Great Basin pluvial basins even when these areas are separated by only tens of kilometers (Britten and Rust 1996, Epps et al. 1998). Evidence from several other dune-obligate beetle species suggests that genetic isolation necessary for speciation occurs at a spatial scale larger than individual dunes or dune complexes (Porter and Rust 1996).

Silver State Dune Complex within the Great Basin consists of 3 sand-filled valleys, Paradise Valley, Silver State Valley, and Desert Valley, spanning 2 pluvial Lake Lahontan subbasins, Blackrock and Humboldt (Fig. 1). Sand in these valleys originated from a delta of the Humboldt River entering Black Rock subbasin of Lake Lahontan at Jungo Flat ending approximately 11,000 BP (years before present) at the end of the last high stand (Benson et al.

[1]Biology Department/MS 314, University of Nevada–Reno, Reno, NV 89557.
[2]Present address: Department of Biology, University of South Dakota, Vermillion, SD 57069.
[3]Address correspondence to this author.

Fig. 1. Silver State Dune Complex in north central Nevada with *Eusattus muricatus* collection locations. The high stand of Lake Lahontan (1390 m), 20-m contours, geographical locations, and highways are marked.

1990, Eissmann 1990, Morrison 1991). As lake levels decreased after 9000 BP, sand was exposed and blown throughout the area in an east-northeasterly fashion (Eissmann 1990).

We used allozymes to examine population structure of *E. muricatus* within Silver State Dune Complex. Levels of variability within populations were estimated as were levels of gene flow within and among populations in these 3 sand-filled valleys. An increased understanding of the small-scale population structure of this representative dune-obligate insect may be useful in managing unique dune systems of the Great Basin (Britten and Rust 1996).

METHODS AND MATERIALS

Study Site

Adult *Eusattus muricatus* were sampled from 4 locations within Silver State Valley, 7 within Desert Valley, and 2 within Paradise Valley using pitfall traps and direct capture of adults (Fig. 1, Table 1). Three outgroup populations from outside the Lahontan Basin, 2 from the Mojave Desert and 1 from the Bonneville Basin, were sampled in a similar manner (Fig. 1, Table 1).

Eusattus muricatus populations from Silver State Dune Complex occur in a vast area of apparently continuous habitat comprising 3 adjacent sand-filled valleys in the northeastern portion of the Lahontan Basin. Desert Valley and Silver State Valley dunes are in Blackrock subbasin of Lake Lahontan, and Paradise Valley dune occurs in Humboldt subbasin (Benson 1991). The last high stand of Lake Lahontan occurred approximately 14,000 BP and lasted about 200 yr (Benson 1991). Because of the relatively low elevation of the sill separating Blackrock and Humboldt subbasins (Fig. 1), the 2 were not separated by lake dry-down until about 6000 BP (Morrison 1991). This was a time of high eolian activity in which exposed deltaic deposits were formed into large sand dunes present across the Great Basin today (Born 1972, Eissmann 1990, Morrison 1991). Wind in the northern Lahontan Basin during the Holocene was greater than at present,

TABLE 1. Location, position, and sample sizes of all sampled populations of *Eusattus muricatus*.

Location	Position	Code	Sample size
Crescent Dune[a]	38°14′N, 117°19′W	CD	40
Desert Valley North West 1	41°5′N, 118°9′W	DVNW1	40
Desert Valley North West 2	41°6′N, 118°9′W	DVNW2	40
Desert Valley 1	41°4′N, 118°5′W	DV1	38
Desert Valley 2	41°4′N, 118°3′W	DV2	35
Desert Valley 3	41°5′N, 118°1′W	DV3	55
Jungo Road	40°57′N, 118°12′W	JR	40
Little Sahara Dune[a]	39°37′N, 112°24′W	LSD	40
Mormon Dan Well	41°8′N, 118°10′W	MD	40
Paradise Valley Dune	41°6′N, 117°42′W	PVD	40
Paradise Valley Feedlot	41°13′N, 117°39′W	PVDFL	37
Silver State Valley Central	41°7′N, 117°50′W	SSVC	38
Silver State Valley East	41°11′N, 117°46′W	SSVE	40
Silver State Valley North	41°11′N, 117°58′W	SSVN	40
Silver State Valley West	41°4′N, 117°59′W	SSVW	40
Teels Marsh[a]	38°11′N, 118°21′W	TM	40

