

NATURAL SELECTION DRIVES ALTITUDINAL DIVERGENCE AT THE ALBUMIN LOCUS IN DEER MICE, *PEROMYSCUS MANICULATUS*

JAY F. STORZ^{1,2} AND JEAN M. DUBACH³

¹Department of Ecology and Evolutionary Biology, University of Arizona, Biosciences West, Tucson, Arizona 85721

³Department of Conservation Biology, Brookfield Zoo, Brookfield, Illinois 60513

Abstract.—In populations that are distributed across steep environmental gradients, the potential for local adaptation is largely determined by the spatial scale of fitness variation relative to dispersal distance. Since altitudinal gradients are generally characterized by dramatic ecological transitions over relatively short linear distances, adaptive divergence across such gradients will typically require especially strong selection to counterbalance the homogenizing effect of gene flow. Here we report the results of a study that was designed to test for evidence of adaptive divergence across an altitudinal gradient in a natural population of deer mice, *Peromyscus maniculatus*. We conducted a multilocus survey of allozyme variation across a steep altitudinal gradient in the southern Rocky Mountains that spanned several distinct biomes, from prairie grassland to alpine tundra. As a control for the effects of altitude, we also surveyed the same loci in mice sampled along a latitudinal transect through the prairie grassland that ran perpendicular to the east-west altitudinal transect. We used a coalescent-based simulation model to identify loci that deviated from neutral expectations, and we then assessed whether locus-specific patterns of variation were nonrandom with respect to altitude. Results indicated that the albumin locus (*Alb*) reflects a history of diversifying selection across the altitudinal gradient. This conclusion is supported by two main lines of evidence: (1) *Alb* was characterized by levels of divergence across the altitudinal transect that exceeded neutral expectations in two consecutive years of sampling (in contrast to the spatial pattern of variation across the latitudinal transect), and (2) levels of divergence at the *Alb* locus exhibited a positive association with altitudinal distance in both years (in contrast to the pattern observed at unlinked loci). We conclude that clinal variation at the *Alb* locus reflects a balance between gene flow and diversifying selection that results from elevational changes in fitness rankings among alternative genotypes.

Key words.—Adaptation, allozymes, clinal variation, coalescent, elevational gradient, interlocus contrasts, mice.

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Can diversifying selection drive adaptive differentiation in the face of countervailing gene flow? This question is of fundamental importance to our understanding of local adaptation and the determinants of geographic variation (Endler 1977). When a population is distributed across a steep environmental gradient, clinal variation in allele frequencies can be maintained by the interplay between diversifying selection and gene flow (Haldane 1948; Slatkin 1973; Endler 1977). In natural populations, clines across latitudinal and altitudinal gradients are often attributed to this type of selection-migration balance (Endler 1986). Indeed, latitudinal clines in allele frequencies provide the basis for some of the most compelling case studies of how positive selection shapes patterns of adaptive genetic variation in natural populations (e.g., Powers and Place 1978; Oakshott et al. 1981, 1982; Berry and Kreitman 1993; Schmidt et al. 2000; Verrelli and Eanes 2001; Duvernell et al. 2003). Altitudinal clines in allele frequencies are of particular interest since similar gradients of environmental variation will typically span a much shorter physical distance relative to the spatial scale of dispersal. Thus, relative to latitudinal clines, the stable maintenance of altitudinal clines will typically require especially strong selection to counterbalance the homogenizing effect of gene flow.

In the absence of barriers to dispersal, steep clines in allele frequencies may often reflect a history of diversifying selection. However, clines can also be produced by the interplay between drift and spatially restricted gene flow (isolation by

distance) or by admixture between previously isolated populations that have come into secondary contact. One means of distinguishing between adaptive versus nonadaptive causes of clinal variation at specific genes is to compare relative levels of spatial differentiation at multiple, unlinked loci. The premise of this approach is that the effects of selection are generally locus-specific, whereas demographic processes are expected to have relatively uniform effects across the entire genome (Cavalli-Sforza 1966; Lewontin and Krakauer 1973).

The purpose of this study was to test for evidence of adaptive divergence across an altitudinal gradient in a natural population of deer mice, *Peromyscus maniculatus*. Specifically, we analyzed patterns of allozyme variation across a steep altitudinal gradient in the southern Rocky Mountains that spanned several distinct biomes, from prairie grassland to alpine tundra. *Peromyscus maniculatus* has the broadest altitudinal distribution of any North American mammal and thus represents an ideal taxon for investigating the process of adaptive divergence across environmental gradients.

A previous analysis identified the albumin locus (*Alb*) as a candidate gene for local adaptation in four different species of *Peromyscus* (Storz and Nachman 2003). Evidence for diversifying selection on *Alb* polymorphism was particularly strong in *P. maniculatus* from Colorado. The *P. maniculatus* dataset analyzed by Storz and Nachman (2003) was based on population samples from a range of different altitudes, suggesting that selection may have been mediated by some factor or combination of factors related to altitude. To investigate this pattern in more detail, and to test for evidence of adaptive divergence across the altitudinal gradient, we augmented the dataset analyzed by Storz and Nachman (2003) by adding data from mice that were collected from three localities at

² Present address: Department of Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, California 94132; E-mail: storz@sfsu.edu.

lower altitudes during the same year. This expanded sample allows us to examine spatial patterns of variation over a more continuous range of altitudes. We also surveyed allozyme variation in mice sampled from the same altitudinal transect in a second consecutive year (to assess the temporal consistency of the spatial pattern) and in mice sampled from a latitudinal transect through the prairie grassland (to control for the effects of altitude on patterns of differentiation). If *Alb* polymorphism is in fact subject to diversifying selection across the altitudinal gradient, levels of divergence at the *Alb* locus should exceed neutral expectations in temporal replicates of the same altitudinal transect (but not in the latitudinal transect), and allele frequencies should exhibit a consistent, nonrandom association with altitude (in contrast to the patterns of variation at unlinked loci).

METHODS

Sampling the Altitudinal Transect

A total of 614 *P. maniculatus* was live-trapped from 11 localities in Colorado during each of two consecutive years. Sampling localities were distributed along an altitudinal transect that spanned approximately 3050 m of vertical relief over a linear distance of 500 km (Fig. 1A). Sampling localities were spaced along the transect at intervals of about 300 m elevation, except for site 6 (Black Hawk, 2697 m) and site 7 (Echo Lake, 3200 m) which were separated by an altitudinal distance of 503 m. Also, site 8 (Kingston Peak, 3475 m) and site 9 (Mt. Evans [I], 3505 m) were sampled at approximately the same elevation. Mice were sampled in early summer, prior to the onset of summer breeding activity. Only overwintering adults were used in the analysis of allozyme variation. Populations of *P. maniculatus* are characterized by extremely high rates of turnover (e.g., O'Farrell 1978), so it is unlikely that our samples from consecutive years contained mice that were born in the same generation.

