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ALPHA-CHAIN HEMOGLOBIN POLYMORPHISMS ARE CORRELATED WITH ALTITUDE IN THE DEER MOUSE, *PEROMYSCUS MANICULATUS*

LEE R. G. SNYDER, JACK P. HAYES,[1] AND MARK A. CHAPPELL
Department of Biology, University of California, Riverside, CA 92521 and White Mountain Research Station, Bishop, CA 93514

Abstract.—In deer mouse (*Peromyscus maniculatus*) populations in the western United States, alpha-globin haplotype frequency, beta-globin haplotype frequency, and base-line blood oxygen affinity (measured after acclimation to low altitude) show strong correlations with native altitude. The correlations improve when an average regional altitude is substituted for the local altitude at collection sites. This substitution roughly compensates for the effects of gene exchange between populations in areas of highly variable topography. When subspecific effects are removed with covariate analyses a significant ($P < 0.05$) relationship remains only for alpha-globin haplotype frequency and altitude. Thus, alpha-globin haplotype frequency, beta-globin haplotype frequency, and base-line blood oxygen affinity may be explained by either subspecific or altitudinal effects, but subspecific effects explain a larger proportion of the variance. Part of the subspecific effect may be attributable to an underlying relationship of subspecies with altitude. The analyses for the alpha-globins in conjunction with other data on the effects of alpha-globins on blood oxygen affinity and whole-animal physiological performance are consistent with the hypothesis that the frequency of the alpha-globins evolved in response to selection resulting from the stress of high-altitude hypoxia.

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The deer mouse (*Peromyscus maniculatus*) has one of the most complex and extensive hemoglobin polymorphisms of any mammal (Snyder, 1978*a*, 1978*b*, 1980*a*). It also inhabits one of the broadest ranges of altitude of any North American mammal. Within Inyo County, California, a single subspecies (*P. m. sonoriensis*) ranges from below sea level to above 4,300 m in the Sierra Nevada and White Mountains (Dunmire, 1960). An obvious question is whether the hemoglobin polymorphisms result from natural selection on the oxygen-transport system. One approach to the question is to examine the altitudinal distribution of different polymorphisms.

Preliminary data on alpha-chain haplotype frequency indicated a strong correlation with native altitude (Snyder, 1981). Alpha-chain polymorphisms affect both blood oxygen affinity and maximal aerobic metabolic rate (Chappell and Snyder, 1984; Chappell et al., 1988), so it is not surprising that blood oxygen affinity is also correlated with native altitude (Snyder et al., 1982; Snyder, 1985). Despite this evidence, the correlations of haplotype frequency with altitude are somewhat puzzling because they are significant only when the data are pooled across subspecies. Within any subspecies (even those whose distributions encompass considerable altitudinal variation), the correlation is not significant (Snyder, 1981, 1985; Snyder et al., 1982). Slatkin (1973) has shown that the response of populations to changes in environmental conditions can only occur over a spatial scale greater than the dispersal distance divided by the square root of the strength of selection. Accordingly, Snyder (1981) suggested that the lack of significant correlation within subspecies may result from gene flow between populations along steep altitudinal gradients.

This paper presents new data and reexamines the relationships of blood oxygen affinity, alpha-chain haplotype frequency, and beta-globin genotype frequency with altitude for deer mice from a wide range of native altitudes. The present study differs from preliminary studies in that we use analysis of covariance to partition subspecific effects from altitudinal effects.

MATERIALS AND METHODS

Collection Sites for Peromyscus maniculatus.—Population samples of deer mice

[1] Address correspondence to Jack P. Hayes, Department of Biology, University of California, Riverside, CA 92521.

TABLE 1. Summary of collection sites, sample sizes, altitudes, hemoglobin-haplotype class frequencies, and $P_{50,7.4}$ in western subspecies of *Peromyscus maniculatus*. Subspecies symbols are as follows: AR = *artemisiae*, AU = *austerus*, BA = *bairdii*, GA = *gambelii*, LU = *luteus*, NE = *nebrascensis*, RB = *rubidus*, RF = *rufinus*, SE = *serratus*, and SO = *sonoriensis*. See text for explanations of regional altitude and haplotype classes. $P_{50,7.4}$ = the partial pressure of oxygen necessary for 50% hemoglobin saturation at pH 7.4.

