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HEMOGLOBIN POLYMORPHISMS IN DEER MICE (*PEROMYSCUS MANICULATUS*): PHYSIOLOGY OF BETA-GLOBIN VARIANTS AND ALPHA-GLOBIN RECOMBINANTS

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Abstract.—Wild populations of deer mice (*Peromyscus maniculatus*) contain hemoglobin polymorphisms at both alpha-globin (*Hba, Hbc*) and beta-globin (*Hbd*) loci. Population gene frequencies of beta-globin variants (d^0 and d^1 haplotypes) are not correlated with altitude, whereas a^1c^1 alpha-globin haplotypes are fixed in low-altitude populations, and a^0c^0 haplotypes reach near fixation at high altitudes. We examined the effects of alpha- and beta-globin variants on blood oxygen affinity and on aerobic performance, measured as maximum oxygen consumption ($\dot{V}O_2max$). Exercise and cold exposure were used to elicit $\dot{V}O_2max$. Experiments were performed at low (340 m) and high (3,800 m) altitude to include the range of oxygen partial pressures encountered by wild deer mice. Beta-globin variants had little effect on blood oxygen affinity or $\dot{V}O_2max$. Oxygen-dissociation curves from a^0c^0 and a^1c^1 homozygotes and heterozygotes had similar shapes, but the P_{50} of a^0c^0 homozygotes was significantly lower than that of other genotypes. Mice carrying a^1c^1/a^1c^1 genotypes had the highest $\dot{V}O_2max$ at low altitude, but mice with a^0c^0/a^0c^0 genotypes had the highest $\dot{V}O_2max$ at high altitude. Mice carrying rare recombinant alpha-globin haplotypes (a^0c^1) had lower $\dot{V}O_2max$ than nonrecombinant genotypes as a whole but in most cases were not significantly different from nonrecombinant heterozygotes (a^0c^0/a^1c^1). We conclude that genetic adaptation to different altitudes was important in the evolution of deer mouse alpha-globin polymorphisms and in the maintenance of linkage disequilibrium in the alpha-globin loci but was not a significant factor in the evolution of beta-globin polymorphisms.

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The extent to which protein polymorphisms affect fitness is a central question in population genetics. Many allozymes and other proteins are differentiated at the biochemical level, but in relatively few cases are they known to have differential effects on whole-animal physiology. Chappell and Snyder (1984) have described functional differences at the biochemical and organismal level for several alpha-globin polymorphisms in the hemoglobins of North American deer mice (*Peromyscus maniculatus*). Polymorphisms occur at the *Hba* and *Hbc* loci and can be tabulated into four allele classes (a^0 and a^1, and c^0 and c^1), based on similarity of isoelectric points. Snyder (1981) proposed that these polymorphisms evolved as a result of selection regimes that vary with altitude. Population gene frequencies of common a^1c^1 and a^0c^0 haplotypes are correlated with native altitude (Snyder, 1981; Snyder et al., 1988). The P_{50} (the oxygen partial pressure at which blood is 50% saturated) decreases with increasing native altitude (Snyder, 1985), although differences among P_{50}'s of populations may also be partially explained by subspecific differentiation. In laboratory strains, P_{50} is a function of alpha-globin genotype (Chappell and Snyder, 1984). Moreover, alpha-globin genotype affects an important whole-animal character, the maximum rate of oxygen consumption or $\dot{V}O_2max$ (Chappell and Snyder, 1984).

These findings support the hypothesis that natural selection has played a significant role in the evolution of deer mouse hemoglobin polymorphisms, but many important aspects of the system have not been investigated. We address two interesting problems here. Strong linkage disequilibrium between the *Hba* and *Hbc* loci is present in deer mice, such that a^0 alleles are almost always associated with c^0 alleles and a^1 alleles are almost always associated with c^1 alleles (more than 98% of haplotypes in natural populations are either a^0c^0 or a^1c^1; Snyder, 1979, 1980). An immediate question is whether the observed disequilibrium is maintained by selection, i.e., are recombinant alpha-globin haplotypes rare because they adversely affect the physiology of oxygen transport? A second question concerns the functional properties of polymorphisms at the beta-globin loci (*Hbd*). These polymorphisms are fairly common in natural

populations and are differentiated with respect to altitude. However, the correlation is much less striking than for the alpha-globins and can be attributed to differences between subspecies rather than to altitude effects (Snyder et al., 1988). Is the weak correlation with altitude a reflection of lack of functional differentiation among the beta-globin alleles?

