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HEMOGLOBIN FUNCTION AND PHYSIOLOGICAL ADAPTATION TO HYPOXIA IN HIGH-ALTITUDE MAMMALS

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Understanding the biochemical mechanisms that enable high-altitude animals to survive and function under conditions of hypoxic stress can provide important insights into the nature of physiological adaptation. Evidence from a number of high-altitude vertebrates indicates that modifications of hemoglobin function typically play a key role in mediating an adaptive response to chronic hypoxia. Because much is known about structure–function relationships of mammalian hemoglobins and their physiological role in oxygen transport, the study of hemoglobin variation in high-altitude mammals holds much promise for understanding the nature of adaptation to hypoxia from the level of blood biochemistry to the level of whole-organism physiology. In this review I 1st discuss basic biochemical principles of hemoglobin function and the nature of physiological adaptation to high-altitude hypoxia in mammals. I then discuss a case study involving a complex hemoglobin polymorphism in North American deer mice (*Peromyscus maniculatus*) that illustrates how integrative studies of protein function and fitness-related physiological performance can be used to obtain evolutionary insights into genetic mechanisms of adaptation.

Key words: adaptation, altitude, deer mouse, ecological physiology, evolutionary physiology, hemoglobin, hypoxia, natural selection, oxygen transport, *Peromyscus maniculatus*

High-altitude environments present a number of physiological challenges for endothermic animals, as they are characterized by a lower partial pressure of oxygen (P_{O_2}) and lower ambient temperatures compared to low-altitude environments at similar latitudes. The reduced P_{O_2} at high altitude results in reduced oxygen loading in the lungs such that the blood may not carry a sufficient supply of oxygen to the cells of respiring tissues (Bencowitz et al. 1982; Bouverot 1985; Turek et al. 1973). This reduced level of tissue oxygenation can impose severe constraints on aerobic metabolism and may therefore influence an animal's food requirements, water requirements, the capacity for sustained locomotor activity, and the capacity for internal heat production.

Although the genetic basis of hypoxia tolerance has yet to be fully elucidated in any vertebrate species, evidence from a number of mammals, birds, and amphibians indicates that modifications of hemoglobin function often play a key role in mediating an adaptive response to high-altitude hypoxia (Perutz 1983). In all vertebrates other than cyclostomes, the hemoglobin protein is a heterotetramer, composed of 2 α-chain and 2 β-chain polypeptides. In mammals and birds, the different subunit polypeptides are encoded by different sets of duplicated genes that are located on different chromosomes (Hardison 2001). Because much is known about structure–function relationships of mammalian hemoglobins and their role in oxygen transport (reviewed by Perutz 1983, 2001; Poyart et al. 1992; Weber and Fago 2004), the study of hemoglobin variation in species that are native to high altitude provides a unique opportunity to understand the nature of genetic adaptation to hypoxic stress from the level of blood biochemistry to the level of whole-organism physiology. In this review I 1st provide some background information about hemoglobin function and the nature of physiological adaptation to high-altitude hypoxia. I then discuss a case study involving a complex hemoglobin polymorphism in deer mice (*Peromyscus maniculatus*) that illustrates how integrative studies of protein function and fitness-related physiological performance can be used to obtain evolutionary insights into genetic mechanisms of adaptation.

CIRCULATORY ADJUSTMENTS TO HYPOXIC STRESS

When atmospheric air is drawn into the alveoli of the lungs, oxygen is under a higher partial pressure than in the pulmonary capillaries, and it therefore diffuses across the respiratory membrane into the arterial bloodstream. Once oxygen has entered the bloodstream, it is immediately bound to hemoglobin in the red blood cells for transport to the oxygen-consuming

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cells of respiring tissues. The gas exchange ends at the tissue capillaries as oxygen, released by hemoglobin, diffuses across the capillary walls through the interstitial fluid to the cells. At the same time, CO_2 and other metabolic end-products enter the bloodstream and are transported to the lungs by the opposite route.

At high altitude, the arterial P_{O_2} is reduced compared to what it would be in an oxygen-rich sea-level environment and it becomes critically important to minimize the corresponding reduction in tissue oxygenation. In the cascade of P_{O_2} across different compartments of the gas-exchange system, there are 2 main steps where circulatory adjustments can help minimize the inevitable reduction in tissue P_{O_2}: the gradient between alveolar gas and arterial blood, and that between capillary blood and the tissues. The P_{O_2} gradient between alveolar gas and arterial blood is normally attributable to a small amount of venous admixture and unequal matching of ventilation to perfusion in the lungs (that is, a mismatch between the diameter of the airways and the diameter of the pulmonary blood vessels). The P_{O_2} gradient between capillary blood and the tissues results from unloading of oxygen in the tissue capillary bed. Tissue gas exchange begins at the arterial inlet to the capillary bed, and the P_{O_2} falls rapidly from the arterial side to the venous side as oxygen diffuses from the high P_{O_2} of the blood to the low P_{O_2} of the interstitial fluid. A meaningful estimate of mean capillary P_{O_2} and the gradient to the cells can be obtained from measurements of arterial and mixed venous P_{O_2}. The arterial-mixed-venous P_{O_2} gradient can be minimized by increasing the circulatory conductance of oxygen in the blood. In high-altitude mammals, one of the primary mechanisms for increasing the circulatory conductance of oxygen involves increasing the oxygen-binding affinity of hemoglobin.