[a]Outgroup populations

facilitating movement of sand from the delta of the Humboldt River to each of the 3 valleys of Silver State Dune Complex (Eissmann 1990, Morrison 1991). Unconsolidated sand is continuously distributed across the 2 passes separating the 3 valleys. Satellite imagery also indicates active sand movement among the 3 valleys at present (Eissmann 1990). These populations have probably existed in the area since the warming and drying of the early Holocene that marked the end of the last pluvial episode in the Great Basin.

Laboratory Procedures

We transported specimens live to the laboratory where they were stored at –80°C until prepared for electrophoresis. Preparation included homogenizing the whole beetle in 0.25 mL of cold extraction buffer (0.05 M tris HCL, pH 7.0; May 1992). After 20 min of cold incubation, samples were centrifuged at 12,000 rpm for 5 min. The supernatant was pipetted into 1.5-mL microcentrifuge tubes and stored at –80°C. We thawed the resulting liquid and applied it to filter paper wicks (Whatman No. 3) that were inserted into 14% starch gels (1:1 Sigma and Connaught starches) for electrophoresis. Gels were run approximately 4 h. All informative gels were photographed for a permanent record.

Allozyme variability was assayed at 27 presumptive loci from 20 enzymes (Table 2). Stains, buffer systems, and electrophoretic procedures followed May (1992). Genotypic frequencies were obtained by direct count of phenotypes on the gels, with a common electromorph being scored as C, faster ones scored as B and then A, slower ones scored as D, etc.

Data Analysis

Using Levene's (1949) method, we calculated genotypic frequencies expected under Hardy-Weinberg equilibrium at 17 polymorphic loci. These were compared to observed frequencies using a chi-square goodness-of-fit test in BIOSYS-1 (Swofford and Selander 1981). The sequential Bonferroni method was used to test the strength of *P*-values associated with Hardy-Weinberg expectations (Rice 1989).

A hierarchical chi-square test for heterogeneity of allele frequencies was performed for sites within the same valley. Nonhierarchical *F*-statistics and hierarchical *F*-statistics were also calculated for estimates of among-population divergence using BIOSYS-1 (Swofford and Selander 1981). *F*-statistics were $(1 - F_{it}) = (1 - F_{is})(1 - F_{st})$ where F_{it} is overall inbreeding, F_{is} is subpopulation inbreeding, and F_{st} is the fixation index. Hierarchical *F*-statistics were put in terms of variance components as $(1 - F_{pt}) = (1 - F_{pv})(1 - F_{vt})$. That is, F_{pt} estimates variance in allele frequencies between populations in the total sample, F_{pv} estimates variance in allele frequencies between populations within the same valleys, and F_{vt} accounts for variance in allele frequencies due to differences between valleys within the total sample. Because *F*-statistics are a measure of inbreeding, they are useful in examining relative gene flow among populations

TABLE 2. Enzyme and buffer conditions used with populations of *Eusattus muricatus.*

Locus	Enzyme	Buffer[a]	E.C. number
AAT-1,2	Aspartate Aminotransferase	C	2.6.1.1
ADA	Adenosine Deamoinase	C	3.5.4.4
ADH	Alcohol Dehydrogenase	R	1.1.1.1
DIA	Dihydrolipoamide Dehydrogenase	R	1.8.1.4
ESTF-1,2	Flourescent Esterase	R	3.1.1.–
GAM	Flu. Galactoseaminidase	R	3.2.1.23
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	C	1.2.1.12
GDA-1,2	Guanine Deaminase	C	3.5.4.3
GK	Glucokinase	C	2.7.1.2
A-GLU	Flourescent Alpha-Glucosidase	R	3.2.1.20
GPI-1,2	Glucophosphate Isomerase	C	5.3.1.9
G3P-2,3	Glycerol-3-Phosphate Dehydrogenase	C	1.1.1.8
HA	Flu. Hexoseaminase	R	3.2.1.52
HBDH	3-Hydroxybutyrate Dehydrogenase	C	1.1.1.30
IDH	Isocitrate Dehydrogenase (NADP)	TC1	1.1.1.42
LDH	Lactate Dehydrogenase	R	1.1.1.27
ME-1,2	Malic Enzyme	TC1	1.1.1.40
MUP	Flu. Methylumbelliferyl Phosphate	R	
PGM-2,3	Phosphoglucomutase	C	5.4.2.2
SOD	Superoxide Dismutase	R	1.15.1.1

[a]Buffer recipes from May (1992)

within valleys and among populations within the total sample (Hartl and Clark 1989, Britten et al. 1994).