In this paper, we describe studies of the effects of recombinant alpha-globin haplotypes on whole-animal performance in deer mice acclimated to both high and low altitude. We also report results from measurements of biochemical and physiological characteristics of several beta-globin variants and discuss the possible role of selection in their evolution.

Materials and Methods

Animals.—Procedures for determining hemoglobin genotype in deer mice have been previously described (Snyder, 1978*a*, 1978*b*, 1980). To avoid the confounding effects of adaptation and acclimation in characters other than hemoglobin, we developed strains of deer mice that carried hemoglobin haplotypes in identical-by-descent (IBD) condition. IBD strains allowed study of the effects of discrete haplotypes in as standardized a genetic background as possible for a species that does not tolerate high levels of inbreeding. For example, an IBD strain for an a^1c^1 haplotype is produced by mating a single a^1c^1/a^0c^0 heterozygote to several a^0c^0 homozygotes from the source population. Progeny carrying the a^1c^1 haplotype are bred to each other and to unrelated a^0c^0 homozygotes derived from the source population until adequate numbers of all three genotype classes are available for testing. These animals are closely matched for sex, age, parentage, and developmental history; they differ (as far as can be controlled) only in hemoglobin genotype.

We worked with five beta-globin strains and three alpha-globin strains. The beta-globin strains (designated 220, 1383, 1936, 7019, and 7131) originated from subspecies *rufinus* from Colorado and Utah. In strains 220, 1936, 7019, and 7131, the three genotypes (d^0/d^0, d^0/d^1, and d^1/d^1) were arrayed against a^0c^0 homozygote alpha-globin backgrounds. In strain 1383, the two genotypes tested (d^0/d^0 and d^0/d^1) were arrayed against an a^1c^1 homozygote background. Two recombinant alpha-globin strains (designated 6941 and 7092; subspecies *nebrascensis* from Colorado) carried an a^0c^1 haplotype. Each recombinant strain contained six genotypes: three nonrecombinant (a^0c^0/a^0c^0, a^1c^1/a^1c^1, a^1c^1/a^0c^0) and three recombinant (a^0c^0/a^0c^1, a^1c^1/a^0c^1, a^0c^1/a^0c^1). A single strain (4229; subspecies *sonoriensis* from eastern California) was used to examine the oxygen-dissociation curve of a^0c^0 and a^1c^1 homozygotes and a^1c^1/a^0c^0 heterozygotes. The beta-globin background was d^0 homozygous for all alpha-globin strains.

Mice were housed in standard laboratory cages with wood shavings and cotton for bedding. Food (commercial rodent chow) and water were available ad libitum. Whenever possible, mice were tested at both low altitude (340 m; at Riverside, California) and high altitude (3,800 m; at the Barcroft Laboratory of the White Mountain Research Station in eastern California). Mean barometric pressures were 737 torr at Riverside and 485 torr at Barcroft. Mice were acclimated to local altitude for at least two months before testing. Animal care was similar at both altitudes, except that room temperature at Riverside was maintained at 22°–23°C, while at Barcroft the temperature varied between 12° and 22°C.

Blood Oxygen Affinity.—Blood-gas measurements were performed on the 1383, 1936, 4229, and 7019 strains (for beta-globin strains, only P_{50} was determined; for the alpha-globin strain 4229, measurements were obtained over a range of oxygen saturations). For each strain, we used a total of 3–5 ml of fresh blood pooled from 10–15 mice within each genotype group. Aliquots were equilibrated in a 37°C tonometer against gases with identical CO_2 content but containing either 30% or 0% O_2. Carefully measured volumes of oxygenated and deoxygenated blood were mixed anaerobically to obtain different degrees of oxygen saturation (Edwards and Martin, 1966; Snyder et al., 1982). At a given P_{O_2}, the pH and P_{CO_2} were varied by using three pairs of calibration gases with different CO_2 content. Values of P_{O_2}, P_{CO_2}, and pH were obtained using an Instrument Laboratories IL-813

blood gas analyzer. For P_{50} measurements, three subsamples were run for each saturation condition; mean values were computed for blood-gas parameters (for strain 4229, single measurements were made at each saturation, because the amount of blood available was limited). The CO_2-Bohr effect, blood buffering capacity, and P_{O_2} values for pH 7.4 were calculated using linear regression (Snyder et al., 1982).