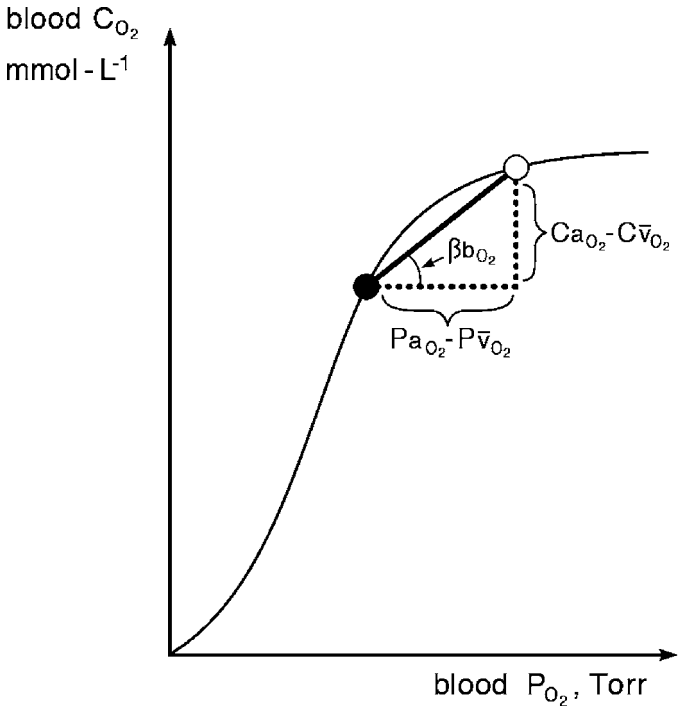

FIG. 1.—A schematic representation of the oxygen dissociation curve under physiochemical conditions prevailing in arterial blood (open circle) and mixed venous blood (solid circle). The y-axis measures the oxygen concentration in the blood (C_{O_2}) and the x-axis measures the partial pressure of oxygen in the blood (P_{O_2}). Ca_{O_2} and Cv_{O_2} are the oxygen concentrations in arterial and mixed venous blood, respectively. Pa_{O_2} and Pv_{O_2} are the partial pressures of oxygen in arterial and mixed venous blood, respectively. The slope of the line joining the arterial and mixed venous points on the curve denotes the blood oxygen capacitance coefficient (βb_{O_2} in equations 2 and 3).

ADAPTIVE MODIFICATION OF HEMOGLOBIN FUNCTION IN HYPOXIA-TOLERANT MAMMALS

When the arterial P_{O_2} is reduced because of high-altitude hypoxia, the transport of oxygen by blood has to serve 2 interrelated functions: it must maintain a sufficient flux of oxygen to meet metabolic demand, and it must also maintain an adequate pressure gradient for oxygen diffusion from the lungs to the cells of respiring tissues (Bouverot 1985; Monge and León-Velarde 1991). The 1st of these 2 functions is described by the following Fick's convection equation:

$$\dot{V}_{O_2} = Qb(Ca_{O_2} - Cv_{O_2}), \quad (1)$$

where $\dot{V}_{O_2}$ is the rate of oxygen consumption, Qb is the total cardiac blood flow, and Ca_{O_2} and Cv_{O_2} are the oxygen concentrations in arterial and mixed venous blood, respectively. This is equivalent to the following:

$$\dot{V}_{O_2} = Qb \times \beta b_{O_2}(Pa_{O_2} - Pv_{O_2}), \quad (2)$$

where $Pa_{O_2} - Pv_{O_2}$ is the arterial-mixed-venous P_{O_2} difference, and βb_{O_2}, called the blood oxygen capacitance coefficient (Dejours et al. 1970), is defined by the ratio

$$\beta b_{O_2} = (Ca_{O_2} - Cv_{O_2})/(Pa_{O_2} - Pv_{O_2}). \quad (3)$$

This capacitance coefficient is defined as the slope of the line connecting the arterial point to the mixed venous point on the oxygen-hemoglobin dissociation curve (ODC; Fig. 1). Because of the nonlinear relationship between oxygen concentration and P_{O_2} in blood (which gives rise to the sigmoid shape of the ODC), the capacitance coefficient βb_{O_2} is not constant.

The maintenance of an adequate pressure gradient for tissue oxygenation can be understood by rearranging equation 2 as follows:

$$Pv_{O_2} = Pa_{O_2} - \{1/[\beta b_{O_2}(Qb/V_{O_2})]\}, \quad (4)$$

where Pv_{O_2} is viewed as the critical pressure at the vascular supply source for oxygen diffusion into the cells of respiring tissues (Bouverot 1985). The product $\beta b_{O_2}(Qb/V_{O_2})$ is the specific oxygen blood conductance. Under hypoxia, an increased oxygen blood conductance helps to maintain a sufficient driving force for oxygen diffusion to the tissues.