We used Nei's (1978) unbiased genetic identity to calculate overall genetic similarities among all pairs of populations. DISPAN (Ota 1993), a genetic distance and phylogenetic analysis program, was used to generate a neighbor-joining (Saitou and Nei 1987) phenogram based on Nei's unbiased (1978) genetic distances (D) with bootstrap values (Felsenstein 1985).

A protein molecular clock calculation (Penney and Zimmerman 1976, Nei 1986, Bilton 1994) was used for all pairwise populations to estimate time of divergence using the following formula:

$$t = D / (2cn_t a)$$

where t is pairwise divergence time, D is Nei's (1978) genetic distance, c is the proportion of amino acid substitutions detectible by electrophoresis (0.3; Lewontin 1974), n_t is the total number of potentially mutable codons comprising a protein (80,367 / 110 = 731; where 80,367 is the mean molecular weight of proteins used in this study, and 110 is the mean molecular weight of an amino acid; Penney and Zimmerman 1976), and a is the mean number of amino acid substitutions per year (2.1×10^{-9}; Nei 1986).

Pairwise geographic distance and estimates of pairwise gene flow (Nm; Wright 1943) were calculated for all populations using a program developed by M. Slatkin. A regression analysis of log pairwise gene flow against log geographic distance was used to test for isolation by distance (Slatkin 1993). A Mantel test (Manley 1991) was used to test the correlation among pairwise gene flow and pairwise geographic distance using 10,000 randomizations.

RESULTS

Allele frequencies for polymorphic loci are reported in Table 3. Of 27 loci, 10 were monomorphic in all populations. Nine of 16 populations examined differed significantly from Hardy-Weinberg expectations for at least 1 locus. Of these, 3 populations within Desert Valley dune differed from Hardy-Weinberg expectations at ≥2 loci. All deviations from Hardy-Weinberg proportions were heterozygote deficiencies. Mean observed heterozygosity ranged from 0.015 to 0.041, and percentage of polymorphic loci ranged from 7.4% to 22.2% using the 95% criterion (Table 4). Ten alleles were found segregating at only 1 sample location within Silver State Dune Complex (Table 3). Probabilities of detecting these unique alleles in other samples at the frequencies in Table 3 where $n = 40$ were 0.55 for ME-1d; 0.59 for PGM-3a and 3b; 0.65 for EST-2g, GAM-1d, and GDA-2b; 0.68 for GK-1b; 0.87 for GLU-1d; and 0.99 for ME-2d and PGM-3d (Table 3).

Table 3. Allele frequencies at all polymorphic loci for *Eusattus muricatus* (unique alleles are underlined).