Maximum Oxygen Consumption.—Maximum rates of oxygen consumption ($\dot{V}O_2$max) for strains 220, 1936, 6941, 7019, 7092, and 7131 were determined with open-circuit respirometry during exercise and/or cold exposure. For strains 1936 and 7019, the same individuals were used for both $\dot{V}O_2$max and blood-gas measurements (in all cases, $\dot{V}O_2$max was determined prior to blood sampling). Untrained mice were run for six minutes on an enclosed treadmill, with tread speed slightly faster than the maximum aerobically sustainable running speed (0.75–1.0 m/sec). Attainment of $\dot{V}O_2$max was judged by evident exhaustion during and after runs and by oxygen debt after runs. Mice were subjected to acute cold exposure (0°–−5°C) for 10–12 min in a closed-loop wind tunnel that generated air speeds of 4–5 m/sec. This regime produced greater heat-loss rates than could be countered by aerobic metabolic-heat production, as indicated by stable and then declining $\dot{V}O_2$ during runs and by hypothermia after runs. For both protocols, gas exchange was monitored by Applied Electrochemistry S-3A O_2 and CD-3A CO_2 analyzers interfaced to a computer. Resolution of the system was ±0.001% for O_2 and ±0.01% for CO_2. The $\dot{V}O_2$max for each test was calculated by having the computer search the stored record for the highest "instantaneous" $\dot{V}O_2$ (Bartholomew et al., 1981) averaged over various time intervals (1, 2, and 5 min for exercise; 2, 5, and 8 min for cold exposure). Details of the protocols are available in Chappell (1984). Performances of genotypes were compared using one- and two-way ANOVAs.

Results

Blood Oxygen Affinity.—No consistent genotypic difference was apparent in measurements of $P_{50,7.4}$ (50% oxygen saturation at pH 7.4) for the three beta-globin strains tested (1383, 1936, and 7019; Fig. 1A), and in no strain were there significant differences among genotype classes. The $P_{50,7.4}$ in the 1383 strain was significantly higher than in the 1936 and 7019 strains, presumably because the alpha-globin background in 1383 is homozygous a^1c^1, which itself confers a higher $P_{50,7.4}$ than the homozygous a^0c^0 background of the 1936 and 7019 strains (Chappell and Snyder, 1984). Significant effects of genotype on other blood parameters ($P_{CO_2\,7.4}$, CO_2-Bohr effect, and blood buffering capacity) were not observed.

One beta-globin strain, 1936, was tested at both low and high altitude. Somewhat surprisingly, acclimation to 3,800 m did not significantly alter the $P_{50,7.4}$ (Fig. 1B). This result is similar to published data for three alpha-globin strains, which also showed negligible change in $P_{50,7.4}$ when tested at high and low altitude (Chappell and Snyder, 1984).

In strain 4229, the hemoglobin oxygen-dissociation curves (ODC's) for the three genotypes (a^0c^0/a^0c^0, a^0c^0/a^1c^1, and a^1c^1/a^1c^1) have very similar shapes over oxygen saturations from 16.7% to 82%, except that the a^0c^0/a^0c^0 curve is left-shifted approximately 3–4 torr from the a^1c^1/a^1c^1 curve at all P_{O_2} (Fig. 2A). The ODC for heterozygotes is intermediate to the ODC's for homozygotes. Hill coefficients ranged from 2.80 to 2.92 (Fig. 2B) and did not differ significantly among genotypes (ANCOVA, $P > 0.07$), indicating similarity of slope. Additional data on the respiratory functions of deer mouse blood are presented by Snyder (1985).