One of the most important mechanisms to compensate for reduced arterial P_{O_2} at high altitude involves shifting the shape and position of the ODC (Luft 1972). The ODC describes how the reversible binding of oxygen by hemoglobin depends on P_{O_2} in the blood. At low P_{O_2} in the bloodstream, the arterial and mixed venous points on the ODC would be shifted leftward to

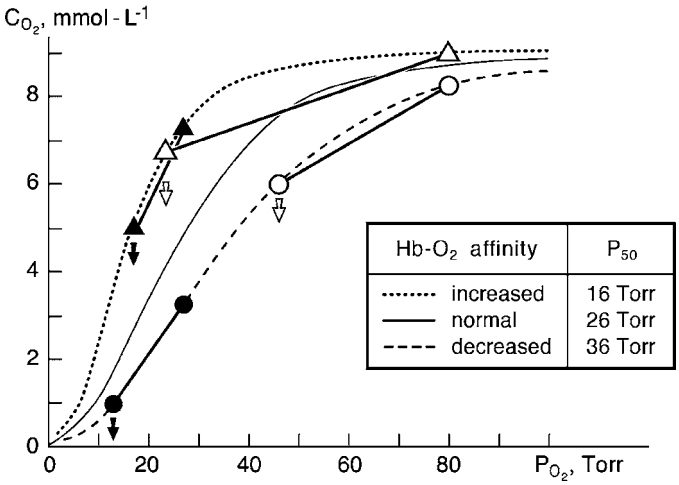

FIG. 2.—Theoretical influence of a change in hemoglobin–oxygen affinity on the oxygen dissociation curve under moderate hypoxia (open symbols) and severe hypoxia (closed symbols). The y-axis measures the oxygen concentration in whole blood (C_{O_2}) and the x-axis measures the partial pressure of oxygen (P_{O_2}). The symbols labeled with arrows denote the values for mixed venous blood, and the unlabeled symbols denote values for arterial blood. For clarity, differences in arterial-mixed-venous oxygen concentrations are identical in each of the 3 oxygen dissociation curves. At a given level of hypoxia, note how changes in hemoglobin–oxygen affinity influence P_{O_2} in mixed venous blood (arrows) and the blood oxygen capacitance coefficient (the slope of the line joining the arterial and mixed venous points on each oxygen dissociation curve). Modified from Bouverot (1985).

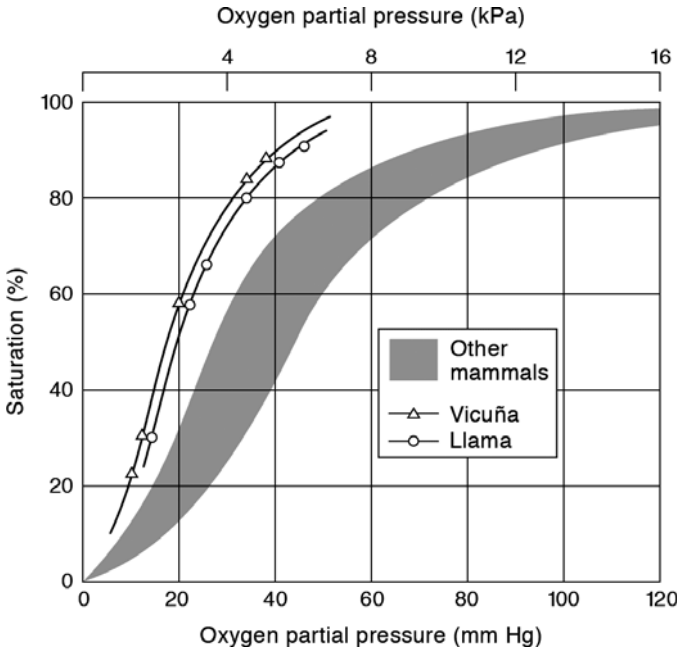

FIG. 3.—The oxygen dissociation curves of llamas and vicuñas are left-shifted relative to the curves of other terrestrial mammals that are not physiologically adapted to chronic hypoxia. The left-shifted curves are attributable to the fact that these 2 high-altitude species have hemoglobins with especially high oxygen-binding affinities (Hall et al. 1936). Modified from Schmidt-Nielsen (1990).

the steeper portion of the curve (Fig. 2). As a result, the slope of the line joining the arterial and mixed-venous points (the capacitance coefficient, βb_{O_2} in equations 2 and 3) would undergo a dramatic increase. This increase in βb_{O_2} would produce an automatic increase in the blood oxygen conductance, thereby preventing P_{O_2} from falling too low under hypoxia. The position and shape of the ODC, and therefore blood oxygen capacitance, can be modified by changes in hemoglobin concentration in the blood or by changes in the oxygen-binding affinity of hemoglobin. In mammals, the former mechanism appears to be more important in the acclimation response to hypoxia in species that are native to lowland environments, whereas the latter mechanism appears to be more important in high-altitude natives that are genetically adapted to chronic hypoxia (Bartels and Baumann 1977; Bullard 1972; Bunn 1980; Hochachka and Somero 2002; Lenfant 1973; Monge and León-Velarde 1991).

