Commentary: Hierarchical reductionism approach to understanding adaptive variation in animal performance

Oliver H. Wearing and Graham R. Scott

Department of Biology, McMaster University, Hamilton ON L8S 4K1, Canada

* Corresponding author

Email address: wearingo@mcmaster.ca

Abstract

Aerobic capacity is a complex performance trait with important consequences for fitness, and is determined by the integrated function of the O_2 transport pathway. The components of the O_2 pathway interact and function as an integrated physiological system, which could strongly influence the contribution of each component to variation in aerobic capacity. In this commentary, we highlight the value of hierarchical reductionism – combining studies of how component parts work in isolation with studies of how components interact within integrated systems – for understanding the evolution of aerobic capacity. This is achieved by focussing on the role of haemoglobin in adaptive increases in aerobic capacity in high-altitude deer mice (*Peromyscus maniculatus*). High-altitude deer mice have evolved increased aerobic capacity in hypoxia, in association with evolved changes in several subordinate traits across the O_2 pathway. This includes an evolved increase in Hb-O_2 affinity – which helps safeguard arterial O_2 saturation in hypoxia – and reductionist approaches have been successful at identifying the genetic, structural, and biochemical underpinnings of variation in this trait. However, theoretical modelling and empirical measurements suggest that increased Hb-O_2 affinity may not augment aerobic capacity on its own. The adaptive benefit of increased Hb-O_2 affinity in high-altitude deer mice appears to have been contingent upon antecedent changes in other traits in the O_2 pathway, particularly an increased capacity for O_2 diffusion and utilization in active tissues. These findings highlight the importance of understanding the interactions between the components of integrated systems for fully appreciating the evolution of complex performance phenotypes.

Key words: Aerobic performance, high-altitude adaptation, biochemical adaptation, oxygen transport pathway, thermogenesis

Introduction

"If we wish to understand how a machine or living body works, we look to its component parts and ask how they interact with each other. If there is a complex thing that we do not yet understand, we can come to understand it in terms of simpler parts that we do already understand." – Richard Dawkins (1986)

Aerobic capacity is a complex organismal trait that often has important consequences for fitness, as it determines the capacity to consume oxygen to support metabolically demanding processes such as locomotion and thermogenesis (Hayes and O'Connor, 1999; Plaut, 2001; Storz et al., 2019). Aerobic capacity is determined by the integrated function of the oxygen transport pathway, the conceptual series of steps that transport O_2 from the environment to mitochondria, namely ventilation, pulmonary diffusion, circulation, tissue diffusion, and mitochondrial O_2 utilization (Scott and Dalziel, in review; Weibel, 1984). Evolved variation in these steps in the O_2 pathway have been associated with adaptive increases in aerobic capacity (McClelland and Scott, 2019; Storz and Cheviron, 2021). However, steps in the O_2 pathway interact and must function as an integrated physiological system. This raises the important question: are the adaptive benefits of changes in some traits contingent on the characteristics of others? If so, the evolution of aerobic performance would be an emergent property of systems-level function that may be hard to fully understand by only studying its component parts (i.e., steps in the O_2 pathway) in isolation. Blind reductionism could therefore leave one unaware of the potential interactions within physiological systems that determine animal performance. Here, we advocate the approach of hierarchical reductionism for examining the evolution of complex traits such as aerobic capacity. As reflected in the above quote, hierarchical reductionism combines studies of how component parts work (e.g., biochemical, molecular, and cellular studies) with studies of how component parts interact within the integrated system in the organism (e.g., systems-level and organismal studies). This approach is practiced successfully by many physiologists, and we will illustrate it here by considering the case study of how evolved changes in haemoglobin function contribute to adaptive variation in aerobic capacity in high-altitude deer mice (*Peromyscus maniculatus*).