Maximum Oxygen Consumption.—ANOVAs on all $\dot{V}O_2$ data indicated that the averaging interval used affected the magnitude of the computed $\dot{V}O_2$max, but did not affect the performance ranking of genotypes. Accordingly, for simplicity we present data only for one-minute intervals for exercise and two-minute intervals for cold exposure (longer intervals yielded qualitatively similar results, with slightly lower $\dot{V}O_2$max values).

Beta-globin genotype had no significant effect on $\dot{V}O_2$max at either high or low altitude (two-way ANOVA; Table 1, Fig. 3), but differences among strains explained a significant amount of variation ($P < 0.001$)

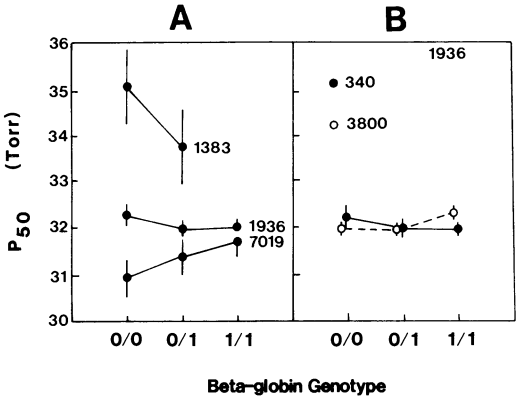

FIG. 1. A) Blood oxygen affinity, measured as $P_{50,7.4}$, in three beta-globin strains (1383, 1936, and 7019). Vertical lines represent 2 (SE); 0/0 = d^0/d^0 genotypes, 0/1 = d^0/d^1 genotypes, and 1/1 = d^1/d^1 genotypes. N = 3 repeat measurements for each point (see text for details). Significant differences among genotypes were not observed. B) $P_{50,7.4}$ at 340 m and 3,800 m for strain 1936. No significant differences were observed among genotypes at either altitude.

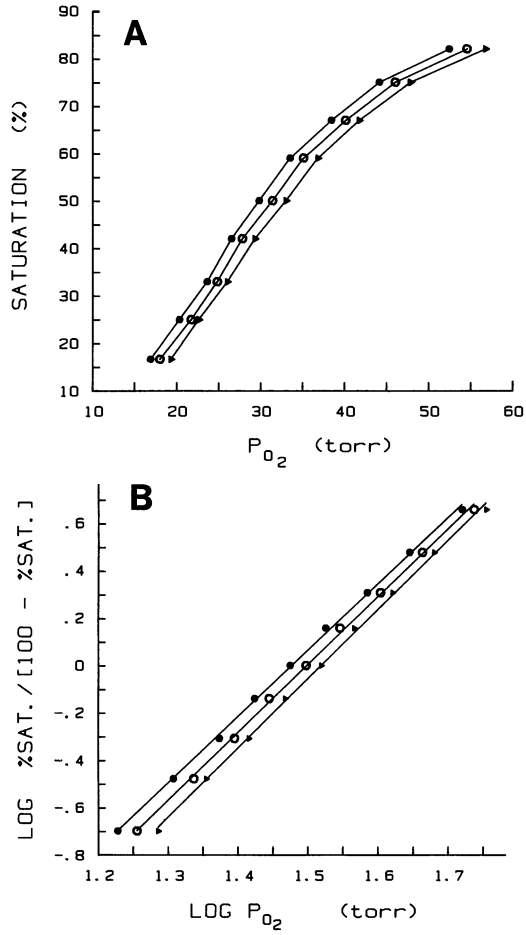

FIG. 2. A) Oxygen dissociation curves (ODC) for three alpha-globin genotypes in strain 4229. Left curve = a^0c^0/a^0c^0 genotypes; middle curve = heterozygotes; right curve = a^1c^1/a^1c^1 genotypes. B) Hill plots for the three ODC's.

for exercise and cold exposure at both altitudes. The mean $\dot{V}O_2$max during exercise was 8–10% higher than $\dot{V}O_2$max during cold exposure at low altitude. At high altitude, exercise $\dot{V}O_2$max was 15–20% higher than $\dot{V}O_2$max during cold exposure. After two months of acclimation to 3,800 m, exercise $\dot{V}O_2$max was reduced by 12% compared to the 340-m value, and the cold-exposure-induced $\dot{V}O_2$max was reduced by 19% compared to the 340-m value.