Under conditions of moderate hypoxia, a right-shifted ODC positions the arterial and mixed venous points on a steeper slope, thereby increasing βb_{O_2} (Fig. 2). This increase in βb_{O_2} is expected to increase Pv_{O_2}, the overall index of tissue oxygenation. By contrast, under severe hypoxia, a left-shifted ODC positions the arterial and mixed venous points on a steeper slope (Shappell and Lenfant 1975; Turek et al. 1973; Fig. 2). The rightward shift in the ODC involves an increase in the P_{O_2} required for half-saturation of hemoglobin (P_{50}), whereas the leftward shift involves a decrease in P_{50}. Physiological experiments by Banchero and Grover (1972) clearly demonstrate the advantage of a right-shifted ODC (high P_{50}) under conditions of moderate hypoxia and the advantage of a left-shifted ODC (low P_{50}) under conditions of severe hypoxia. In comparisons between llamas (*Lama glama*; a high-altitude native with low P_{50}) and sheep (*Ovis aries*, a species that has a comparatively high P_{50}), blood oxygen capacitance was higher in sheep at simulated altitudes of 1,600–2,800 m, but was much higher in llamas at the maximal simulated altitude of 6,400 m. Over the full range of altitudes, the decline in Pv_{O_2} was only 8 torr in llamas compared to 26 torr in sheep.

For any given species it is difficult to specify the exact altitude at which a left-shifted ODC becomes advantageous. However, because small mammals are generally characterized by higher mass-specific metabolic rates (and therefore, intrinsically high oxygen demands), the altitude at which a left-shifted curve becomes advantageous should be lower than in the case for large mammals (Snyder 1981; Turek et al. 1973).

Based on theoretical considerations and experimental results, alpine mammals that are genetically adapted to high-altitude hypoxia might be expected to have chronically left-shifted ODCs (low P_{50}). This prediction is borne out by surveys of blood oxygen affinity in a diverse range of terrestrial mammals, including species that inhabit high-altitude environments and those that live in the hypoxic conditions of subterranean burrows (Bullard 1972; Hall et al. 1936; Monge and León-Velarde 1991). For example, high-altitude natives such as llamas (*L. glama*) and vicuñas (*Vicugna vicugna*) are characterized by left-shifted ODCs relative to other terrestrial

TABLE 1.—Comparison of blood oxygen affinities (as indexed by P_{50}) and amino acid differences in the α- and β-globin subunits of hemoglobin in 1 lowland camelid (*Camelus dromedarius*) and 4 high-altitude camelid species (*Lama guanicoe*, *L. glama*, *L. pacos*, and *Vicugna vicugna*). Data for humans are provided for comparison. P_{50} is the partial pressure of oxygen in the bloodstream at which hemoglobin is 50% saturated. Differences in the sequence of α- and β-globin polypeptides among the different species are from Piccinini et al. (1990). The position of the amino acid residues in the primary structure of the globin polypeptides is given in parentheses. Modified from Poyart et al. (1992).

	Camelus dromedarius	*Lama guanicoe*	*L. glama*	*L. pacos*	*Vicugna vicugna*	*Homo sapiens*
P_{50}[a]	21.5	22.2	20.3	20.3	17.5	26.0
Amino acid differences						
α-chain						
8(A6)	Thr	Ala	Ala	Ala	Ala	Thr
0(A8)	Val	Ile	Ile	Ile	Val	Val
23(B4)	Glu	Asp	Asp	Asp	Asp	Glu
120(H3)	Ser	Ala	Ala	Ala	Ala	Ala
122(H5)	His	His	Asp	His	His	His
130(H13)	Ala	Ala	Ala	Ala	Thr	Ala
β-chain						
2(NA2)	His	Asn	Asn	Asn	Asn	His
76(E20)	Asn	Ser	Ser	Ser	Ser	Ala

[a] See Piccinini et al. (1990) for details regarding the experimental measurement of P_{50}.

mammals that are not physiologically adapted to chronic hypoxia (Fig. 3). Similarly, birds that fly at extremely high altitudes typically have left-shifted ODCs relative to their lowland sister taxa (Black and Tenny 1980; Monge and León-Velarde 1991; Petschow et al. 1977). The high blood oxygen affinity of high-altitude mammals is generally attributable to the possession of hemoglobin with an intrinsically high oxygen-binding affinity or a reduced responsiveness toward organic phosphates such as 2,3-biphosphoglycerate that stabilize the low-affinity, deoxygenated conformation of hemoglobin (Brewer and Eaton 1971).