Endotherms native to high altitude are ideal for studying the mechanisms underlying the evolution of aerobic performance. At high altitude, cold temperatures increase the O_2 demands of thermogenesis to support thermoregulation (Hayes, 1989), which must be maintained concurrent with the metabolic demands of foraging, reproduction, and the performance of other important activities that could affect fitness (Sears et al., 2006). Such increases in O_2 demand must be met despite a low ambient O_2 availability ('hypoxia'), such that animals residing at high altitudes can be exposed to significant selection pressure for increased aerobic capacity for thermogenesis (i.e., thermogenic $\dot{V}O_2max$) and/or exercise in hypoxia (Hayes and O'Connor, 1999). Indeed, several high-altitude taxa have evolved increased aerobic capacity in hypoxia relative to their lowland counterparts (Brutsaert, 2007; Monge and Leon-Velarde, 1991; Moore, 2017; Storz and Scott, 2019). Studies of humans, non-human mammals, and birds have shown that adaptive variation in aerobic performance is underlain by evolved changes across the O_2 pathway (Brutsaert, 2007; Ivy and Scott, 2015; McClelland and Scott, 2019; Monge and Leon-Velarde, 1991; Storz et al., 2019; Storz and Scott, 2019).

One of the most pervasive features of mammals and birds that have adapted to high altitude are genetically based increases in haemoglobin(Hb)-O_2 affinity (Storz, 2016; Storz, 2019; Weber, 2007; Winslow, 2007). While it may initially seem intuitive that changes in Hb protein function should safeguard arterial O_2 saturation in hypoxia and thus improve O_2 transport, the benefits of increasing Hb-O_2 affinity for aerobic capacity in hypoxia are controversial (Dempsey, 2020). On the one hand, mathematical models of the O_2 pathway have suggested that increases in Hb-O_2 affinity are not sufficient on their own to increase aerobic capacity in humans (Wagner, 1996a, b, 1997). On the other hand, for example, a recent study demonstrated that humans possessing a rare, high-affinity Hb variant maintain higher aerobic capacities in hypoxia than subjects with normal Hb (Dominelli et al., 2020). The reasons for such discrepancies remain unclear, but they suggest that the benefit of increasing Hb-O_2 affinity for improving aerobic performance in hypoxia are context dependent and may be contingent upon other traits in the O_2 pathway (the characteristics of which may differ between studies and experimental subjects/taxa). It is also possible that in some cases increases in Hb-O_2 affinity do not serve to increase aerobic capacity and have an alternative role in the organism. Unfortunately, work in comparative genomics sometimes ascribes adaptive significance to variation in haemoglobins and other genes without any empirical functional evidence, which can

sometimes lead to faulty conclusions, as previously shown (Cheviron et al., 2014). Therefore, consideration of the role of haemoglobin in adaptive variation in aerobic capacity is useful for appreciating the value of a hierarchical reductionism approach.

The North American deer mouse (*Peromyscus maniculatus*) presents a compelling opportunity to examine these issues. This species can be found across a wide elevational range, from around sea level to over 4300m elevation in the Rocky Mountains (Natarajan et al., 2015; Snyder et al., 1982). Thermogenic $\dot{V}O_2max$ has been shown to be under strong directional selection in high-altitude populations (Hayes and O'Connor, 1999), leading to evolved increases in aerobic capacity in hypoxia (Cheviron et al., 2013; Lui et al., 2015). Highland deer mice have also evolved increased Hb-O_2 affinity compared to lowland conspecifics (Chappell et al., 1988; Chappell and Snyder, 1984; Ivy et al., 2020; Jensen et al., 2016; Natarajan et al., 2015; Natarajan et al., 2013; Snyder et al., 1988; Snyder, 1981; Snyder et al., 1982; Storz et al., 2010; Storz et al., 2009). In this commentary, we will consider how the evolution of increased Hb-O_2 affinity may contribute to adaptive increases in thermogenic $\dot{V}O_2max$ in high-altitude deer mice, using it as an instructive case study to illustrate how hierarchical reductionism can be used to provide an in-depth understanding of the evolution of animal performance.