In contrast to beta-globin genotype, alpha-globin genotype had large and significant effects on $\dot{V}O_2$max. Among nonrecombinant genotypes in both the 7092 and 6941 strains, a^1c^1 homozygotes had the highest $\dot{V}O_2$max at low altitude, while a^0c^0 homozygotes were the best performers at high altitude (Fig. 4A, B). These differences were significant in five of six comparisons (P = 0.032 to P = 0.0007); the single exception involved low-altitude cold exposure for the 6941 strain (P = 0.77). Qualitative results were similar for exercise and cold exposure in strain 7092 (unfortunately, we obtained no exercise data for strain 6941, because our mouse colony was vandalized). The ranking of genotypes we observed corresponds to data for other alpha-globin strains (Chappell and Snyder, 1984).

Comparisons (one- and two-way ANOVAs) of average $\dot{V}O_2$max of the three recombinant genotypes with that of the three nonrecombinant genotypes indicate that mice carrying recombinant genotypes suffer a deficit in aerobic capacity of 5–10% (P = 0.038 to $P < 0.0001$ for all cases; Table 2). Much of the difference can be attributed to the presence of "optimal" genotypes for particular altitudes (i.e., a^0c^0/a^0c^0 at high altitude and a^1c^1/a^1c^1 at low altitude) among the nonrecombinant classes. When recombinant mice were compared to nonrecombinant heterozygotes (which carry the same overall combination of alleles but in a different chromosomal arrangement), a significant difference was observed in only one out of six experiments (P = 0.0002; high-altitude cold exposure for the 6941 strain;

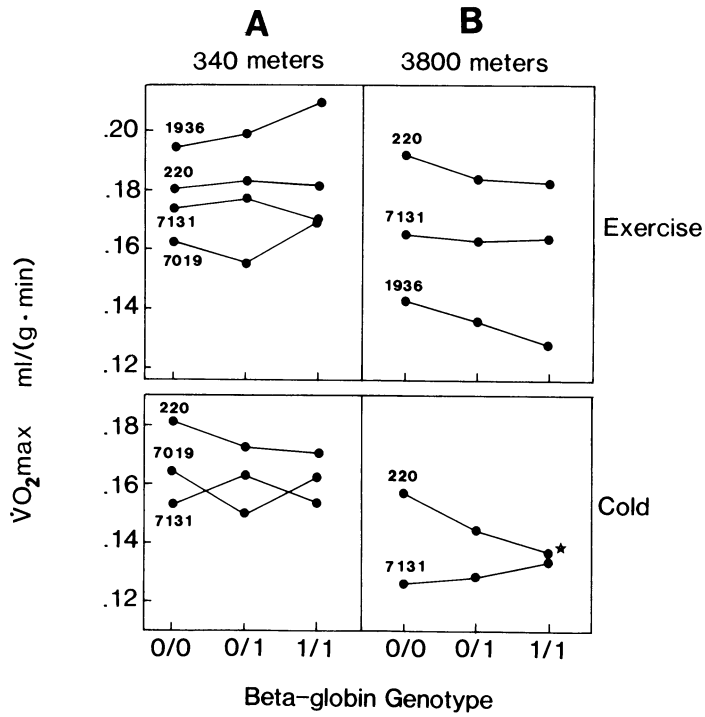

FIG. 3. Maximum oxygen consumption ($\dot{V}O_2max$) measured during exercise and acute cold exposure for several beta-globin strains (220, 1936, 7019, and 7137). Labels for genotypes are as in Figure 1. Each point is a mean obtained from 9–14 (mean 12.6) individuals. The star indicates a significant difference among genotypes of strain 220. A) Data obtained at 340 m altitude; B) data obtained at 3,800 m altitude.

Table 2A). Two-way ANOVAs for cold exposure (combining data from both strains) revealed a significant difference between recombinants and heterozygotes at high altitude ($P < 0.0001$) but not at low altitude ($P = 0.19$; Table 2B).