The family Camelidae provides an interesting case study of hemoglobin variation in relation to altitude. The camelid family comprises 6 extant species that are distributed in South America, North Africa, and Central Asia. The Asian and African forms, *Camelus bactrianus* and *C. dromedarius*, are restricted to lowland deserts, whereas the South American forms, *Lama glama*, *L. guanicoe*, *L. pacos*, and *V. vicugna*, live at altitudes of 2,000–5,000 m in the Andes. The P_{50} values of all 6 camelid species are in the range of 17–22 torr (Table 1), which is low relative to other mammals of comparable size (Jürgens 1989; Piccinini et al. 1990). It thus appears that camelids of the high Andes were preadapted to high-altitude hypoxia in the sense that they possessed a blood biochemistry that allowed them to colonize alpine environments without extensive modifications of the ancestral condition. However, the Andean camelids have hemoglobins with even higher oxygen affinities than those of the Asian or African camels. This biochemical difference appears to be related to a His→Asn amino acid substitution at β2 (the 2nd residue of the β-globin polypeptide), which suppresses 2 binding sites for 2,3-biphosphoglycerate per tetramer. Among the Andean camelids, the vicuña inhabits the highest elevational zone (4,500–5,000 m) and it also exhibits the highest blood oxygen affinity ($P_{50} = 17.5$). The especially high oxygen affinity of vicuña hemoglobin appears to be attributable to an Ala→Thr substitution at α130 and a His→Asn substitution at β2 (Clementi et al. 1994; Piccinini et al. 1990; Poyart et al. 1992). One important conclusion of these molecular studies is that a small number of amino acid substitutions at key positions may be sufficient to adapt the functional properties of hemoglobin to the hypoxic conditions of high altitude (Poyart et al. 1992).

With respect to possible adaptive modifications of hemoglobin function, we generally know what to expect in the case of mammalian species such as the vicuña that are exclusively restricted to high-altitude environments. In contrast, it is not clear what we should expect in the case of species that inhabit a broad range of different altitudes across their geographic range. The deer mouse (*P. maniculatus*) is one such species. *P. maniculatus* has one of the broadest altitudinal distributions of any North American mammal, occurring in lowland prairie and deserts as well as alpine environments at elevations over 4,300 m (Hall 1981; Hock 1964). Because deer mice are characterized by one of the most complex and extensive hemoglobin polymorphisms of any mammal, this species is an exemplary study organism for elucidating how modifications of hemoglobin function contribute to adaptive variation in physiological performance under hypoxic stress (reviewed by Snyder 1981). Indeed, the adaptive significance of hemoglobin polymorphism in deer mice served as the focus for a brief but prolific research program in physiological genetics by the late Lee R. G. Snyder and his colleagues during the 1970s and 1980s.

HEMOGLOBIN POLYMORPHISM IN DEER MICE AND ITS ROLE IN PHYSIOLOGICAL ADAPTATION TO HIGH-ALTITUDE HYPOXIA

Given that high-altitude species generally have hemoglobins with higher oxygen binding affinities than those of their lowland relatives, an obvious question is whether high- and low-altitude populations of wide-ranging species such as *P. maniculatus* are similarly characterized by divergent fine-tuning of hemoglobin function. Available evidence for *P. maniculatus* suggests that modifications of hemoglobin function do indeed play a role in physiological adaptation to different elevational zones. Biochemical studies (Snyder 1985; Snyder et al. 1982, 1988) revealed a strong, negative correlation between P_{50} values and the native altitude of different populations of *P. maniculatus* across western North America (Fig. 4). Thus, mice that are native to alpine and subalpine environments tend to have higher blood oxygen affinities (lower P_{50} values) than their lowland counterparts. Given that the oxygen-binding affinity of hemoglobin is largely determined by variation in the primary structure of the individual subunit polypeptides, this presumably adaptive altitudinal variation in blood oxygen affinity may be associated with allelic variation in the α- or β-globin genes.

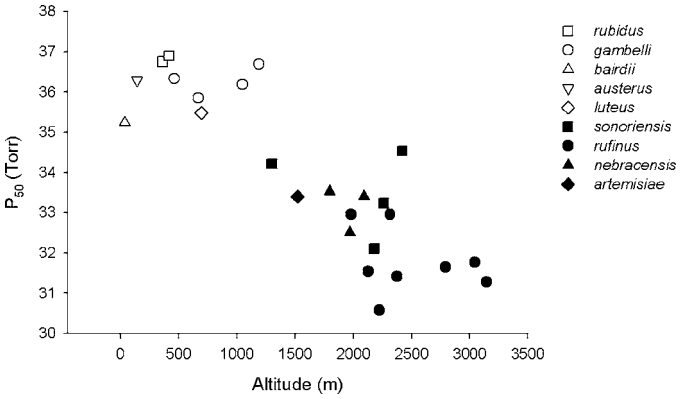

FIG. 4.—Variation in blood oxygen affinity (as measured by half-saturation of hemoglobin [P_{50}]) among subspecies of *Peromyscus maniculatus* from different altitudes across western North America. Based on data compiled by Snyder et al. (1988).