Sampling the Latitudinal Transect

In a similar fashion, a total of 112 mice were live-trapped from five localities along a latitudinal transect that ran parallel to the Front Range of the Colorado Rockies (Fig. 1B). The latitudinal transect was intended as a control for the effects of altitude on the spatial patterning of allozyme variation. Sampling localities were all situated in prairie grassland habitat at roughly the same altitude (about 1960–1980 m). The latitudinal transect spanned 200 km and intersected the lower end of the east-west altitudinal transect at Golden, the only locality that was common to both transects. Samples from the latitudinal transect were collected during the same season as the year 1 altitudinal samples, so the same sample of mice from Golden was used in both analyses.

Allozyme Analysis

Liver, kidney, blood plasma, or hemolysate preparations were subjected to electrophoretic analysis following the procedures of Selander et al. (1971). Horizontal starch-gel electrophoresis was used to measure variation at a total of 18 enzymatic and non-enzymatic proteins (all data were collected by JMD). The enzymes (and associated Enzyme Com-

mission numbers) included alcohol dehydrogenase (*Adh*-1; 1.1.1.1), aspartate aminotransferase (*Aat*-1 and *Aat*-2; 2.6.1.1), esterase (*Est*-4; 3.1.1.1), glucose-6-phosphate dehydrogenase (*G6pd*; 1.1.1.49), glycerol-3-phosphate dehydrogenase (α -*Gpd*; 1.1.1.8), isocitrate dehydrogenase (*Idh*; 1.1.1.42), lactate dehydrogenase (*Ldh*-1 and *Ldh*-2; 1.1.1.27), malate dehydrogenase (*Mdh*-1 and *Mdh*-2; 1.1.1.37), phosphoglucomutase (*Pgm*-1; 2.7.5.1), 6-phosphogluconate dehydrogenase (*6-Pgd*; 1.1.1.44), sorbitol dehydrogenase (*Sdh*; 1.1.1.14), and superoxide dismutase (*Sod*; 1.15.1.1). The non-enzymatic proteins included albumin (*Alb*), hemoglobin (*Hb*), and transferrin (*Trf*). For all of the allozyme loci included in this survey of *P. maniculatus*, codominant segregation of electrophoretic variants has been confirmed by breeding experiments (e.g., Baccus et al. 1980; Nadeau and Baccus 1981; Aquadro and Avise 1982; Dawson 1982; Dawson et al. 1983). A subset of the year 1 data (localities 4–10 from the altitudinal transect) was included in a previous analysis by Storz and Nachman (2003).

Allelic and Genotypic Disequilibria

We used a randomization procedure to test for evidence of linkage disequilibrium between each pairwise combination of loci. Genotypes at pairs of loci were associated at random and the log-likelihood *G*-statistic was calculated for each randomized data set. *P*-values for each pairwise combination of loci were calculated as the proportion of randomized datasets with values of the *G*-statistic that were equal to or greater than the observed value. For each year, null distributions of the *G*-statistic were generated from $[20L(L - 1)]/2$ randomizations where *L* is the number of polymorphic loci. In a similar fashion, we used a randomization procedure to test for departures from Hardy-Weinberg genotypic proportions using Weir and Cockerham's (1984) estimators of the inbreeding coefficients: $f(=F_{IS})$ and $F(=F_{IT})$. Null distributions were generated from 10,000 randomizations of alleles among individuals within samples (for F_{IS}) and among individuals from the total set of samples (for F_{IT}). Ninety-five percent confidence intervals were obtained by bootstrapping over loci. The program FSTAT version 2.9.3 (updated from Goudet 1995) was used for all calculations.

Testing for Evidence of Selection

Previous efforts to develop an F_{ST} -based neutrality test (e.g., Lewontin and Krakauer 1973) were criticized on the grounds that parametric expectations for the interlocus variance in F_{ST} did not account for patterns of migration or population history that could induce a correlation in allele frequencies among subpopulations (Nei and Maruyama 1975; Robertson 1975a,b). An alternative approach is to use a coalescent simulation model to generate null distributions of F_{ST} (conditional on heterozygosity) under a neutral model of population structure (Beaumont and Nichols 1996; see also McDonald 1994; Vitalis et al. 2001; Porter 2003).

Considering each of the three datasets separately (the latitudinal transect and two replicates of the altitudinal transect), we obtained single-locus estimates of F_{ST} using the β statistic of Cockerham and Weir (1993):