	Population															
	Desert Valley							Silver State Valley				Paradise		Outgroups		
Locus	DV1	DV2	DV3	DVNW1	DVNW2	JR	MD	SSVW	SSVC	SSVE	SSVN	PVD	PVDFL	TM	CD	LSD
AAT-1																
A	0.014	0.014	0.000	0.000	0.000	0.000	0.000	0.014	0.013	0.000	0.000	0.000	0.027	0.000	0.000	0.000
B	0.750	0.857	0.800	0.650	0.750	0.875	0.744	0.797	0.705	0.756	1.000	0.888	0.878	0.310	0.577	0.000
C	0.000	0.000	0.091	0.150	0.038	0.000	0.128	0.000	0.064	0.013	0.000	0.000	0.000	0.678	0.385	1.000
D	0.236	0.129	0.109	0.200	0.212	0.125	0.128	0.189	0.218	0.231	0.000	0.112	0.095	0.012	0.038	0.000
AAT-2																
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.974	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000
EST-2																
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	1.000
C	0.013	0.029	0.027	0.056	0.013	0.000	0.030	0.013	0.000	0.000	0.000	0.015	0.000	0.532	0.962	0.000
D	0.132	0.171	0.255	0.167	0.158	0.224	0.197	0.138	0.158	0.276	0.105	0.132	0.135	0.424	0.038	0.000
E	0.855	0.786	0.718	0.777	0.816	0.776	0.773	0.749	0.829	0.671	0.869	0.779	0.770	0.011	0.000	0.000
F	0.000	0.014	0.000	0.000	0.013	0.000	0.000	0.100	0.013	0.053	0.013	0.074	0.095	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
GAM-1																
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.987	1.000	1.000	1.000	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
GDA-1																
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000
GDA-2																
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.380	0.000	0.053
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.987	1.000	1.000	1.000	0.620	1.000	0.947
GK-1																
B	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	0.986	0.981	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.956	1.000	0.982
D	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.000	0.018
GLU-1																
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	0.988	0.987	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.012	0.013	0.000
GPDH2																
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.987	1.000	1.000

TABLE 3. Continued.

	Population															
	Desert Valley							Silver State Valley				Paradise		Outgroups		
Locus	DV1	DV2	DV3	DVNW1	DVNW2	JR	MD	SSVW	SSVC	SSVE	SSVN	PVD	PVDFL	TM	CD	LSD
GPI-2																
A	0.013	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.013
B	0.948	0.943	0.946	1.000	0.987	0.962	1.000	0.950	0.987	0.988	0.987	0.974	1.000	1.000	0.987	0.882
C	0.039	0.057	0.045	0.000	0.013	0.013	0.000	0.050	0.000	0.013	0.013	0.013	0.000	0.000	0.000	0.092
D	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.013	0.013
G3P-2																
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
HBDH2																
B	0.224	0.200	0.091	0.000	0.000	0.014	0.000	0.386	0.000	0.000	0.000	0.000	0.000	0.000	0.205	0.592
C	0.776	0.800	0.909	1.000	1.000	0.986	1.000	0.614	1.000	1.000	1.000	1.000	1.000	1.000	0.795	0.408
IDH-1																
B	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.013	0.000
C	0.987	0.986	1.000	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.948	0.987	0.987
D	0.013	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.013
ME-01																
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.990	1.000	0.842
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.158
ME-2																
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.821	1.000	1.000	1.000	1.000	1.000	1.000	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.179	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-2																
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.041	0.000	0.000	0.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.908	0.808	1.000	0.918	0.957	0.948	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.079	0.192	0.000	0.041	0.043	0.026	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000
PGM-3																
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.018
C	1.000	1.000	0.931	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.978	1.000	0.982
D	0.000	0.000	*0.069*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 4. Percent polymorphic loci and mean expected heterozygosity for all populations of *Eusattus muricatus*.

Population	% polymorphic loci	Mean heterozygosity
Crescent Dune	29.6	0.038
Desert Valley North West 1	7.4	0.015
Desert Valley North West 2	14.8	0.022
Desert Valley 1	18.5	0.035
Desert Valley 2	22.2	0.029
Desert Valley 3	22.2	0.032
Jungo Road	14.8	0.02
Little Sahara Dune	25.9	0.031
Mormon Dan Well	7.4	0.029
Paradise Valley Dune	14.8	0.018
Paradise Valley Feedlot	11.1	0.024
Silver State Valley Central	14.8	0.041
Silver State Valley East	18.5	0.035
Silver State Valley North	11.1	0.015
Silver State Valley West	18.5	0.031
Teels Marsh	40.7	0.036