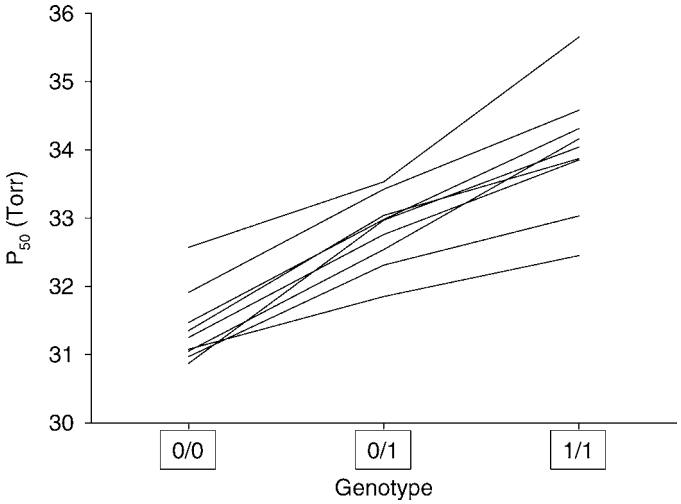

FIG. 5.—Variation in blood oxygen affinity, as measured by half-saturation of hemoglobin (P_{50}), among congenic strains of deer mice that carry different α-globin haplotypes in identical-by-descent condition (i.e., homologous alleles at each gene were derived from a single allele carried by the common ancestor of each strain). The 9 congenic strains were derived from wild-caught samples of 2 deer mouse subspecies (*Peromyscus maniculatus nebracensis* from Mesa County, Colorado, and *P. m. sonoriensis* from Mono County, California) that have the broadest altitudinal distributions. In each case, P_{50} values were measured after acclimation to a uniform altitude of 340 m. The same genotypic rank-order of P_{50} values was observed for population samples of subspecies *nebracensis*, *rufinus*, and *sonoriensis*, and for congenic strains of *nebracensis* and *sonoriensis* tested at high altitude (3,800 m). Two-locus α-globin genotypes are abbreviated as follows: $0/0 = a^0c^0/a^0c^0$, $0/1 = a^0c^0/a^1c^1$, and $1/1 = a^1c^1/a^1c^1$. Based on data compiled by Chappell and Snyder (1984).

Making the connection between genotype and phenotype.—Genetic analysis of 3 subspecies of *P. maniculatus* with the broadest altitudinal ranges (*nebracensis*, *sonoriensis*, and *rufinus*) revealed that variation in blood oxygen affinity is strongly associated with allelic variation at 2 closely linked gene duplicates that encode the α-chains of adult hemoglobin (Chappell et al. 1988; Chappell and Snyder 1984). In *P. maniculatus*, the 2 loci encoding the α-chains of adult hemoglobin, *Hba* and *Hbc*, are each polymorphic for 2 main classes of electrophoretically detectable protein alleles, Hba^0, Hba^1, Hbc^0, and Hbc^1 (Snyder 1978a, 1978b, 1980, 1981). Alleles at the 2 genes are characterized by a highly nonrandom pattern of association: the a^0 and c^0 alleles almost always occur together on the same haplotype, and likewise for the a^1 and c^1 alleles. In the parlance of population genetics, the 2 α-globin genes are characterized by nearly complete linkage disequilibrium. Consequently, alleles at the 2 genes most commonly occur in the following diploid combinations: a^0c^0/a^0c^0, a^0c^0/a^1c^1, and a^1c^1/a^1c^1. The 3 genotypes exhibited a highly consistent rank-order of P_{50} values when tested under both high- and low-altitude conditions: mice with the a^0c^0/a^0c^0 genotype exhibited the lowest P_{50} value (the most left-shifted ODC), mice with the a^1c^1/a^1c^1 genotype exhibited the highest P_{50} value (the most right-shifted ODC), and the a^0c^0/a^1c^1 double heterozygotes had an intermediate blood oxygen affinity (Fig. 5). To control for genetic background, these experiments were based on congenic strains of mice that carried different 2-locus α-globin haplotypes in identical-by-descent condition (i.e., homologous alleles at each gene were derived from a single allele carried by the common ancestor of each strain—Chappell and Snyder 1984; Chappell et al. 1988).

In addition to the effects on blood biochemistry, the phenotypic effects of these α-globin genes also were manifest at the level of whole-organism physiology. In the context of adaptation to high-altitude hypoxia, one especially important measure of physiological performance is $\dot{V}_{O_{2max}}$, which is defined as the maximal rate of oxygen consumption elicited by aerobic exercise or cold exposure. $\dot{V}_{O_{2max}}$ sets the upper limit on 2 important types of physiological performance: capacity for sustained activity (aerobic capacity) and internal heat production (thermogenic capacity). This measure of aerobic metabolism showed a striking pattern of variation among mice with different α-globin genotypes: $\dot{V}_{O_{2max}}$ was highest for a^0c^0/a^0c^0 mice when tested at an altitude of 3,800 m, whereas $\dot{V}_{O_{2max}}$ was highest for a^1c^1/a^1c^1 mice when tested at 340 m (Chappell et al. 1988; Chappell and Snyder 1984; Fig. 6). Effects of the α-globin genotypes on the distal physiological phenotype ($\dot{V}_{O_{2max}}$) appear to stem directly from effects on blood O_2 affinity, because no genotypic effects were detected for other aspects of blood biochemistry such as the CO_2-Bohr effect (the regression coefficient of log-P_{50} on pH), P_{CO_2} (the partial pressure of CO_2 at 50% oxygen saturation in the blood), blood buffering capacity (the regression coefficient of log-P_{CO_2} on pH), erythrocyte 2,3-biphosphoglycerate concentration, hematocrit, or hemoglobin concentration (Chappell et al. 1988; Chappell and Snyder 1984).