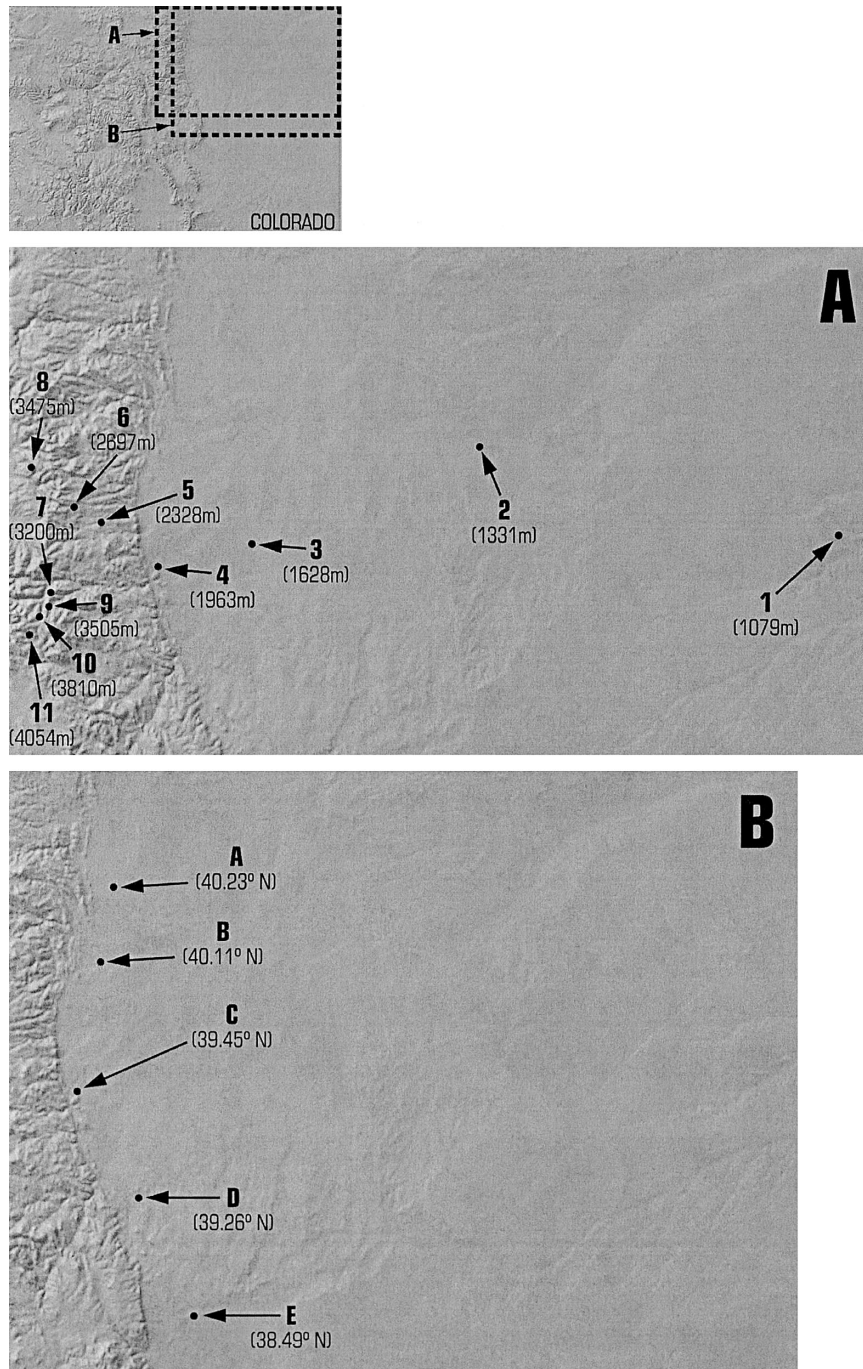


FIG. 1. Map of northeastern Colorado showing localities where deer mice were sampled for the analysis of allozyme variation. (A) Map of the altitudinal transect. Names of sampling localities, elevation, and sample size (no. of mice sampled in year 1/year 2) are as follows: 1, Wray, 1079 m (28/23); 2, Fort Morgan, 1331 m (49/45); 3, Northglenn, 1628 m (52/43); 4, Golden, 1963 m (42/34); 5, Golden Gate State Park, 2328 m (0/29); 6, Black Hawk, 2697 m (6/22); 7, Echo Lake, 3200 m (14/14); 8, Kingston Peak, 3475 m (16/23); 9, Mt. Evans (I), 3505 m (40/30); 10, Mt. Evans (II), 3810 m (30/23); and 11, Mt. Evans (III), 4054 m (31/20). (B) Map of the latitudinal transect. Names of sampling localities, degrees north latitude, and sample size (no. of mice) are as follows: A, Carter Lake, 40.23°N (17); B, Longmont, 40.11°N (14); C, Golden, 39.45°N (42); D, Sedalia, 39.26°N (14); E, Colorado Springs, 38.49°N (25).

$$\beta = \frac{F_0 - F_1}{1 - F_1}, \quad (1)$$

where $1 - F_1$ is the average probability of identity-by-descent for two alleles sampled randomly from separate subpopulations, and $1 - F_0$ is the average probability of identity-by-descent for two alleles sampled randomly from the same subpopulation. We estimated heterozygosity, H , as $1 - F_1$. For each data set, we calculated the expected F_{ST} as the among-locus average weighted by H (Weir and Cockerham 1984). We then tested for evidence of selection by comparing observed F_{ST} values to a null distribution generated by a coalescent-based simulation model. Specifically, we used the structured coalescent model of Beaumont and Nichols (1996) to generate a null distribution of F_{ST} conditional on H . We performed coalescent simulations using a symmetrical 100-island model of population structure and two different mutation models: the infinite alleles model (IAM; Kimura and Crow 1964) and the stepwise mutation model (SMM; Ohta and Kimura 1973). Sample sizes were set equal to the median of actual sample sizes in each of the three datasets. After generating a heterozygosity value uniformly on (0, 1), values of $N_e\mu$ (the mutation rate scaled to effective population size) were derived from the following relationships:

$$H = \frac{4N_e\mu}{1 + 4N_e\mu} \quad (2)$$

for the IAM (Kimura and Crow 1964), and

$$H = 1 - \frac{1}{(8N_e\mu + 1)^{0.5}} \quad (3)$$

for the SMM (Ohta and Kimura 1973). Coalescent simulations were used to generate a total of 50,000 paired values of F_{ST} and H . To compute the conditional probability density of F_{ST} , we used a computer program written by M. A. Beaumont (available at <http://www.rubic.rdg.ac.uk/~mab/software.html>).

To assess the statistical significance of departures from the neutral expectation, two-tailed probabilities were expressed as the proportion of 50,000 simulated values that were more extreme than the observed values. For each dataset, the α -level was Bonferroni-adjusted for multiple comparisons (0.05/number of loci).

Testing for Evidence of Adaptive Divergence across the Altitudinal Gradient

After identifying loci that deviated from neutral expectations, we tested for nonrandom associations with altitude. For each pairwise combination of sampling localities, arcsine-transformed values of $\sqrt{F_{ST}}$ were calculated separately for each putatively selected locus ($F_{ST(\text{selected})}$) and the remaining putatively neutral loci ($F_{ST(\text{neutral})}$). Because oxygen tension, ambient temperature, and climatic variability covary with altitude, pairwise difference in altitude can be considered as a proxy measure for overall environmental dissimilarity between sampling localities. Altitudinal distances were computed as $\sqrt{(x_i - x_j)^2}$, where x_i and x_j represent altitudes for the i th and j th localities.

If altitudinal clines in allele frequencies are simply attrib-

utable to stochastic differentiation via drift, a positive association between $F_{ST(\text{selected})}$ and altitudinal distance would not be expected to remain statistically significant after controlling for $F_{ST(\text{neutral})}$. The null hypothesis of neutral divergence can be tested using a multiple regression model (Storz 2002). A significant partial regression of $F_{ST(\text{selected})}$ on altitudinal distance would indicate that migration-drift equilibrium is not a sufficient explanation for the observed pattern of differentiation. We used partial Mantel tests to assess the association between $F_{ST(\text{selected})}$ and altitudinal distance. These tests involve simultaneous comparisons of one dependent variable matrix and multiple independent variable matrices. For the partial Mantel tests, a matrix of $F_{ST(\text{selected})}$ (**S**) was related to a matrix of $F_{ST(\text{neutral})}$ (**N**) and a matrix of pairwise measures of altitudinal distance (**A**). For the three respective matrices, let s_{ij} , n_{ij} , and a_{ij} denote distances between localities i and j . We then fit the following regression equation:

$$s_{ij} = c_{v(i)v(j)} + \beta_1 n_{ij} + \beta_2 a_{ij}, \quad (4)$$

where β_2 measures the association between s_{ij} and a_{ij} while controlling for the effects of n_{ij} , and $c_{v(i)v(j)}$ is a nuisance parameter that depends on the group identities of the localities under consideration. Specifically, $v(i)$ denotes the group identity of locality i , and c_{rs} takes into account the overall difference in $F_{ST(\text{selected})}$ between localities belonging to groups r and s .

To assess the statistical significance of the tests, we used a matrix randomization procedure in which permutations of the response matrix were restricted to two spatially defined groups of localities along the altitudinal transect: prairie grassland localities (1–4) and montane localities (5–11; Fig. 1A). After constructing a null distribution of regression coefficients by iteratively randomizing s_{ij} within the high- and low-altitude groups, probability values for the null hypothesis of no association were then expressed as the proportion of 10,000 matrix randomizations that yielded t -values greater than or equal to the observed value.