Sub-species	Collection site (county, state)	2N	Local altitude	Regional altitude		Frequency		$P_{50,7.4}$
				20 km	50 km	a^1c^1	Hbd^1	
AR	Cascade, MT	50	1,465	1,607	1,523	1.000	0.020	33.40
AU	Thurston, WA	28	6	63	145	1.000	0.000	36.29
	Lewis, WA	88	150	236	294	1.000	0.000	—
BA	Beadle, SD	54	425	389	411	1.000	0.037	35.24
GA	Riverside, CA	28	410	601	466	1.000	0.000	36.33
	Amador, CA	38	550	600	664	0.947	0.000	—
	Riverside, CA	18	365	430	670	1.000	0.000	35.85
	Trinity, CA	14	550	921	972	1.000	0.000	—
	Riverside, CA	36	2,255	1,188	1,049	1.000	0.028	36.18
	Kittitas, WA	20	610	973	1,089	1.000	0.000	—
	Los Angeles, CA	92	1,570	1,592	1,193	0.989	0.065	36.69
	Tulare, CA	20	2,330	1,956	1,896	0.950	0.000	—
LU	Phelps, NE	68	685	693	695	0.985	0.059	35.48
NE	Mesa, CO	92	1,355	1,577	1,795	0.272	0.250	33.52
	Larimer, CO	36	1,830	1,849	1,972	0.472	0.056	32.50
	Carbon, WY	116	2,195	2,150	2,094	0.647	0.086	33.40
	Fremont, WY	22	2,865	2,675	2,416	0.591	0.045	—
	Sublette, WY	30	2,440	2,960	2,743	0.500	0.067	—
RB	Curry, OR	44	3	306	364	1.000	0.000	36.75
	Humboldt, CA	28	3	273	418	1.000	0.000	36.90
RF	Wayne, UT	96	2,680	2,339	1,983	0.250	0.083	32.95
	Coconino, AZ	26	2,895	2,416	2,129	0.192	0.038	31.54
	Wasatch, UT	70	2,560	2,295	2,172	0.429	0.290	—
	Mesa, CO	58	2,990	2,553	2,225	0.155	0.207	30.58
	Senpete, UT	60	2,925	2,713	2,315	0.317	0.050	32.96
	Montezuma, CO	18	2,375	2,485	2,377	0.111	0.222	31.42
	Garfield, CO	124	2,513	2,604	2,538	0.298	0.089	—
	Summit, UT	18	3,110	2,950	2,682	0.500	0.000	—
	Boulder, CO	26	3,050	2,963	2,735	0.231	0.154	—
	Gunnison, CO	124	2,320	2,594	2,790	0.113	0.065	31.64
	Gunnison, CO	76	2,895	3,294	3,044	0.105	0.079	31.76
	Clear Creek, CO	66	3,980	3,303	3,064	0.167	0.197	—
	San Miguel, CO	62	2,895	3,300	3,146	0.097	0.048	31.28
	Lake, CO	66	3,363	3,380	3,180	0.091	0.106	—
	Hinsdale, CO	26	3,415	3,316	3,211	0.115	0.038	—
SE	Custer, ID	24	2,285	2,375	2,294	1.000	0.000	—
SO	Lincoln, ID	106	1,175	1,271	1,302	0.849	0.019	34.21
	Nevada, CA	70	1,980	2,033	1,906	0.657	0.000	—
	Inyo, CA	222	1,535	1,895	2,179	0.617	0.018	32.10
	Mono, CA	244	3,448	2,446	2,182	0.505	0.025	32.11
	Kane, UT	140	2,805	2,598	2,264	0.436	0.014	33.23
	Mono, CA	16	1,980	2,301	2,421	0.625	0.063	34.53

representing ten nominal subspecies were obtained from 42 sites in the central and western United States (Table 1). Mean sample size was 31.7 mice (range: 7–122). Identification of subspecies was based on the range maps given by Hall (1981), except for several southern California populations, which were judged to be subspecies *gambelii* instead of *sonoriensis* on the basis of external morphology and the population arrays of hemoglobin phenotypes.