The chi-square test for population heterogeneity in allele frequencies was significant over all loci and sample locations (χ^2 = 5660.18, $P < 0.001$, df = 465). Chi-square heterogeneity tests were carried out individually for each dune. Samples from both Silver State Valley and Desert Valley dunes were significantly heterogeneous (χ^2 = 242.89, $P < 0.001$, df = 45; χ^2 = 187.93, $P < 0.001$, df = 90, respectively), while those from the 2 Paradise Valley Dune sample locations were not (χ^2 = 12.79, $P < 0.236$, df = 10). Mean *F*-statistics for all 16 populations included in the study were: F_{ST} = 0.364, F_{IS} = 0.240, and F_{IT} = 0.517. Hierarchical *F*-statistics revealed only weak population structuring due to sampling in different dunes within the complex; F_{vt} = 0.143, F_{pv} = 0.248, and F_{pt} = 0.356. Expressed as components of total variance in allele frequencies (i.e., percentage of the variance component for F_{pt}), populations within valleys (F_{pv}) contributed 60% of the estimated total variance in allele frequencies while valleys within the total sample (F_{vt}) contributed 40%. Estimated number of migrants exchanged per generation between pairs of sample locations (*Nm*) within Silver State Dune Complex ranged from 2.0 between Silver State Valley Northwest and Silver State Valley West to 51.4 between Mormon Dan Well and Desert Valley Northwest. Regression of log *Nm* against log pairwise geographic distance for all samples included in the study was not significant (R^2 = 0.00, P = 0.38 for which the observed regression coefficient was equaled or exceeded in 37.8% of the 10,000 Mantel pseudoreplicates; Fig. 3). Mean *F*-statistics and *Nm* estimates for each dune are given in Table 5.

Topology of a neighbor-joining tree (Saitou and Nei 1987) based on unbiased genetic distances did not correspond to the expected topology predicted from relative locations of sample sites within Silver State Dune Complex (Fig. 2). For example, Silver State Valley Northwest clustered closest to the 2 Paradise Valley Dune populations, not with nearer Silver State Valley Dune populations (Fig. 2). Divergence times estimated from genetic distances were <7000 yr for all pairs of populations within Silver State Dune Complex, with some populations showing divergence times of <1000 yr. All divergence times between populations within Silver State Dune Complex and outgroup populations were >18,000 yr.

DISCUSSION

Previous work has demonstrated that isolation by distance describes *Eusattus muricatus* population structure over greater distances between sample locations than encountered in Silver State Dune Complex (Britten and Rust 1996, Epps et al. 1998). This finding was attributed to an equilibrium between drift among populations on isolated sand dunes within the same pluvial basin and among those derived from different pluvial basins (Britten and Rust 1996, Epps et al. 1998). It is likely that this equilibrium is due to gene flow in the recent past and that populations will continue to diverge in allele frequencies by drift. While