This restricted randomization procedure permits an assessment of the association between pairwise measures of $F_{ST(\text{selected})}$ and altitudinal distance after allowing for large-scale differences between high- and low-altitude groups of localities, as well as small-scale differences within groups. Since restricted randomizations account for the spatial structure of the data, this procedure should account for any residual source of spatial autocorrelation that is not removed by including the matrix of $F_{ST(\text{neutral})}$ as an independent variable in the regression model (Sokal et al. 1989, 1990; Manly 1997).

We tested for evidence of spatial autocorrelation in the residuals of the fitted equation as follows. For each pair of localities we measured the difference in the residuals from separate regressions of the response matrix **S** on the two independent variable matrices. We calculated the residuals as the differences in observed values of $F_{ST(\text{selected})}$ between localities and the differences predicted by fitting equation (4). Residual differences between localities i and j were calculated as

$$d_{ij} = \sqrt{\sum_{k=1, k \neq i, j}^n \frac{(r_{ik} - r_{jk})^2}{(n-2)}}, \quad (5)$$

where n is the number of localities and r_{ik} is the residual difference between localities i and k . We then assessed whether these pairwise residual differences were positively correlated with pairwise measures of $F_{ST(\text{neutral})}$.

To determine whether the altitudinal and latitudinal transects were characterized by any detectable difference in the spatial scale of gene flow, we tested for evidence of isolation by distance (Wright 1943) by performing a regression of pairwise $F_{ST(\text{neutral})}$ against ln-transformed measures of pairwise geographic distance for each transect. We tested for significance of the regression coefficients using matrix randomizations as described above.

RESULTS

Three of the 18 loci analyzed in *P. maniculatus* from the altitudinal transect were monomorphic in both years (*Mdh-2*, *G6pd*, and *Sod*) and *Ldh-2* was monomorphic in samples from year 1. For the remaining loci, expected heterozygosity averaged 0.206 in year 1 ($n = 308$ mice) and 0.230 in year 2 ($n = 306$ mice; Table 1). Two additional loci were monomorphic in mice sampled from the latitudinal transect (*Aat-2* and *Ldh-1*) and expected heterozygosity at the remaining 13 loci averaged 0.201 ($n = 112$ mice; Table 1).

Tests of Allelic and Genotypic Disequilibria

Randomization tests revealed six significant cases of pairwise linkage disequilibrium in the year 1 altitudinal transect (*Ldh-2* × *Adh*, *6-Pgd* × *α-Gpd*, *Est-4* × *Adh*, *Est-4* × *Pgm*, *Est-4* × *Trf*, and *α-Gpd* × *Adh*), four cases in the year 2 altitudinal transect (*Ldh-1* × *Hb*, *Idh-1* × *Mdh-1*, *Est-4* × *Aat-2*, and *Aat-1* × *Aat-2*), and three cases in the latitudinal transect (*Aat-1* × *Pgm*, *Sdh* × *Trf*, and *Pgm* × *Alb*). However, no pairwise associations were statistically significant after correcting for multiple comparisons. The same was true on a within-locality basis.

Both years of sampling across the altitudinal transect were characterized by a slight deficit of heterozygotes within localities (mean $F_{IS(\text{year 1})} = 0.127$, 95% CI = 0.036 to 0.210; mean $F_{IS(\text{year 2})} = 0.059$, 95% CI = -0.022–0.166) and among localities (mean $F_{IT(\text{year 1})} = 0.171$, 95% CI = 0.093–0.247; mean $F_{IT(\text{year 2})} = 0.097$, 95% CI = 0.019–0.202). Out of 150 locus × locality combinations in year 1, randomization tests revealed 12 cases of a within-sample heterozygote deficit (*6-Pgd* in localities 2, 3, 4, 9, and 10; *Est-4* in locality 1; *Aat-1* in locality 9; and *Adh* in localities 1, 2, 4, 9, and 11) and one case of a heterozygote excess (*Aat-1* in locality 2). Out of 154 locus × locality combinations in year 2, randomization tests revealed eight cases of a heterozygote deficit (*Aat-1* in locality 6; *Alb* in locality 11; and *Hb* in localities 2, 5, 8, 9, 10, and 11) and one case of a heterozygote excess (*6-Pgd* in locality 11). However, none of the single-locus F_{IS} values remained significant after correcting for multiple comparisons. Sampling across the latitudinal transect also revealed a slight deficit of heterozygotes within localities (mean $F_{IS} = 0.109$, 95% CI = 0.032–0.164) and among localities

TABLE 1. Patterns of variation at 15 allozyme loci used in the genetic analysis of deer mice from Colorado. In each row: altitudinal transect, year 1, top; altitudinal transect, year 2, middle; latitudinal transect, bottom. Samples sizes for all localities are given in the legend for Figure 1. N_A = number of alleles.

	N_A	H	F_{IS}	F_{IT}	F_{ST}
<i>Alb</i>	3	0.331	0.000	0.166	0.218
	3	0.378	0.022	0.115	0.107
	2	0.306	-0.005	0.012	0.023
<i>Adh-1</i>	2	0.160	0.545	0.558	0.042
	3	0.380	0.136	0.144	0.015
	3	0.092	0.171	0.170	0.007
<i>Aat-1</i>	3	0.503	0.027	0.043	0.006
	3	0.479	-0.066	-0.023	0.040
	2	0.475	0.001	-0.020	-0.022
<i>Aat-2</i>	2	0.006	0.002	-0.002	-0.002
	3	0.019	-0.031	-0.002	0.024
	1	0.000	—	—	—
<i>Est-4</i>	3	0.483	0.064	0.124	0.071
	4	0.567	0.055	0.082	0.028
	3	0.503	0.129	0.159	0.039
<i>α-Gpd</i>	3	0.031	-0.035	-0.003	0.032
	3	0.020	-0.004	-0.006	-0.002
	3	0.026	-0.014	0.004	-0.004
<i>Hb</i>	2	0.504	0.182	0.234	0.096
	2	0.504	0.333	0.363	0.065
<i>Idh</i>	2	0.492	0.114	0.144	0.043
	3	0.073	-0.025	-0.022	0.001
	3	0.050	-0.025	-0.015	0.007
<i>Ldh-1</i>	3	0.033	-0.002	-0.002	0.012
	2	0.003	0.004	-0.001	0.000
	3	0.011	-0.018	-0.001	0.015
<i>Ldh-2</i>	1	0.000	—	—	—
	3	0.019	-0.003	-0.005	0.014
	1	0.000	—	—	—
<i>Mdh-1</i>	3	0.021	-0.002	-0.005	0.002
	2	0.002	0.004	-0.001	0.000
	3	0.012	-0.004	-0.004	0.001
<i>Pgm-1</i>	3	0.033	-0.019	-0.002	0.012
	4	0.034	-0.018	-0.009	0.010
	3	0.088	-0.042	-0.037	0.006
<i>6-Pgd</i>	3	0.073	-0.067	-0.010	0.053
	4	0.643	0.216	0.233	0.016
	5	0.694	-0.021	0.010	0.039
<i>Sdh</i>	4	0.683	0.215	0.226	0.015
	2	0.048	-0.054	-0.015	0.047
	3	0.054	0.108	0.117	0.007
<i>Trf</i>	2	0.033	-0.025	-0.016	0.011
	3	0.246	0.065	0.093	0.008
	3	0.196	-0.047	0.044	0.068
	3	0.249	0.180	0.181	0.016

(mean $F_{IT} = 0.123$, 95% CI = 0.037–0.178). As in the case with samples from the altitudinal transect, no within-locality departures from Hardy-Weinberg proportions remained significant after correcting for multiple comparisons.