DISCUSSION

Oxygen-Dissociation Curves.—In previous studies of deer mouse hemoglobins (Chappell and Snyder, 1984; Snyder, 1985), blood oxygen affinity was measured only as $P_{50,7.4}$, with the assumption that the shape of the ODC is consistent between genotypes. A priori, that assumption is suspect, because the mixture of hemoglobin tetramers in individual deer mice is complex, particularly in heterozygotes (a typical a^0c^0/a^1c^1 heterozygote with no beta-globin heterozygosity produces ten possible tetramers). Furthermore, tetrameric ratios in deer mice show considerable variation, which is under genetic control (Snyder, 1978*a*, 1978*b*, 1980). In some species with multiple hemoglobins (e.g., bullfrogs and turtles), the ODC does not have a standard sigmoid shape (Maginniss et al., 1980). However, data from the present study (Fig. 2) indicate that ODC's for deer mice with different alpha-globin genotypes have similar shapes, even in heterozygotes. Moreover, slight differences in shape should be much less significant for O_2 transport than the large left-shift of a^0c^0 homozygotes relative to heterozygotes and a^1c^1 homozygotes. Hence, the P_{50} measure-

TABLE 1. Significance table for two-way ANOVA comparing the effects of strain, genotype, and their interaction, on the $\dot{V}O_2max$ of deer mice carrying beta-globin polymorphisms. The strains compared were 220, 1383, 1936, 7019, and 7131. LA = low altitude (340 m); HA = high altitude (3,800 m). Data shown are probability values; see Figure 3 for a graphical presentation of the data.

Treatment	*N*	Strain	Genotype	Interaction
Exercise, LA	147	0.0001	0.3196	0.1515
Cold exposure, LA	115	0.0005	0.5824	0.1351
Exercise, HA	111	0.0001	0.0937	0.7858
Cold exposure, HA	80	0.0008	0.5011	0.0483