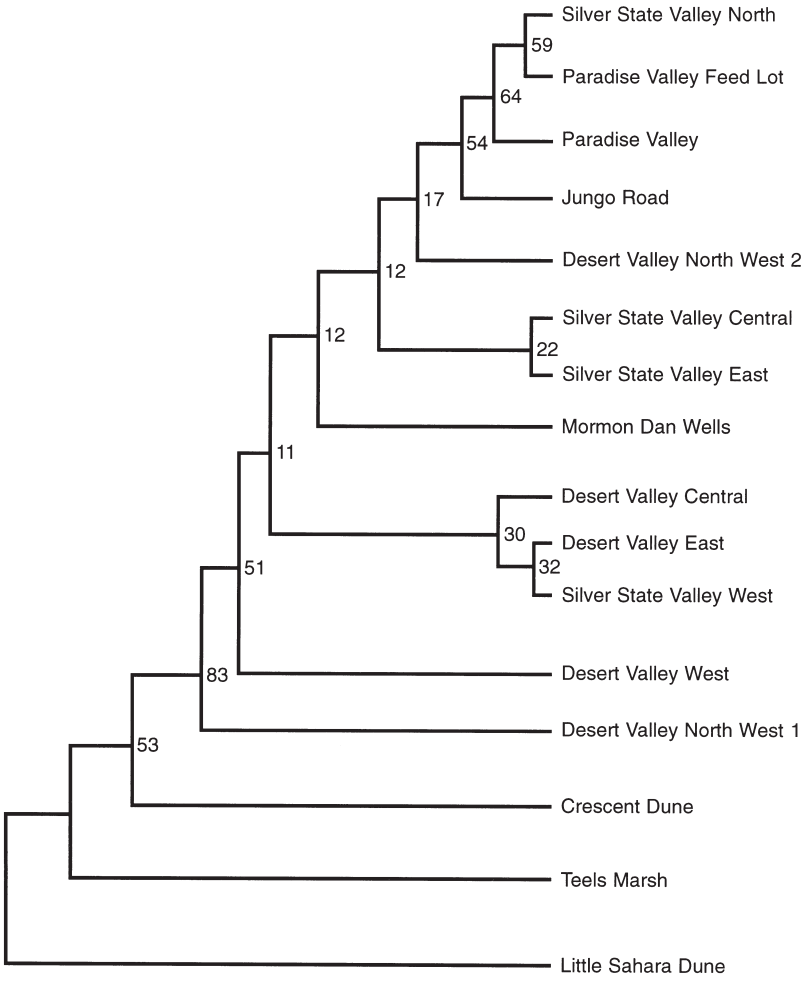

Fig. 2. Neighbor-joining phenogram for *Eusattus muricatus* populations with Little Sahara Dune selected as an outgroup (numbers at nodes indicate bootstrap values).

TABLE 5. Mean *F*-statistics and estimates of numbers of genetically effective migrants exchanged each generation (*Nm*) for *Eusattus muricatus* within the 3 dunes of Silver State Dune Complex.

Dune	F_{IS}	F_{IT}	F_{ST}	*Nm*
Desert Valley	0.238	0.265	0.035	6.9
Silver State Valley	0.183	0.266	0.102	2.2
Paradise Valley	0.180	0.184	0.005	49.7

isolation by distance suggests equilibrium between drift and gene flow at larger geographic scales (e.g., basin-wide), a nonequilibrium situation in which gene flow occurs with little drift is still apparent among populations at smaller scales in the relatively continuous habitat of Silver State Dune Complex. At yet smaller geographic scales (e.g., sample sites within a dune), we see evidence of inbreeding and/or Wahlund effect within populations. It is possible that individuals from the same broods were inadvertently sampled at some, or all, locations. This would be consistent with our observation that *E. muricatus* is somewhat patchily distributed on dunes at this small scale. Similar results have been reported for *Aegialia* species,

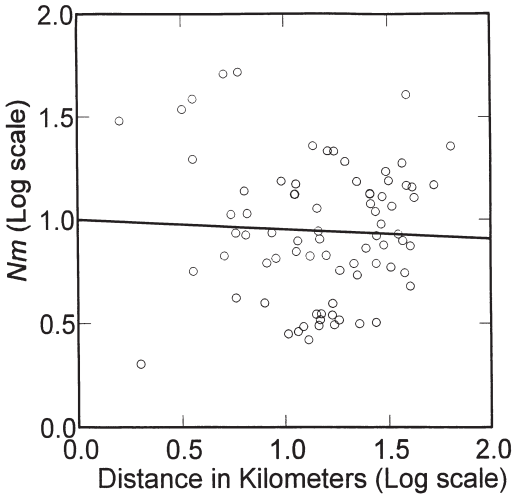

Fig. 3. Regression of log pairwise dispersal (*Nm*) on log pairwise geographic distance for Silver State Dune Complex samples of *Eusattus muricatus* ($R^2 = 0.00$, $P = 0.70$, $y = 1.2 - 0.0x$).

another sand-obligate beetle genus from many of the same dunes sampled for *E. muricatus* (Porter and Rust 1996).

Estimates of pairwise divergence times based on a molecular clock reflect the geologic history of the 3 valleys of Silver State Dune Complex. Divergence times of <7000 yr were estimated for several pairs of populations within the complex while divergence times among Silver State Dune Complex populations and our 3 outgroups (Teels Marsh, Crescent Dune, and Little Sahara Dune) show divergence times >18,000 yr.