Tests for Evidence of Selection

Analysis of the year 1 data revealed an extremely low degree of genetic subdivision across the altitudinal gradient (weighted mean $F_{ST} = 0.059$, $n = 15$ loci). The expected distribution of F_{ST} as a function of H gave a poor fit to the data under both mutation models, since more points fell below the 0.025 quantile than expected by chance (Fig. 2A). The conditional probability distributions were virtually identical under both mutation models, consistent with the sim-

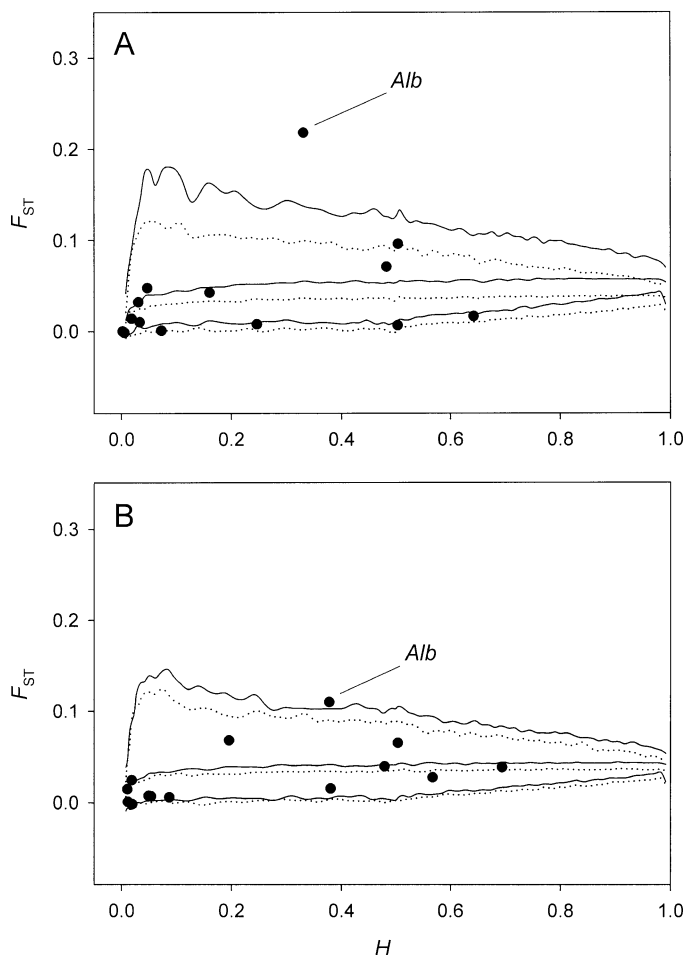


FIG. 2. Estimated F_{ST} values from allozyme loci plotted as a function of heterozygosity in *P. maniculatus* from the altitudinal transect. Results are shown for (A) year 1 and (B) year 2. Lines denote the 0.975, 0.500, and 0.025 quantiles of the conditional distribution. Simulation-based expectations were obtained under the infinite alleles model of mutation. Dotted lines denote quantiles of the distribution that were recalculated after removing *Alb*.

ulation results of Beaumont and Nichols (1996) and Storz and Nachman (2003). Only results obtained under the IAM will be presented in the remainder of the paper. The poor fit to the data was attributable to a single locus (*Alb*) that was characterized by an unusually high level of differentiation relative to the remaining 14 loci (Table 1). The departure from neutral expectations at the *Alb* locus remained statistically significant after correcting for multiple comparisons ($P = 0.0018$; Bonferroni-adjusted α -level = 0.0033). The weighted mean F_{ST} was reduced to 0.039 after removing this outlier, and we used this new average to recalculate the expected neutral distribution. The F_{ST} values for almost all remaining loci fell within the 0.025 and 0.975 quantiles of the recalculated distribution (Fig. 2A).

We observed a similar pattern in the year 2 data. Again, the data revealed an extremely low degree of genetic subdivision across the altitudinal gradient (weighted mean $F_{ST} = 0.045$, $n = 14$ loci). The majority of loci exhibited a remarkably close fit to the expectations of the neutral model

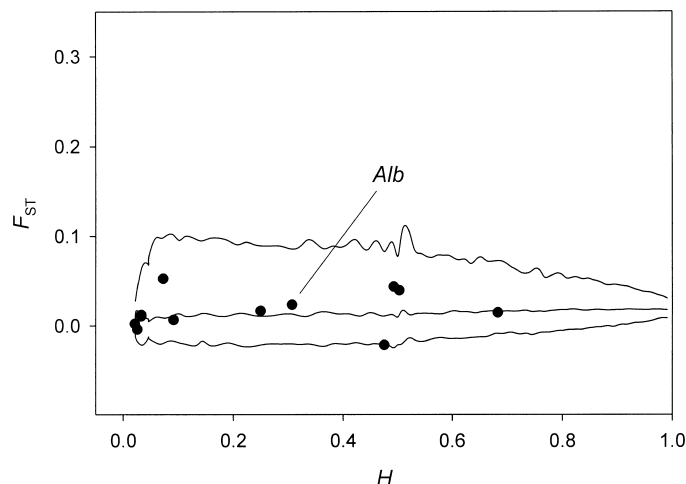


FIG. 3. Estimated F_{ST} values from allozyme loci plotted as a function of heterozygosity in *Peromyscus maniculatus* from the latitudinal transect. Lines denote the 0.975, 0.500, and 0.025 quantiles of the conditional distribution. Simulation-based expectations were obtained under the infinite alleles model of mutation.

(Fig. 2B). The only exception was *Alb*, which was again characterized by the highest F_{ST} value of all loci (Table 1). The deviation from the neutral expectation did not remain statistically significant after correcting for multiple comparisons ($P = 0.0293$; Bonferroni-adjusted α -level = 0.0036). The weighted mean F_{ST} was reduced to 0.038 after removing this outlier, but this adjustment did not dramatically alter the fit of the data to the conditional distribution (Fig. 2B).

As with the altitudinal transects, data from the latitudinal transect revealed an extremely low degree of genetic subdivision (weighted mean $F_{ST} = 0.019$, $n = 13$ loci). In contrast to the altitudinal patterns of variation, data from the latitudinal transect revealed no evidence of selection on *Alb* polymorphism (Fig. 3). All loci closely conformed to expectations of the neutral model.