Determinants of adaptive variation in aerobic capacity across the O_2 pathway

The enhanced thermogenic $\dot{V}O_2max$ of highland deer mice results in large part from an accentuation of the plastic response to chronic hypoxia. Specifically, chronic exposure to hypoxia increases thermogenic $\dot{V}O_2max$ in hypoxia (relative to normoxic controls) by a much greater magnitude in highland deer mice than in lowland deer mice or white-footed mice (Tate et al., 2017; Tate et al., 2020). As a result, thermogenic $\dot{V}O_2max$ in hypoxia is up to 70% greater in highlanders than in lowlanders after hypoxia acclimation. Measurements across the O_2 pathway suggest that this superior aerobic capacity of highlanders is attributable to the evolution of accentuated plasticity in some steps in the O_2 pathway, in conjunction with evolved changes in the trait means for other steps in the O_2 pathway. Cardiac output at $\dot{V}O_2max$ increases in chronic hypoxia by a greater magnitude in highlanders than in lowlanders, primarily as a result of increases in stroke volume (Tate et al., 2017; Tate et al., 2020). Blood haemoglobin content increases in chronic hypoxia, but the magnitude tends to be less in highlanders than in lowlanders (Lui et al., 2015; Tate et al., 2020). Arterial O_2 saturation (the proportion of Hb in

arterial blood that is bound by O_2) is greater in highlanders than in lowlanders, but is unaffected by hypoxia acclimation (Tate et al., 2017; Tate et al., 2020). The combined influence of each of these traits leads to an accentuated increase in circulatory O_2 delivery after chronic hypoxia in highlanders compared to lowlanders (Tate et al., 2020). In contrast, several other traits in the O_2 pathway are greater in highlanders than in lowlanders but they do not exhibit greater plasticity in highlanders, namely pulmonary O_2 extraction and O_2 diffusing capacity, tissue O_2 extraction and muscle capillarity, and muscle oxidative capacity (Dawson et al., 2018; Lui et al., 2015; Mahalingam et al., 2020; Mahalingam et al., 2017; Scott et al., 2015; Scott et al., 2018; Tate et al., 2020; West et al., 2021).

The observation that arterial O_2 saturation is greater in highlanders compared to lowlanders is likely explained at least in part by the evolved increase in Hb-O_2 affinity. Molecular and genetic approaches have provided valuable insights into the biochemical mechanisms underlying this evolved change in Hb function. The specific amino acid replacements that are responsible for evolved changes in Hb-O_2 affinity have been identified (Storz et al., 2012; Storz et al., 2010; Storz et al., 2009) and crystallographic studies have revealed the biophysical mechanisms by which mutations exert their effects (Inoguchi et al., 2017; Inoguchi et al., 2013; Natarajan et al., 2015). Protein engineering has shown that these modifications lead to increases in Hb-O_2 affinity for isolated protein *in vitro*, without affecting the Bohr effect (Jensen et al., 2016; Natarajan et al., 2013). Measurements of Hb-O_2 affinity in erythrocytes and in blood have shown that these biochemical differences are realized at cell and tissue levels (Chappell et al., 1988; Chappell and Snyder, 1984; Ivy et al., 2020; Snyder et al., 1982). However, the key issue we address here in this commentary is the extent to which these biochemical differences in Hb function impact systems-level respiratory traits (i.e., arterial O_2 saturation) and VO_2max, in absence of and/or in combination with the other evolved changes in the O_2 pathway discussed above.