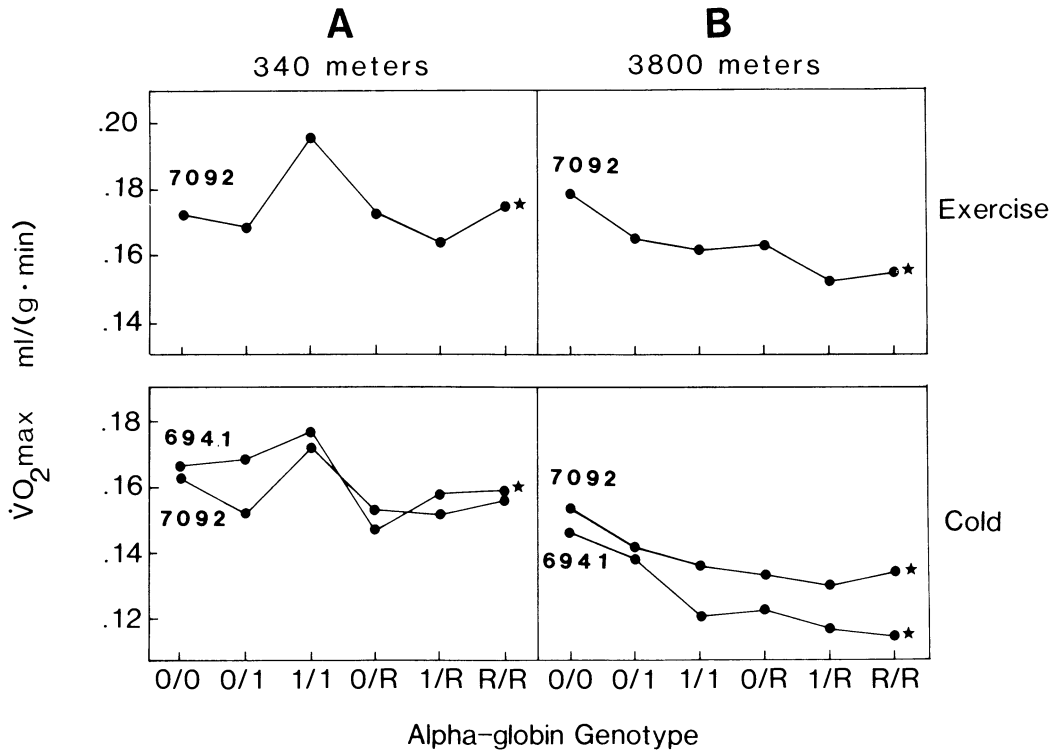

FIG. 4. Maximum oxygen consumption ($\dot{V}O_2max$) during cold exposure and exercise for two alpha-globin strains (6941 and 7092) containing recombinant genotypes. 0/0 = a^0c^0/a^0c^0 genotypes, 0/1 = a^0c^0/a^1c^1 genotypes, 1/1 = a^1c^1/a^1c^1 genotypes, 0/R = a^0c^0/a^0c^1 genotypes, 1/R = a^1c^1/a^0c^1 genotypes, R/R = a^0c^1/a^0c^1 genotypes. Each point is a mean obtained from 7–20 (mean 15.4) individuals. Significant differences among genotypes in a strain are indicated by stars. A) Data obtained at 340 m altitude; B) data obtained at 3,800 m altitude.

TABLE 2. A) Significance table for one-way ANOVA for the $\dot{V}O_2max$ of two strains of deer mice (6941 and 7092) carrying alpha-globin polymorphisms, including recombinant haplotypes. The codes for experimental treatments are: LE = low altitude (340 m), exercise; HE = high altitude (3,800 m), exercise; LC = low altitude, cold exposure; and HC = high altitude, cold exposure. Numbers shown are probabilities; for comparisons with recombinants, numbers of individuals are shown in parentheses. See Figure 4 for a graphical presentation of the data. B) Significance table for two-way ANOVA for the cold-exposure $\dot{V}O_2max$ of the 6941 and 7092 strains. LA = low altitude; HA = high altitude. Numbers shown are probabilities. Total sample sizes were 187 for LA and 179 for HA; for comparisons with recombinants, numbers of individuals are shown in parentheses. See Figure 4 for a graphical presentation of the data.

A.

Treatment	Strain	*N*	All genotypes	Recombinants vs. nonrecombinants	Recombinants vs. heterozygotes
LE	7092	83	0.0009	0.0375 (42, 41)	0.816 (42, 14)
HE	7092	105	0.0002	0.0007 (53, 52)	0.076 (53, 18)
LC	7092	112	0.0199	0.0354 (59, 53)	0.787 (59, 18)
HC	7092	105	0.0012	0.0027 (52, 53)	0.073 (52, 19)
LC	6941	75	0.1964	0.0219 (43, 32)	0.088 (43, 15)
HC	6941	74	0.0001	0.0001 (42, 32)	0.0002 (42, 16)

B.

Treatment	Strain	Genotype	Interaction	Recombinants vs. nonrecombinants	Recombinants vs. heterozygotes
LA	0.3015	0.0092	0.5365	0.0010 (102, 85)	0.1915 (102, 33)
HA	0.0001	0.0001	0.3635	0.0001 (94, 85)	0.0001 (94, 35)

ments of Chappell and Snyder (1984) and Snyder (1985) appear to be valid indices for comparison of blood oxygen affinity between genotypes.

Beta-Globin Polymorphisms.—The d^1 class of beta-globin alleles is rare at low elevations but attains frequencies of 0.1 to 0.2 in populations from regions above 2,000 m. The overall correlation of allele frequency with altitude is significant. However, the distribution is attributable to subspecies differences and not to altitude per se; no correlation of d^1 frequency with altitude occurs within subspecies (Snyder et al., 1988). Thus, it appears from the biogeographic evidence that differences in beta-globin frequencies result from factors not predicated on hypoxia-related selection or other adaptations to altitude. Results from the present study are consistent with that hypothesis. Blood oxygen affinity and $\dot{V}O_2$max are unaffected by beta-globin genotype at either low or high altitude (Table 1; Figs. 1, 3). We conclude that the beta-globin polymorphisms in deer mice arose from drift, weak levels of selection on phenotypic differences too small to be discernible in our protocols, or selection on factors we did not examine.