Calculation of Average Regional Altitudes.—To compensate for gene exchange, two "average regional altitudes" were cal-

culated for each collection site. A grid shaped like a spoked wheel was centered over each collection site on a 1:250,000-scale topographic map. Along 12 radii equally spaced at 30° arcs, the altitude was determined (to the nearest 15 m) at 5-km intervals out to a point 50 km from the collection site. The 20-km regional altitude was based on all points out to 20 km, and the 50-km regional altitude was based on all points out to 50 km. The rationale for the shape of the grid was that areas progressively more distant from the collection site were sampled more sparsely per unit area and hence contributed progressively less to the calculated regional altitude. In similar fashion, the magnitude of gene flow would presumably decline progressively with distance from the sample site.

We used 20 and 50 km radii for calculating regional altitudes for the following reasons. First, earlier analyses showed that correlations with altitude improved with increasing size of regional altitude, suggesting that larger regional altitudes may better describe the evolutionary forces encountered by the mice. Second, our experience indicates that mice are capable of travelling substantial distances (>0.5 km) in a single evening. Thus, it appears likely that during the time selection would be operating mice could move considerable distances. Hence, use of local altitudes or regional altitudes with smaller radii may not be representative of the conditions under which gene frequencies and physiological performance are evolving.

Defining Samples.—For some collection sites, there was overlap in the areas used to calculate 20-km regional altitudes. Accordingly, data were averaged for samples less than 40 km apart, and they were treated as single samples in subsequent analyses. However, we did not lump two samples of subspecies *sonoriensis* that were 32 km apart, because their local altitudes differed by almost 2,000 m.

Determination of Hemoglobin Genotypes.—Alpha-globin genotypes were determined for 1,330 wild-caught deer mice and 969 progeny from 108 laboratory matings between known genotypes, including matings to distinguish alpha- and beta-globin variants on the basis of comparative genetic linkages (Snyder, 1980*b*). The genotypes were determined by thin-layer isoelectric focusing (IEF), as described by Snyder (1980*a*).

In most adult deer mice, the alpha-globins are coded by two tightly linked loci (*Hba* and *Hbc*), which produce subunits in a ratio of approximately 2:1 (Snyder, 1980*a*). In phenotypes involving only alpha-globin variation, the hemoglobin bands fall into three zones on a pH 6–8 IEF gel. Relatively fainter bands in the more anodal zone are attributed to the c^1 class of alleles at the *Hbc* locus; relatively dense bands in the more cathodal zone represent the a^1 class of alleles at the *Hba* locus. Relatively faint versus dense bands in the middle zone are designated as the a^0 and c^0 allele classes, respectively. At least three a^1, three a^0, eight c^1, and four c^0 alleles are distinguishable. Importantly, there is very strong linkage disequilibrium, such that alleles of like superscript are almost always associated. Therefore, it is possible to estimate haplotype frequencies quite accurately from diploid genotypes, even though only a small fraction of the mice can be directly tested for haplotype composition via progeny testing. "Recombinant" haplotypes (i.e., either a^1c^0 or a^0c^1) are rare; their average population frequency is estimated to be 0.015, with a maximum of 0.107. Hence the alpha-globin variation can be conveniently summarized in terms of a single haplotype-class frequency [since $\text{freq}(a^1c^1) \cong 1 - \text{freq}(a^0c^0)$].

Essentially all beta-globin variation in deer mouse populations stems from alleles at the *Hbd* locus, which accounts for about 12% (per allele) of total beta-globin subunit production. We scored beta-globin haplotypes either as d^0 (the common, monomorphic haplotype) or d^1 (a heterogeneous class of haplotypes, including at least four distinct *Hbd* alleles).

Determination of Whole-Blood Oxygen Affinity.—We reanalyzed the blood oxygen affinity data of Snyder (1985) to separate the effects of subspecies from the effects of altitude. The original data were obtained for mice allowed to acclimate to low altitude (340 m) for periods of 3–30 months. The datum for each population was obtained on a blood sample pooled from 10–13 mice, chosen so that their array of hemoglobin genotypes was representative of the popu-

lation as a whole. The partial pressure of oxygen necessary for 50% hemoglobin saturation at pH 7.4 (i.e., $P_{50,7.4}$) was determined by mixing equal volumes of fully oxygenated and deoxygenated blood (Edwards and Martin, 1966). Complete procedural details are given in Snyder et al. (1982).