Tests of Adaptive Divergence across the Altitudinal Gradient

Having determined that the *Alb* locus exhibits a higher-than-expected level of divergence across the altitudinal gradient, we then performed partial Mantel tests to assess whether levels of divergence exhibited a nonrandom pattern with respect to altitude. In both years, F_{ST} for *Alb* ($F_{ST(Alb)}$) exhibited a stronger positive association with altitudinal distance than did the weighted mean F_{ST} for all remaining neutral loci ($F_{ST(neutral)}$; Fig. 4). The partial Mantel test with restricted randomizations revealed a statistically significant association between $F_{ST(Alb)}$ and altitudinal distance in both years. In other words, the increase in pairwise $F_{ST(Alb)}$ as a positive function of pairwise altitudinal distance remained significant even when the effects of neutral divergence (as measured by $F_{ST(neutral)}$) were partialled out. In both years, regression coefficients for both $F_{ST(neutral)}$ and altitudinal distance were significantly greater than zero, and F -values for the extra sum of squares accounted for by adding each var-

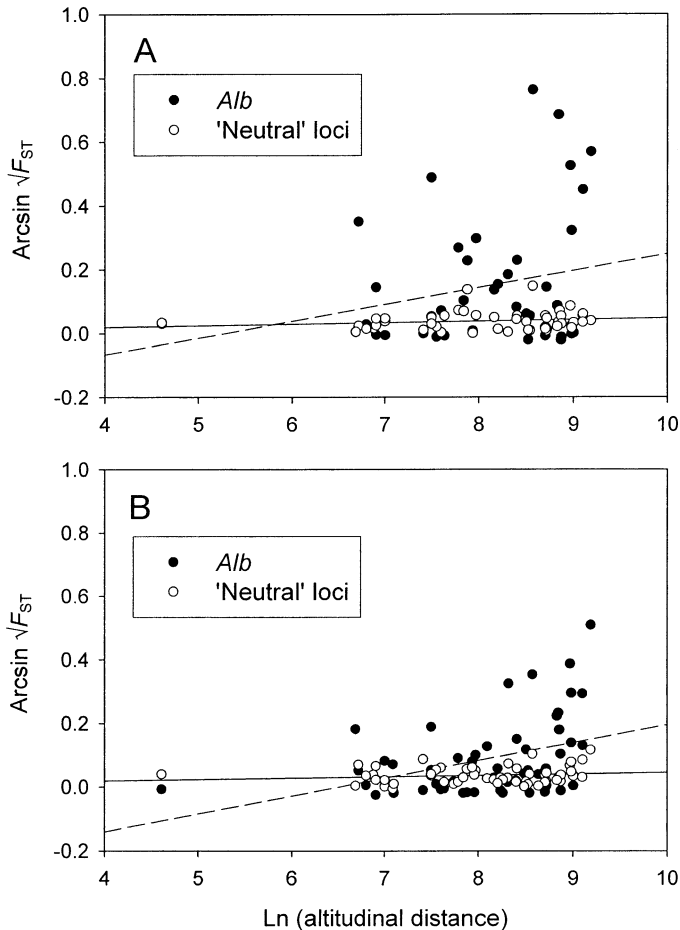


FIG. 4. Linear regression of $\arcsin \sqrt{F_{ST}}$ against \ln (altitudinal distance) for each pairwise combination of sampling localities in (A) year 1 and (B) year 2. The solid line denotes the linear regression line for transformed values of $F_{ST(\text{neutral})}$ versus altitudinal distance, and the dashed line denotes the linear regression line for transformed values of $F_{ST(Alb)}$ versus altitudinal distance.

table into the equation were also significant at an α -level of 0.05 (Table 2).

To test for evidence of autocorrelation in the residuals of the fitted equation, we performed a regression of residual differences between localities (d_{ij}) against pairwise values of $F_{ST(\text{neutral})}$ (n_{ij}). The regressions gave the fitted equations $d_{ij} = 0.206 + 0.171n_{ij}$ for year 1, and $d_{ij} = 0.135 - 0.103n_{ij}$ for year 2. Mantel tests with unrestricted randomizations

showed that the regression coefficients were not significantly greater than zero ($P = 0.583$ and 0.598 in years 1 and 2, respectively), indicating that the positive associations between $F_{ST(Alb)}$ and altitudinal distance cannot be explained as an artifact of spatial autocorrelation.

The striking pattern of spatial differentiation at the *Alb* locus reflects a steep altitudinal cline in *Alb* allele frequencies (Fig. 5A,B). Averaging across both years, the *b* allele shifted from a frequency of 0.359 at the lowest point on the transect (1079 m) to 0.927 at the highest point (4054 m). By contrast, frequencies of this same allele exhibited no systematic pattern of variation across the latitudinal transect (Fig. 5C). None of the other loci exhibited a similar pattern of clinal variation across the altitudinal or latitudinal transects.

A weak pattern of isolation by distance was evident in samples of *P. maniculatus* from the altitudinal transect, as revealed by a positive association between pairwise $F_{ST(\text{neutral})}$ and pairwise geographic distance in both years (Fig. 6A,B). By contrast, there was no evidence for an association between genetic differentiation and geographic distance across the latitudinal transect, which spanned a shorter linear distance (Fig. 6C). If we restrict attention to the range of pairwise distances covered by the latitudinal transect (29.3–124.3 km), the association between neutral differentiation and geographic distance across the altitudinal transect is no longer significant ($P > 0.05$ in both years). Moreover, weighted mean values of $F_{ST(\text{neutral})}$ were not significantly different between samples from the altitudinal and latitudinal transects over this same range of distances. Thus, there does not appear to be any substantial difference in the spatial scale of neutral differentiation across the altitudinal and latitudinal transects.

DISCUSSION

Results of the coalescent simulations indicate that observed levels of divergence at the *Alb* locus reflect a history of diversifying selection across the altitudinal gradient. This conclusion supports the findings of a previous analysis that was based on a subset of the year 1 data (Storz and Nachman 2003), and is further bolstered by two main lines of evidence: (1) *Alb* exhibited a higher-than-expected level of divergence in two consecutive years of sampling across the altitudinal transect (in contrast to the spatial pattern of variation across the latitudinal transect) and (2) levels of divergence at the *Alb* locus were positively associated with altitudinal distance in both years (in contrast to the pattern observed at unlinked loci). Departures from neutrality were highly significant in

TABLE 2. Results of partial Mantel tests with restricted randomizations. The multiple regression model was designed to test for evidence of adaptive divergence across the altitudinal gradient. A matrix of $\arcsin \sqrt{F_{ST(Alb)}}$ was used as the dependent variable, and a matrix of $\arcsin \sqrt{F_{ST(\text{neutral})}}$ was included as an independent variable to control for the effects of neutral genetic divergence. P -values are expressed as the proportion of 10,000 randomizations that yielded values greater than or equal to observed t - or F -values.