This variation in $\dot{V}_{O_{2max}}$ likely has important fitness consequences in high-altitude deer mice. As stated by Chappell and Snyder (1984:5487), "A mouse capable of attaining a higher $\dot{V}_{O_{2max}}$ can exercise more vigorously without incurring debilitating oxygen debt and/or it can maintain body temperature by means of aerobic thermogenesis at lower ambient tempera-

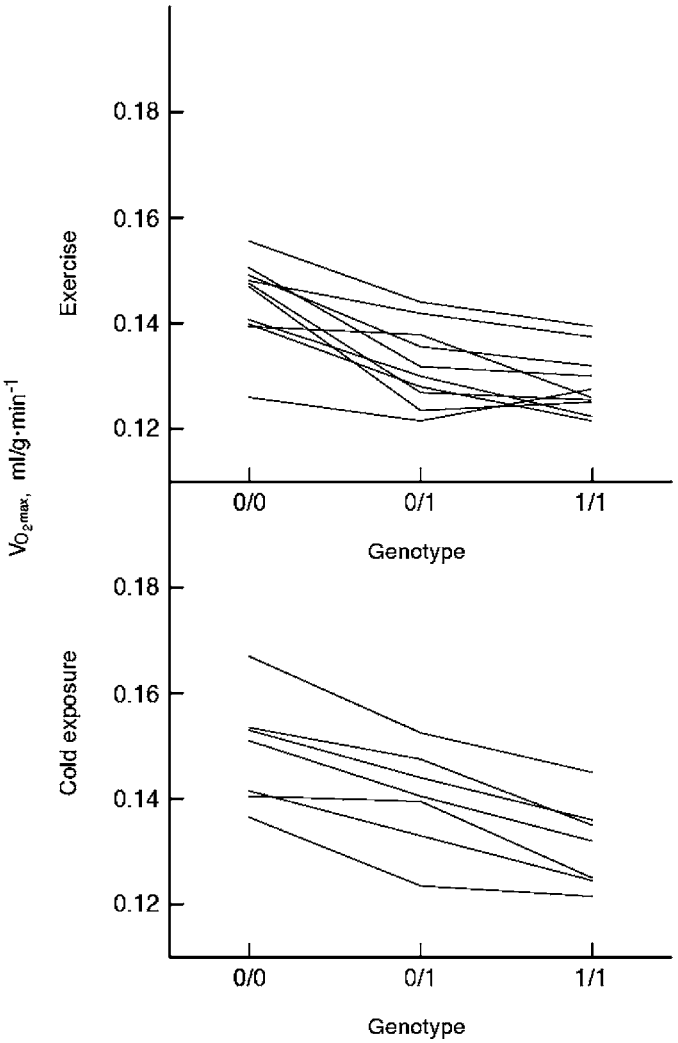

FIG. 6.—Variation in maximal rate of oxygen consumption ($\dot{V}_{O_{2max}}$) among deer mice (*Peromyscus maniculatus*) during treadmill exercise and cold exposure at high altitude (3,800 m). The experiments were based on population samples of subspecies *nebracensis*, *rufinus*, and *sonoriensis*, as well as congenic strains of *nebracensis* and *sonoriensis* that carried different α-globin haplotypes in identical-by-descent condition. Modified from Chappell and Snyder (1984).

tures. Many behavior patterns of deer mice, including foraging, courtship, territorial defense, and predator avoidance probably necessitate substantial exertion ….'' Variation in thermogenic capacity may have especially important fitness consequences in subalpine and alpine environments. Because deer mice do not hibernate, they rely heavily on metabolic heat production to maintain a constant body temperature (Wickler 1980). Thus, severe cold exposure can elicit the maximal rate of heat production (thermogenic capacity), which in an aerobic organism is reflected by $\dot{V}_{O_{2max}}$ (Hayes 1989; Rosenmann et al. 1975; Wickler 1980). Because $\dot{V}_{O_{2max}}$ is markedly impaired under conditions of high-altitude hypoxia (Rosenmann and Morrison 1975; Ward et al. 1995), high-altitude endotherms face a double bind as thermogenic capacity is compromised in an environment where thermoregulatory demands are the most severe. It therefore seems likely that aerobic performance is subject to strong directional selection in high-altitude populations of small-bodied, endothermic animals such as deer mice. In fact, such selection has been empirically documented in a survivorship study of high-altitude deer mice in the White Mountains of eastern California (Hayes and O'Connor 1999).

Altitudinal patterns of hemoglobin polymorphism.—The ''high oxygen affinity'' a^0c^0/a^0c^0 genotype is associated with superior physiological performance under hypoxic conditions at 3,800 m above sea level, but is associated with poor performance (relative to the a^1c^1/a^1c^1 genotype) in the oxygen-rich environment at 340 m. In both altitudinal extremes, the a^0c^0/a^1c^1 heterozygotes were generally intermediate with respect to both P_{50} and $\dot{V}_{O_{2max}}$ (Chappell et al. 1988; Chappell and Snyder 1984). This rank order of genotypic effects is probably due to the fact that the possession of high-affinity $a^0{}_2\beta_2$ or $c^0{}_2\beta_2$ hemoglobin isoforms facilitates pulmonary oxygen loading in high-altitude environments with low P_{O_2}, but hinders the release of oxygen in low-altitude environments with relatively high P_{O_2}. On the basis of these physiological tradeoffs between oxygen transport efficiency at different altitudes, the high-affinity a^0c^0/a^0c^0 genotype should be favored in high-altitude populations, whereas the low-affinity a^1c^1/a^1c^1 genotype should be favored in low-altitude populations. In fact, electrophoretic surveys of α-globin variation in deer mice from western North America have revealed a pattern consistent with this idea: the high-affinity a^0c^0 haplotype is present at relatively high frequency in high-altitude environments (>2,750 m), whereas the a^1c^1 haplotype is either fixed or nearly fixed in low-altitude environments (<1,750 m; Fig. 7).