Statistical Analyses.—Standard least-squares linear regressions were used to examine the relationship between $P_{50,7.4}$ and various measures of altitude in the nine subspecies for which we have $P_{50,7.4}$ data. Simple regressions may give inflated indications of the association of $P_{50,7.4}$ and hemoglobin gene frequencies with altitude, because the subspecies tend to occur over different altitudinal ranges. As a result, significant regressions may be due to differences among subspecies instead of differences among altitudes. Analyses of covariance (ANCOVA) were used to remove subspecific effects in order to avoid this problem. Thus, subspecific effects were entered into the ANCOVA first, and altitude was entered second, allowing an evaluation of the significance of altitude after accounting for the differences due to subspecies. Analogous ANCOVAs were performed with altitude entered into the model first and subspecies second to evaluate the relative importance of altitude and subspecies. The ANCOVA were performed using only the data for subspecies *gambelii, nebrascensis, rufinus,* and *sonoriensis,* because we lacked sufficient data for other subspecies.

The raw hemoglobin gene frequencies showed deviations from normality and/or heteroscedasticity. Angular transformations (arcsine[square root]), with analyses weighted by sample size should normalize the variances of binomial data with unequal sample sizes (Snedecor and Cochran, 1967 p. 327). However, unweighted analyses with simple arcsine rather than angular transformations proved most satisfactory for the alpha-globin data. The analyses for alpha-globin frequency and local altitude and for alpha-globin frequency and 20-km regional altitude indicated a borderline significant heterogeneity of slopes necessitating the removal of subspecies *nebrascensis* for these analyses. Analyses weighted by sample size with angular transformations improved the residual pattern for the beta-globin analyses, but there were still problems with deviations from normality and heteroscedasticity. For these data, a weighted analysis using the arcsine(cube root)-transformation proved satisfactory.

The ANCOVAs presented here are more powerful tests of the relationship with altitude than examining the correlations within subspecies individually. Testing subspecies individually reduces the sample sizes too much to detect weak correlations. In addition, some subspecies such as *gambelii* do not occur at altitudes that typically result in physiological adjustments (i.e., altitudinal acclimatization) by this species or other mammals. Therefore, one would not expect to find any relationship of hemoglobin frequency or $P_{50,7.4}$ with altitude within those subspecies.

Results

Correlation of $P_{50,7.4}$ with Local and Regional Altitudes.—$P_{50,7.4}$ decreased with increasing altitude. A simple regression for all nine subspecies for which data were available indicated a highly significant negative correlation between $P_{50,7.4}$ and local altitude ($r^2 = 0.646$, $F_{[1, 24]} = 43.88$, $P < 0.0001$). The regression involving 20-km regional altitude showed a greater correlation ($r^2 = 0.738$, $F_{[1, 24]} = 76.65$, $P < 0.0001$), and the 50-km regional altitude showed the highest correlation ($r^2 = 0.774$, $F_{[1, 24]} = 82.22$, $P < 0.0001$).

The ANCOVA indicated that none of the measures of altitude was significantly correlated with $P_{50,7.4}$ ($P > 0.05$) after accounting for subspecific effects, but the altitude indices were always statistically significant ($P < 0.0001$) if they were entered into the model first. Subspecies effects were always significant ($P < 0.05$) regardless of their order of entry into the analysis. Overall, the two variables explained about 80% of the variance in the data.

Alpha-Globin Haplotype Frequencies and Altitude.—The a^1c^1 haplotype frequencies decreased with increasing altitude (Fig. 1). The correlation of alpha-globin frequency with both local altitude and 20-km regional altitude showed borderline significant heterogeneity of slopes with the slope for sub-