Recombinant Alpha-Globins.—There is considerable theoretical and empirical interest in the role of selection in creating and/or maintaining linkage disequilibrium between polymorphic protein-coding loci (e.g., Asmussen and Clegg, 1982; Clegg, 1984). The strong gametic-phase linkage disequilibrium in the *Hba* and *Hbc* loci of deer mice (Snyder, 1979, 1980) provides a good opportunity for examining this problem. One hypothesis is that recombinants are rare (about 1–1.5% of haplotypes in natural populations) because they produce highly aberrant oxygen-transport properties compared to those of nonrecombinant genotypes and are consequently eliminated by strong selection. A second hypothesis predicts that recombinant genotypes produce blood properties similar to those of nonrecombinant heterozygotes. According to this scenario, the physiology of oxygen transport in recombinants is not "abnormal." Nevertheless, recombinants are selected against, because they are less fit than mice carrying an "optimal" nonrecombinant genotype for best aerobic performance (i.e., a^1c^1 homozygous at low altitude or a^0c^0 homozygous at high altitude). A third (null) hypothesis is that the blood properties and whole-animal performance of recombinants are similar to those of nonrecombinants and that the observed linkage disequilibrium is not a result of selection on hemoglobin function per se.

We lack adequate data on the blood oxygen affinities of mice carrying recombinant haplotypes, although preliminary measurements by one of us (L.R.G.S.) on a small number of animals carrying an a^1c^0 haplotype indicate that the P_{50} of recombinants is only slightly elevated relative to that of nonrecombinants (unpubl.). Nevertheless, results from $\dot{V}O_2$max measurements (Fig. 4) suggest that selection is potentially important in maintaining linkage disequilibrium. Mice carrying one or two recombinant haplotypes suffer a modest (5–10%) but significant reduction in $\dot{V}O_2$max compared to mice with normal genotypes. The difference is greater when recombinants are compared to animals with the "optimal" alpha-globin genotype for a particular altitude. Whether or not there is a difference between the $\dot{V}O_2$max of recombinants and nonrecombinant heterozygotes is uncertain (Fig. 4, Table 2). Significant differences between recombinants and heterozygotes within strains occurred in only one of six comparisons (6941 strain; high-altitude cold exposure). A two-way ANOVA for cold-exposure tests showed a significant difference between heterozygotes and recombinants at high altitude but not at low altitude. Thus, our data are most consistent with the second of the three hypotheses proposed above, and we conclude that recombinants occur at low frequencies at least partially as a result of selection.

Selection on Hemoglobin Polymorphisms.—In this paper and elsewhere (Chappell and Snyder, 1984), we have demonstrated that alpha-globin genotype affects $\dot{V}O_2$max, which is an important factor in whole-animal performance. However, $\dot{V}O_2$max measurements are not direct indices of fitness. Therefore, it is necessary to make indirect arguments concerning the possible influence of $\dot{V}O_2$max on the Darwinian fitness of deer mice and, hence, on

the evolution of their hemoglobins. We are aware of no data linking $\dot{V}O_2$max to survival or reproduction in a natural population. Nevertheless, high $\dot{V}O_2$max is presumably important during territorial defense, courtship, escape from predators, gestation and lactation, and especially thermogenesis in cold weather. Mean daily field metabolic rates of deer mice in the White Mountains of California are equivalent to 61% of $\dot{V}O_2$max in summer and autumn (Hayes, unpubl.). Since mice are active primarily at night and rest in nests during the day, these data imply that $\dot{V}O_2$'s during the nocturnal activity period are close to $\dot{V}O_2$max. Winter-acclimated *Peromyscus* have elevated thermogenic capacity (Wickler, 1980; Hayes and Chappell, 1986), but environmental temperatures are considerably colder as well. The metabolic costs of exercise and thermogenesis are additive in small mammals (i.e., "waste" heat from muscular exercise cannot be substituted for thermogenic requirements; Bartholomew, 1977). In cold conditions, the bulk of a deer mouse's aerobic capacity is dedicated to thermogenesis. Therefore, small increases in $\dot{V}O_2$max, such as those attained from possessing the "correct" hemoglobin genotype, may disproportionately expand the fraction of aerobic scope available for exercise or other nonthermogenic activities and have a correspondingly disproportionate effect on survival and fecundity.

Acknowledgments

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