	Regression coefficient	Estimate	t^1	Extra SS	F^2
Year 1	$\arcsin \sqrt{F_{ST(\text{neutral})}}$	$b_1 = 2.804$	3.16 ($P < 0.01$)	0.371	11.87 ($P < 0.01$)
	\ln (altitudinal distance)	$b_2 = 0.042$	1.45 ($P < 0.05$)	0.066	2.10 ($P < 0.05$)
Year 2	$\arcsin \sqrt{F_{ST(\text{neutral})}}$	$b_1 = 1.892$	3.68 ($P < 0.01$)	0.167	16.80 ($P < 0.01$)
	\ln (altitudinal distance)	$b_2 = 0.052$	3.10 ($P < 0.01$)	0.096	9.59 ($P < 0.01$)

¹ Associated P -values are for one-sided tests.

² F -values are for the extra sum of squares accounted for by adding each variable into the equation.

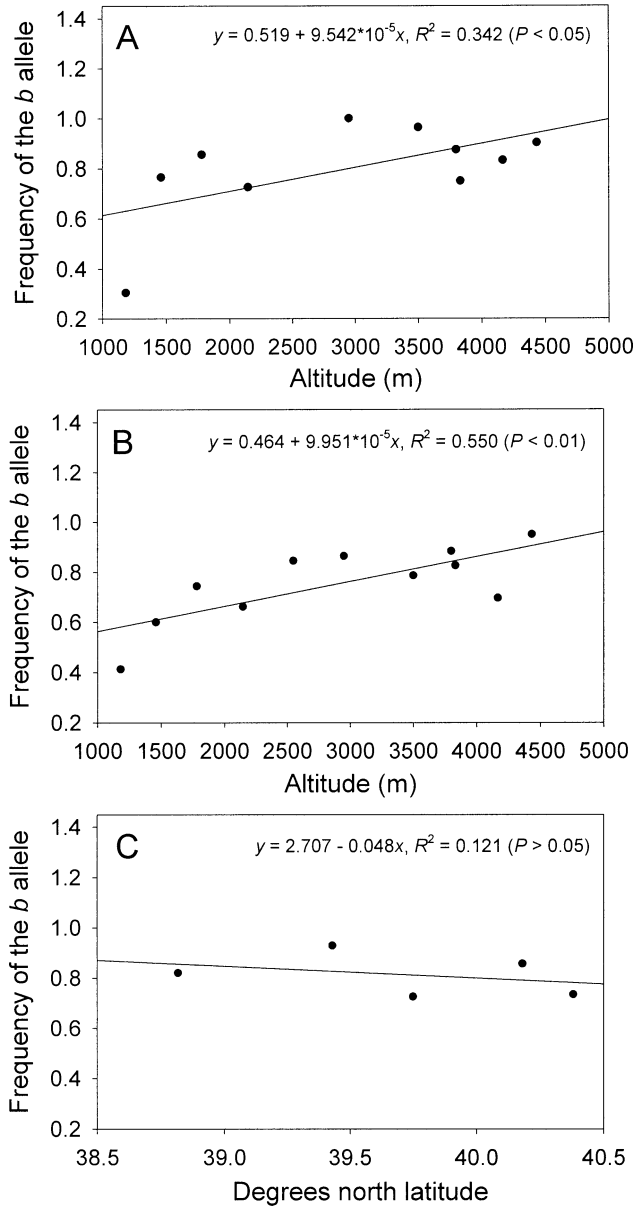


FIG. 5. Linear regression of *Alb* allele frequency against altitude (m) in (A) year 1 and (B) year 2, and regression of *Alb* allele frequency against latitude (C). *P*-values are one-tailed.

year 1, and marginally significant in year 2 (after correcting for multiple comparisons), and the positive association between locus-specific divergence and altitudinal distance was highly significant in both years, even after controlling for the effects of neutral divergence. Taken together, these two lines of evidence strongly suggest that diversifying selection on the *Alb* locus is mediated by some environmental factor, such as ambient temperature or oxygen tension, that covaries with altitude.

The contrasting pattern of altitudinal divergence between *Alb* and the remaining loci indicates that diversifying selection has driven adaptive divergence in the face of extremely high levels of gene flow. All of the putatively neutral loci

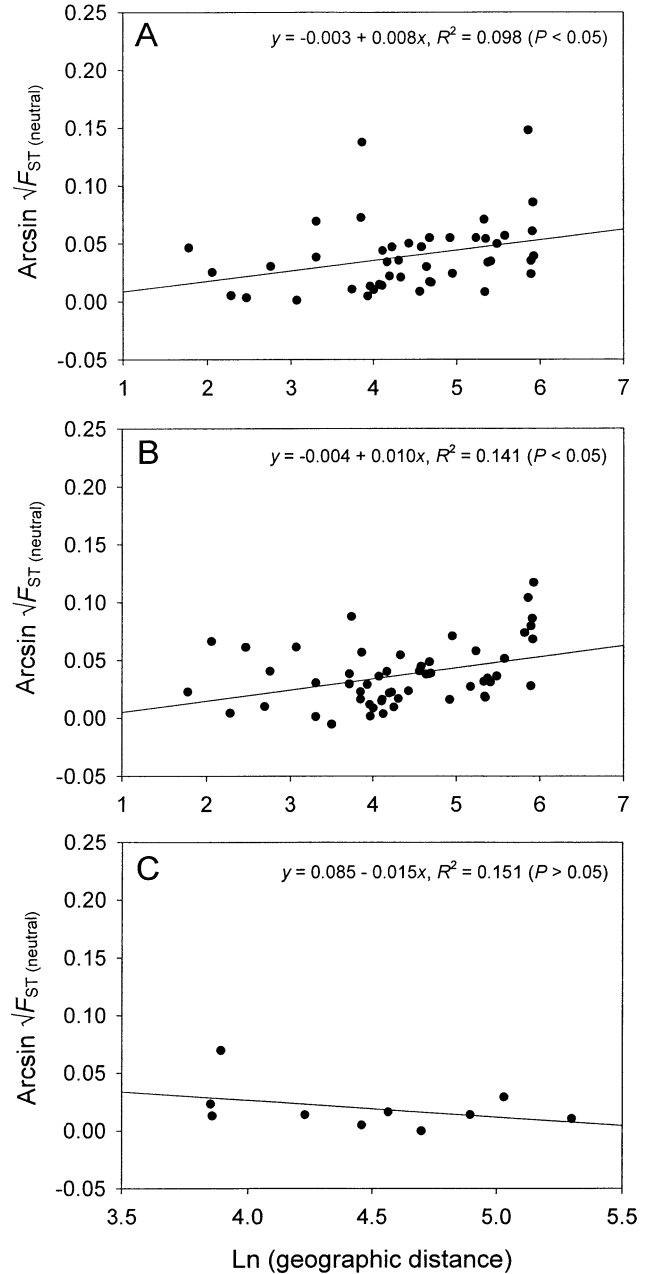


FIG. 6. Linear regression of $\arcsin \sqrt{F_{ST(\text{neutral})}}$ against \ln (geographic distance) for each pairwise combination of sampling localities across the altitudinal transect (year 1, A; year 2, B) and across the latitudinal transect (C). *P*-values are from one-tailed randomization tests.