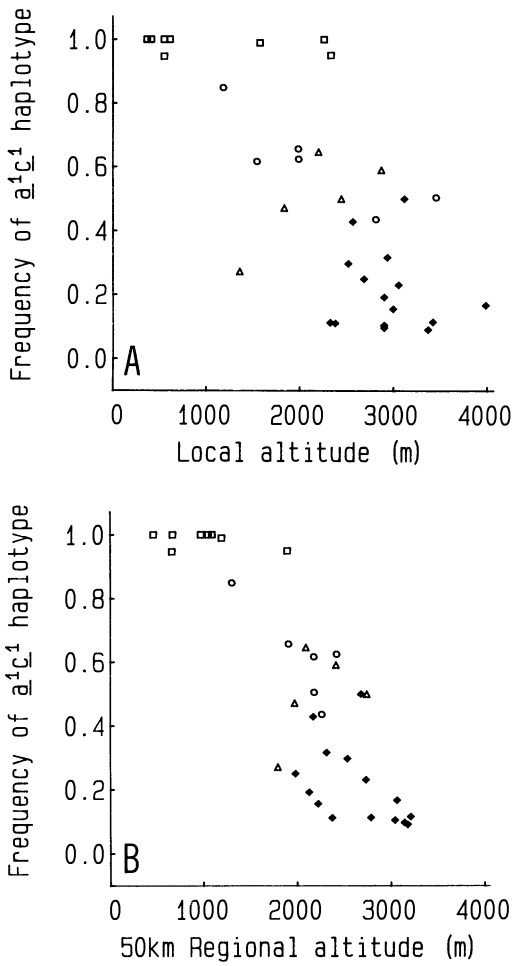

FIG. 1. Frequency of a^1c^1 alpha-globin haplotypes versus A) local or B) 50-km regional altitude in 34 samples. Open squares are *gambelii,* open triangles are *nebrascensis,* open circles are *sonoriensis,* and filled diamonds are *rufinus.* Data for (A) are taken in part from Snyder (1981).

species *nebrascensis* most different from the other slopes. With subspecies *nebrascensis* deleted from the model, local altitude was significantly correlated with alpha-globin haplotype frequency ($F_{[1, 25]} = 5.28$, $P = 0.0302$). The relationship for 20-km regional altitude and alpha-globin frequency was even stronger ($F_{[1, 25]} = 10.84$, $P = 0.0030$). The analysis for 50-km regional altitude (again using all subspecies) was also statistically significant after the removal of subspecific effects ($F_{[1, 29]} = 5.80$, $P = 0.0226$). When the three altitude indices were entered into the model first, all three explained an even larger part of the variation. As with $P_{50,7.4}$, subspecific effects were always significant ($P < 0.05$) regardless of their order of entry into the model. All of the alpha-globin analyses explained 90–95% of the total variance in the data.

Beta-Globin Genotype Frequencies and Altitude.—After removing subspecific effects, none of the measures of altitude showed a significant correlation with beta-globin genotype frequency. However, when entered into the model first, each altitude index did explain a significant ($P < 0.05$) part of the variation. Subspecific effects were significant when entered first or second into the model. In general, the analyses for the beta-globins explained a much smaller portion of the variance in the data (about 50%) than did the alpha-globins analyses.

DISCUSSION

The better fit of the models using regional instead of local altitude suggests that there is substantial gene flow. However, which regional altitude is most appropriate for describing the evolutionary pressures on alpha-globin frequency, beta-globin frequency, and $P_{50,7.4}$ of a particular population is problematic. Compared to local altitudes, regional altitudes presumably reflect more accurately the altitude over which the progenitors evolved, because they better incorporate the influences of selection, gene flow, and drift. However, the appropriate regional altitude will probably vary from population to population, and any regional altitude we define will necessarily be somewhat arbitrary.

The analyses for $P_{50,7.4}$, alpha-globin haplotype, and beta-globin genotype all indicate a significant relationship with altitude before accounting for subspecific effects. Only alpha-globin haplotype frequencies show a significant altitudinal correlation after accounting for subspecies. The proportion of variance explained by subspecific effects is larger than that explained by altitude, and subspecific effects are always significant even when they are entered into the model last. Thus, subspecific effects are clearly important correlates of $P_{50,7.4}$ and beta-globin genotype frequency, while altitude may or

may not be. Both regional altitude and subspecies are significant correlates with alpha-globin haplotype frequency. It is conceivable (even likely) that some of the explanatory power of subspecies is due to altitude-related selection during the differentiation of the various subspecies.