Geologic evidence also supports the apparent anomalous placement of the Jungo Road (JR) population, which is in Desert Valley (Blackrock subbasin), with 2 Paradise Valley (PV and PVFL; Humboldt subbasin) populations on the neighbor-joining phenogram (Fig. 2). The course of the Humboldt River has changed from late Pliestocene to the present (Eissmann 1990). The mouth of the river was near the south end of Desert Valley (Jungo Road site) between 22,000 and 13,500 BP. At present, the Humboldt River turns south in Paradise Valley and flows further southward, ending in Humboldt Sink. This changing course of the Humboldt River since the last high stand (14,000 BP) provides sand habitat contact in river bank sand deposits between Jungo Road and Paradise Valley populations. The other apparently anomalous grouping of Silver State Valley West (SSVW) and 2 Desert Valley sites (DVE and DVC) on the neighbor-joining phenogram (Fig. 2) is expected as SSVW and DVE are only 3 km apart and within the wind flow and sand drift patterns suggested by Eissmann (1990).

Although flightless, *E. muricatus* is capable of moving distances of 500 m in a single night (Rust personal observation). Gene flow estimates suggest there is enough genetic exchange among distant locations within the dunes of Silver State Dune Complex to prevent genetic divergence and that geographic distance and gene flow are not statistically related. The presence of unique alleles in high frequencies would be contrary evidence for this assertion. Probabilities of detecting 7 of 10 unique alleles found segregating at various sample locations are low (≤0.68) due to low frequencies for these alleles and relatively large sample sizes ($n = 40$) for each site in Silver State Dune Complex. The remaining 3 unique allles with higher detection probabilities (≥0.87) suggest that some genetic isolation between sample locations has occurred.

Allozyme results from Silver State Dune Complex and previous work (Britten and Rust 1996) suggest that there is, or has been in the recent past, substantial gene flow among sample locations in different dunes within the same pluvial basin. Hierarchical *F*-statistics suggest only a weak influence on population structure due to sampling from different dunes within the complex. Divergence time estimates were within the range expected for the sample locations given the known history of Silver State Dune Complex.

ACKNOWLEDGMENTS

We thank the Theodore Roosevelt Memorial Fund of the American Museum of Natural History for partial funding and the Nevada Biodiversity Initiative and Dr. Peter F. Brussard for partial funding and use of laboratory equipment; Dr. Nick Lancaster, Desert Research Institute University of Nevada, for valuable information on geologic history of the Great Basin sand dunes and for review of this paper; Lynn Riley and Robert Bamfort for pairwise distances and map preparation; and John Carpenter, Jeff Knight, Richard Duncan, and Janene Auger for assistance in fieldwork.

LITERATURE CITED

BECHTEL, R.C., L.M. HANKS, AND R.W. RUST. 1983. Coleoptera of Sand Mountain and Blow Sand Mountain, Nevada. Southwestern Naturalist 28:473–478.

Benson, L.V. 1991 Timing of the last highstrand of Lake Lahoatan. Journal of Paleolimnology 5:115–126.

BENSON, L.V., D.R. CURREY, R.I. DORN, K.R. LAJOIE, C.G. OVIATT, S.W. ROBINSON, G.I. SMITH, AND S. STINE. 1990. Chronology of expansion and contraction of four Great Basin lake systems during the past 35,000 years. Palaeogeography, Palaeoclimatology, Palaeoecology 78: 241–286.

BILTON, D.T. 1994. Phylogenetic and recent historical biogeography of *Hydroporous glabriusculus* Aube (Coleoptera: Dytiscidae) in the British Isles and Scandinavia. Biological Journal of the Linnean Society 51:293–307.

BORN, S.M. 1972. Late Quaternary history, deltaic sedimentation, and mudlump formation at Pyramid Lake Nevada. Center for Water Resources Research, DRI, Reno.

BRITTEN, H.B., P.F. BRUSSARD, D.D. MURPHY, AND G.T. AUSTIN. 1994. Colony isolation and isozyme variability of the western seep fritillary, *Speyeria nokomis apacheana* (Nymphalidae), in the western Great Basin. Great Basin Naturalist 54:97–105.

BRITTEN, H.B., P.F. BRUSSARD, D.D. MURPHY, AND P.R. EHRLICH. 1995. A test for isolation-by-distance in central Rocky Mountain and Great Basin populations of Edith's checkerspot butterfly (*Euphydryas editha*). Journal of Heredity 86:204–210.

BRITTEN, H.B., AND R.W. RUST. 1996. Population structure of a sand dune-obligate beetle, *Eusattus muricatus*, and its implications for dune management. Conservation Biology 10:647–652.