This system represents a unique case where fitness-related variation in whole-organism physiology can be related to a relatively simple biochemical phenotype (blood oxygen affinity) that has a well-characterized genetic basis. The study of deer mouse hemoglobins at the DNA sequence level thus provides a unique opportunity to elucidate the molecular underpinnings of physiological adaptation. In fact, a recent study of sequence variation in the α-globin gene duplicates of *P. maniculatus* has identified the specific amino acid changes that are responsible for the divergent fine-tuning of hemoglobin function between different elevational zones (J. F. Storz, in litt.) By comparing the DNA sequences of functionally distinct α-globin alleles that exhibit frequency differences between high- and low-altitude populations, this study revealed that adaptive modifications of protein function are primarily attributable to the independent or joint effects of 5 amino acid replacement mutations in the *Hba* gene that modulate steric hindrance to oxygen binding: 50(CD15)His/Pro, 57(E6)Gly/Ala, 60(E9)Ala/Gly, 64(E13)Asp/Gly, and 71(EF1)Gly/Ser. Detailed functional studies are now required to assess whether the additive or epistatic effects of all 5 *Hba* mutations are required to produce the adaptive shift in hemoglobin–oxygen affinity. Alternatively, if just 1 or 2 mutations are directly responsible for the shift in hemoglobin–oxygen affinity, then the remaining mutations may have undergone altitudinal changes in allele frequency simply as a result of genetic hitchhiking.

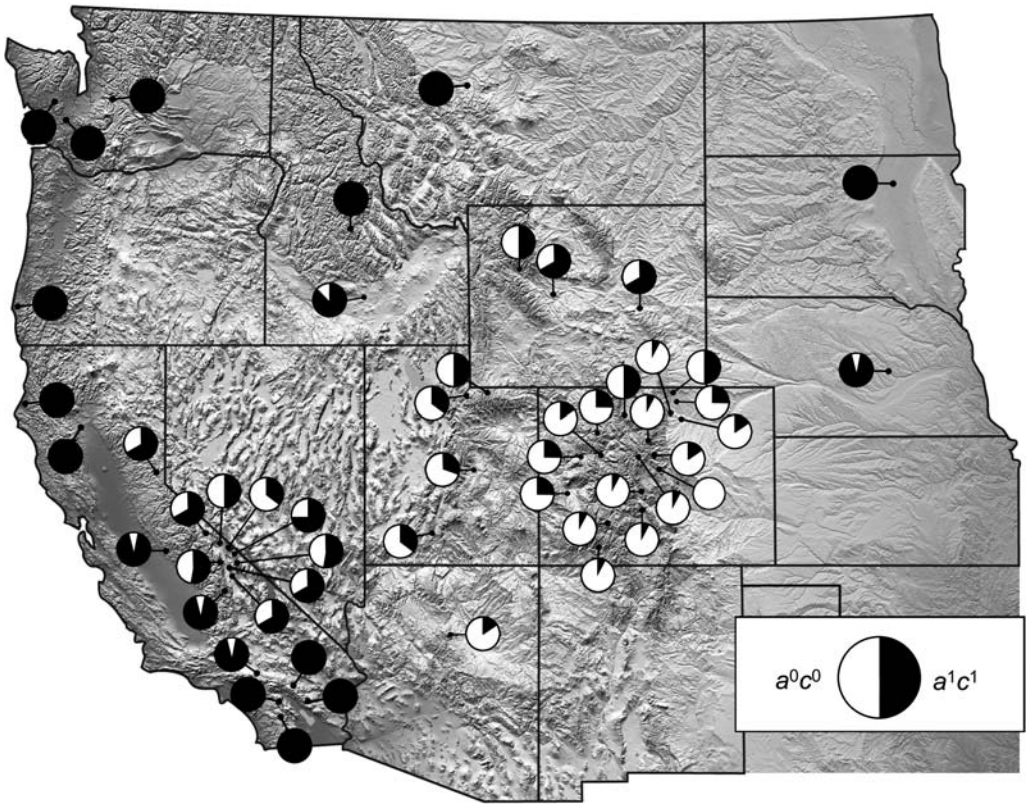

FIG. 7.—Geographic variation in the frequency of 2-locus α-globin haplotypes of *Peromyscus maniculatus*. The a^0c^0 haplotype is present at relatively high frequency in samples from high-altitude localities (e.g., the Southern Rockies of Colorado and other montane regions). By contrast, the a^1c^1 haplotype is present at relatively high frequency in low-altitude localities along the West Coast and in the plains. Based on data compiled by Snyder et al. (1988).

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