As in many studies examining the relationship of a trait with an environmental variable (Felsenstein, 1985), the statistical analyses presented in this paper must be viewed with caution. We treated samples of mice as independent. Several scenarios, including drift with isolation by distance, may result in correlated deviations of the gene frequencies of our samples. An analysis on such data would then assume more degrees of freedom than are appropriate, and the significance levels of the regressions would be erroneously exaggerated. We attempted to counter this potential problem by lumping samples that were geographically close (i.e., less than 40 km apart). To deal with this more rigorously, we would need detailed information on current and historical gene flow over large geographic areas. We acknowledge the potential and intractable problem (Slatkin, 1987) with the significance levels of our analyses. We also note that, while gene flow may produce accidental correlations under some scenarios, positive and negative correlations would be equally likely (i.e., the direction of the accidental correlations should vary randomly between subspecies). This was not true for the majority of the analyses we conducted.

The significant correlations with altitude suggest that there may be altitude-related selection on alpha-globins, but, as with other studies of the causes of clinal variation in gene frequencies (Powers et al., 1986 [and references therein]), the correlations alone are not compelling. Below we discuss a variety of supporting evidence that, in conjunction with the correlations, strongly suggests that the alpha-globin polymorphism results at least in part from differential selection over altitude.

Hypoxia and Hemoglobin Evolution.—Snyder (1981) proposed that selection exerted by different O_2 partial pressures at different altitudes is an important determinant of hemoglobin haplotype frequencies in deer mice. This hypothesis rests on the assumption that hemoglobin haplotypes influence the fitness of deer mice and that the fitness of the haplotypes varies with altitude.

Alpha-globin haplotypes detected by IEF have a significant and consistent effect on P_{50}. We have tested for an alpha-globin effect in laboratory lines of deer mice that carry either an a^1c^1 or an a^0c^0 haplotype in identical-by-descent condition on an otherwise outbred, unselected genetic background from the original population (Snyder, 1981; Chappell and Snyder, 1984). Thus far, in all 16 lines tested, the ranking of genotypes according to $P_{50,7.4}$ has been $a^1c^1/a^1c^1 > a^1c^1/a^0c^0 > a^0c^0/a^0c^0$ (Chappell and Snyder, 1984; Chappell et al., 1988). In the present study, there was no significant correlation of $P_{50,7.4}$ with altitude after accounting for subspecies effects. Nevertheless the covariance of subspecies with alpha-globin haplotypes, combined with the clear effect of the haplotype on $P_{50,7.4}$, suggests that altitude may be an important determinant of baseline $P_{50,7.4}$. The analyses also make it clear that factors other than altitude are influencing $P_{50,7.4}$.

The adaptive significance of different P_{50}'s and the optimal characteristics for hemoglobin at high altitude are controversial topics (Snyder et al., 1982; Snyder, 1985). Several theoretical models of blood gas transport (Turek et al., 1973; Shappell and Lenfant, 1975; Bencowitz et al., 1982) predict that, relative to the sea level value, in situ P_{50} (i.e., P_{50} measured at the native altitude) should increase at moderately high altitudes, then decline at higher altitudes. Unfortunately, for any given species, it is difficult to specify the "inversion altitude" (i.e., the altitude above which a decrease rather than an increase in P_{50} becomes advantageous).

How well the P_{50}'s of deer mice conform to theoretical predictions is not clear. The theoretical models all apply to in situ P_{50} whereas the data reported here are base-line P_{50}'s. In deer mice native to a variety of altitudes but acclimated to either low (340 m) or high (3,800 m) altitude, $P_{50,7.4}$ changes only negligibly upon acclimation (Snyder, 1985). In other words, at those altitudinal extremes deer mice conform to the prediction that in situ P_{50} should be lower at high altitude. We do not now whether in situ P_{50}

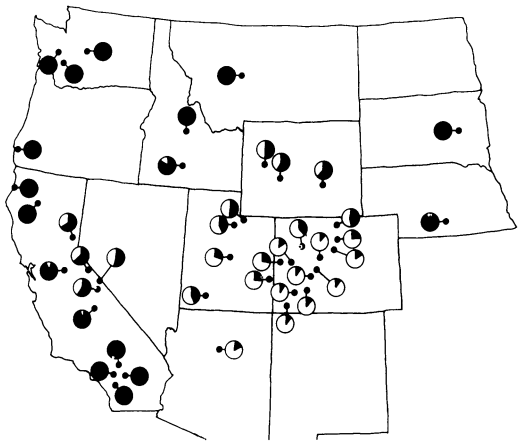

FIG. 2. Frequency of a^1c^1 haplotypes (black segments).

increases at intermediate altitudes, as the models predict.