DOYEN, J.T. 1984. Systematics of *Eusattus* and *Conisattus* (Coleoptera: Tenebrionidae; Coniontini; Eusatti). Occasional Papers of the California Academy of Science 141:1–104.

EISSMANN, L.J. 1990. Eolian sand transport in western Nevada. Master's thesis, University of Nevada, Reno.

EPPS, T., H.B. BRITTEN, AND R.W. RUST. 1998. Historical biogeography of *Eusattus muricatus* (Coleoptera: Tenebrionidae) within the Great Basin, western North America. Journal of Biogeography 25:957–968.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies, an approach to using the bootstrap. Evolution 39: 783–791.

FOSTER R.F., AND P. KNOWLES. 1990. Genetic variation in five species and 12 populations of *Cicindela* (Coleoptera: Cicindelidae) in northwestern Ontario. Annals of the Entomological Society of America 83: 838–845.

HARTL, D.L., AND A.G. CLARK.1989. Principles of population genetics. Sinauer, Sunderland, MA. 682 pp.

HIGBY, P.K., AND M.W. STOCK. 1982. Genetic relationships between two sibling species of bark beetle (Coleoptera: Scolytidae), Jeffrey pine beetle and mountain pine beetle, in northern California. Annals of the Entomological Society of America 75:665–674.

LARSON, A., D.B. WAKE, AND K.P. YANEV. 1984. Measuring gene flow among populations having high levels of genetic fragmentation. Genetics 106:293–308.

LEVENE, H. 1949. On a matching problem arising in genetics. Annual Mathematical Statistics 20:91–94.

LEWONTIN, R.C. 1974. The genetic basis of evolutionary change. Columbia University Press, New York. 346 pp.

MANLEY, B.F.J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, New York. 281 pp.

MAY, B. 1992. Starch gel electrophoresis of allozymes. Pages 1–27 *in* A.R. Hoezel, editor, Molecular genetic analysis of populations: a practical approach. Oxford University Press, Oxford, U.K.

MORRISON, R.B. 1991. Quaternary stratigraphic, hydrologic, and climatic history of the Great Basin, with emphasis on lakes Lahontan, Bonneville, and Tecopa. Pages 283–320 *in* R.B. Morrison, editor, The geology of North America. Volume K-2. The Geological Society of America, Golden, CO.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 98:583–590.

_____. 1986. Molecular evolutionary genetics. Columbia University Press, New York.

OTA, T. 1993. DISPAN: genetic distance and phylogenetic analysis. Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park.

PENNEY, D.F., AND E.G. ZIMMERMAN. 1976. Genetic divergence and local population differentiation by random genetic drift in the pocket gopher genus *Geomys.* Evolution 30:473–483.

PORTER, J.L., AND R.W. RUST. 1996. Allozyme variation within five species of *Aegialia* (Coleoptera: Scarabaeidae). Annals of the Entomological Society of America 89:710–721.

RICE, W. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

RUST, R.W. 1986. Seasonal distribution, trophic structure, and origin of sand-obligate insect communities in the Great Basin. Pan-Pacific Entomologist 62:44–52.

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

SLATKIN, M. 1985. Rare alleles as indicators of gene flow. Evolution 39:53–65.

_____. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

SMITH, R.S.U. 1982. Sand dunes in the North American deserts. Pages 481–524 *in* G.L. Bender, editor, Reference handbook of the deserts of North America. Greenwood Press, Westport, CT.

STOCK, M., AND P. CASTROVILLO. 1981. Genetic relationships among representative populations of five *Choristoneura* species: *C. occidentalis, C. retiniana, C. biennis, C. lambertiana,* and *C. fumiferana* (Lepidoptera: Tortricidae). Canadian Entomologist 113:857–865.

SWOFFORD, D.L., AND R.B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and evolution. Journal of Heredity 72:281–283.

WRIGHT, S. 1943. Isolation by distance. Genetics 28: 114–138.

YAMASHITA, T., AND G.A. POLIS. 1995. Geographical analysis of scorpion populations on habitat islands. Heredity 75:495–505.

Received 9 September 1998
Accepted 17 June 1999