exhibited uniformly low F_{ST} values (Table 1). Using Wright's (1943) infinite-island approximation, $F_{ST} = 1/(1 + 4N_e m)$, the weighted mean values of $F_{ST(\text{neutral})}$ for year 1 (0.039) and year 2 (0.038) translate into estimates of $N_e m \approx 6.16$ and 6.33 migrants/generation, respectively (where $N_e m$ is the migration rate scaled to local effective size). Kimura and Weiss (1964) showed that when $N_e m > 4$, the homogenizing effect of gene flow is sufficient to prevent stochastic differentiation of allele frequencies, such that the regional array of subpopulations is effectively panmictic. Under such con-

ditions, local adaptation may be constrained by high levels of gene flow that produce a spatial averaging of fitness variation among different habitats (García-Ramos and Kirkpatrick 1997; Lenormand 2002). We conclude that clinal variation at the *Alb* locus reflects a balance between gene flow and diversifying selection that results from elevational changes in fitness rankings among alternative genotypes.

All available evidence indicates that *P. maniculatus* is continuously distributed across the altitudinal gradient that we surveyed. With the exception of *Alb*, all loci exhibited a remarkably close fit to the expectations of the neutral model (Fig. 2). If our transect had spanned a zone of secondary contact, the effects of admixture would have been readily detectable. Specifically, we would have seen systematic departures from Hardy-Weinberg genotypic proportions in addition to a bimodal distribution of pairwise F_{ST} values (corresponding to comparisons within and between samples from opposite sides of the contact zone). Admixture would also be expected to produce pervasive linkage disequilibria among loci that have different allele frequencies in the separate source populations. None of these patterns were evident. No cases of allelic or genotypic disequilibria remained significant after correcting for multiple comparisons. Moreover, the pattern of isolation by distance across the altitudinal gradient (Fig. 6A,B) indicates that the regional population of *P. maniculatus* has attained migration-drift equilibrium under extremely high levels of gene flow. The fact that all loci other than *Alb* conformed so closely to the null expectations indicates that the simulation model provides a reasonable approximation to the population structure of *P. maniculatus* over the spatial scale considered in this study.

In addition to the effects of selection on the distribution of *Alb* allele frequencies, patterns of variation will also be influenced by the same demographic processes that affect every other gene in the genome. Thus, positive associations between $F_{ST(Alb)}$ and $F_{ST(neutral)}$ in both years (Table 2) are not surprising given the high levels of gene flow.

In allozyme surveys of mice in the genus *Peromyscus*, *Alb* consistently emerges as one of the most highly differentiated loci, and statistically significant departures from neutral expectations have been documented in three other congeners of *P. maniculatus* (*P. gossypinus*, *P. leucopus*, and *P. polionotus*; Storz and Nachman 2003). It thus seems clear that *Alb* (or a closely linked locus) is subject to diversifying selection in multiple species of *Peromyscus*. However, since *P. gossypinus*, *P. leucopus*, and *P. polionotus* do not have very broad altitudinal distributions, the agent of selection in these species is probably not directly related to altitude.

The *Alb* data from the altitudinal and latitudinal transects illustrate how the same locus can exhibit both neutral and non-neutral patterns of variation depending on the environmental context. A similar situation has been documented for the glucose phosphate isomerase locus (*Gpi*) in populations of the northern acorn barnacle (*Semibalanus balanoides*) on the east coast of North America. *Gpi* genotypes in these barnacles are subject to fitness variation between high- and low-tide microhabitats in some coastal regions but not in others, possibly due to regional variation in the interaction between thermal stress and availability of dietary carbohydrates (Rand et al. 2002). In species like *P. maniculatus* that are distributed

across ecologically heterogeneous landscapes, allelic variation that is essentially neutral under most conditions may have hidden adaptive potential that is only exploited in certain environmental circumstances.

It is also possible that the apparent evidence for selection on *Alb* polymorphism is actually attributable to the indirect effects of selection at a closely linked locus. For example, a genetic study of European rat populations (Kohn et al. 2000) revealed that positive selection on *Rw*, a gene that confers rodenticide resistance, induced a high degree of linkage disequilibrium across about 32cM (14%) of rat chromosome 1. Thus, selection at a single locus may have affected patterns of variability at a multitude of linked sites within this chromosomal region (Kohn et al. 2000, 2003). *Alb* is a member of a multigene family consisting of three other genes that encode serum transport proteins: α -fetoprotein, α -albumin, and vitamin D binding protein. In rodents, the genes for albumin, α -fetoprotein, and α -albumin are closely linked within a 100-kb block that is syntenic with the region centered on map position 50 cM of *Mus* chromosome 5. The gene for the vitamin D binding protein is situated further downstream at map position 44 cM (Mouse Genome Informatics, available at <http://www.informatics.jax.org>). Even if the observed patterns of variability at the *Alb* locus reflect the indirect effects of selection at linked sites, closely linked members of the serum albumin gene family may be the most likely candidates for being the actual target of selection. If selection is sorting variation at a linked locus, then the expected level of differentiation at the unobserved polymorphism would be even higher than that observed at *Alb*, because the covariance in allele frequencies between the two loci would be dissipated at a rate proportional to the recombinational distance between them (Stephan and Mitchell 1992; Stephan 1994).

It is not clear what environmental factors may be mediating selection on the *Alb* locus in *P. maniculatus*. In mammals, albumin is a highly abundant serum protein that plays an important role in maintaining biochemical equilibria in the bloodstream and extravascular fluids. Albumin supplies most of the acid/base buffering action of the plasma proteins (Figge et al. 1991) and it binds and transports a wide diversity of endogenous anions, particularly bilirubin, hemein, and long-chain fatty acids that are critical intermediates in lipid metabolism (Peters 1996). Albumin also binds nitric oxide (NO), an oxidizing gas with vasodilatory, antiplatelet, and neurotransmitting properties. Of the total NO in blood plasma, over 80% is carried as *S*-nitrosoalbumin (Keaney et al. 1993). The NO-binding properties of the protein suggest the possibility that high-altitude protein variants may mediate a vasodilatory response to hypoxic conditions. DNA sequence data may permit some inferences about functional differences among the allelic variants that predominate at different altitudes, but biochemical and/or physiological experiments will ultimately be required to determine the mechanistic basis of fitness differences among alternative *Alb* genotypes. By integrating surveys of geographic variation with detailed molecular-functional analyses, the study of allozyme variation can provide much insight into genetic mechanisms of physiological adaptation (Watt 1991; Mitton 1997; Eanes 1999; Watt and Dean 2000; Schmidt and Rand 2001).

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Corresponding Editor: R. Harrison