More importantly, Chappell and Snyder (1984) and Chappell et al. (1988) have obtained direct evidence that the alpha-globin haplotypes result in enhanced physiological performance at native altitude. Matched groups of mice from both identical-by-descent laboratory lines and population samples were tested for maximal oxygen consumption ($\dot{V}O_2max$) during intense exercise or during acute cold exposure after acclimation to 340 m or 3,800 m. There was a clear a priori prediction: since a higher rate of oxygen consumption is presumably advantageous during exercise or cold exposure, mice carrying the "low-altitude-adapted" genotype (a^1c^1/a^1c^1) should have the highest $\dot{V}O_2max$ at low altitude, whereas mice with the "high-altitude-adapted" genotype (a^0c^0/a^0c^0) should have higher $\dot{V}O_2max$ at high altitude. Heterozygotes should generally be intermediate. The combined results were highly statistically significant and matched the a priori predictions (Chappell and Snyder, 1984). Deer mice in their natural environments may be subjected at least occasionally to exercise stress, and those at high altitude are almost certainly subjected to cold stress, which suggests that alpha-globin haplotypes undergo differential selection as a function of altitude.

As previously suggested, gene flow may limit differentiation along localized altitudinal gradients. If that supposition is correct, than all aspects of the data can be rationalized by considering the average altitude (and hence the average selective regime) over broader geographic areas. Subspecies *artemisiae, austerus, bairdii, gambelii, luteus,* and *rubidus* inhabit regions of relatively low altitude (mean 50-km regional altitude of the sample sites = 790 m) with relatively low variation in topographic range. The a^1c^1/a^1c^1 genotypes confer the best physiological performance at low altitude, and since all populations experience approximately the same selective regime in terms of altitude, the populations are essentially fixed for a^1c^1 haplotypes. Subspecies *nebrascensis* and *sonoriensis* inhabit regions where the average altitude is intermediate (mean 50-km regional altitude of sampled sites = 2,114 m), and the variation in topography (especially for *sonoriensis* in eastern California) is high. Because of the abrupt altitudinal variation in these regions, populations at high or low altitudes exchange genes with an array of populations at intermediate altitudes. The net result is that all populations show intermediate levels of polymorphism for alpha-globin haplotypes. Despite gene flow, there is enough selection to produce a significant correlation of the arcsine of alpha-globin genotype frequency with local and both regional altitudes for subspecies *sonoriensis.* Subspecies *rufinus* inhabits the region with the highest average altitude (mean 50-km regional altitude of sampled sites = 2,639 m) and with a fairly high variation in topography. Populations of *rufinus* are generally polymorphic, but a^0c^0 haplotypes predominate. Presumably, the a^0c^0 haplotype class does not reach fixation because of gene exchange with populations at intermediate local altitudes.

There is another intriguing aspect to the haplotype-frequency data. Viewed on a broad scale, both the alpha-globin frequencies (Fig. 2) and beta-globin frequencies (data not shown) reveal a consistent geographic pattern. The apex of a^0c^0 frequencies occurs in central Colorado. The frequency of the a^0c^0 haplotype declines in all directions away from central Colorado. The biogeographical trend corresponds on a coarse scale to altitudinal trends, at least for altitudes averaged over relatively large areas. Altitude and

biogeography are, therefore, confounded variables.

However, any hypothesis involving biogeographic factors must explain why those factors affect only the hemoglobin loci and not the genome as a whole. The geographic pattern of the hemoglobin polymorphisms in *P. maniculatus* is distinctly different from the geographic patterns for polymorphic allozyme loci (Avise et al., 1979), for mitochondrial DNA polymorphisms (Lansman et al., 1983), and for variation in gross morphology (Blair, 1950). Lansman et al. (1983) point out that the last three types of variation differ strikingly from one another in their geographic patterns, suggesting that different elements of the genome are influenced by markedly different evolutionary forces.

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