As a first step in considering the role of Hb adaptations on $\dot{V}O_2$max, we have examined the combined influence of evolved differences in the several traits underpinning circulatory O_2 delivery and tissue O_2 extraction, using a graphical analysis with what has become known as a 'Johansen Plot' in reference to the late physiologist Kjell Johansen (Fig. 1) (Milsom et al., 2021). The left panel shows O_2 equilibrium curves for highland deer mice and lowland white-footed mice in chronic hypoxia, created using our previous measurements of blood haemoglobin

content ([Hb]) (Tate et al., 2020) and erythrocyte O_2 binding characteristics (Ivy et al., 2020). This panel shows the combined effects of highlanders having higher Hb-O_2 affinity (lower P_{50}; reflected by a leftwards shift in the curve) and lower blood [Hb] (reflected by a lower plateau in the curve, due to a lower O_2 carrying capacity). Based on the Fick equation, $\dot{V}O_2max$ is equal to the product of cardiac output and the difference between arterial O_2 content (C_aO_2) and venous O_2 content (C_vO_2), all of which were determined previously (Tate et al., 2020). The right panel shows these relationships for highland and lowland mice in chronic hypoxia, in which the height of each box represents the difference between C_aO_2 and C_vO_2, and the area of each box represents $\dot{V}O_2max$. The height of the box relative to C_aO_2 reflects relative tissue O_2 extraction (E_TO_2). Shown in this manner, population differences in cardiac output appear to be a key determinant of differences in $\dot{V}O_2max$, and the higher arterial O_2 saturation (along with slightly higher arterial O_2 tension) in highlanders appears to help offset the reduction in C_aO_2 caused by lower blood [Hb]. However, this analysis does not determine the influence of variation in individual traits such as Hb-O_2 affinity on $\dot{V}O_2max$. It is also unable to shed light on potential interactions between traits that underlie adaptive increases in $\dot{V}O_2max$, and whether the adaptive benefit of some traits are contingent upon the evolution of some others. Such issues are better addressed by experimental designs and/or theoretical approaches in which individual traits can be altered independently and in combination with others to determine their effects on $\dot{V}O_2max$.

The role of haemoglobin evolution in adaptive variation in VO_2max

To help further examine how $\dot{V}O_2max$ in hypoxia is affected by adaptations in Hb-O_2 affinity on their own, we again make use of a Johansen Plot (Fig. 2). The left panel shows O_2 equilibrium curves for highland deer mice and lowland white-footed mice, but in this case both were generated using the same blood [Hb] of lowlanders in chronic hypoxia. The panel on the right shows the $\dot{V}O_2max$, as reflected by the areas of the boxes, that would result for each Hb-O_2 affinity for similar lowland values of cardiac output and O_2 tensions in arterial blood (P_aO_2) and venous blood (P_vO_2). When all else is equal in this scenario, P_{50} has no effect on $\dot{V}O_2max$. Reducing C_vO_2 would increase $\dot{V}O_2max$, such as would be expected to result from an increase in tissue O_2 diffusing capacity, and is calculated in Fig. 2 for the C_vO_2 achieved in lowlanders. However, this graphical analysis cannot account for the potential effects of changing P_{50} on

178 P_vO_2, and the results could also vary at greater magnitudes of tissue O_2 extraction as P_vO_2
179 approaches the flatter region of the O_2 equilibrium curve.
180 Direct empirical insights into the influence of Hb adaptations on $\dot{V}O_2max$ come from
181 studies of inter-population hybrids of deer mouse populations from high and low altitudes
182 (Chappell and Snyder, 1984; Wearing et al., in press). Although the effects of Hb-O_2 affinity on
183 its own cannot be studied by comparing highland versus lowland deer mice, because many other
184 respiratory traits co-vary between populations, controlled breeding approaches provide an
185 opportunity to study the effects of genetically based variation in Hb function on a common
186 genetic background. In one previous study, Chappell and Snyder (1984) backcrossed highland or
187 lowland α-globin genotypes into a highland genetic background. Mice homozygous for highland
188 α-globin were thus shown to have a higher Hb-O_2 affinity and significantly higher $\dot{V}O_2max$ in
189 high-altitude hypoxia. In a second study, we and colleagues developed F_2 interpopulation hybrids
190 to evaluate the effects of α- and β-globin variants on an admixed genetic background, in which
191 each individual mouse has an approximately even mix of genetic material from highland and
192 lowland grandparents (Wearing et al., in press). In contrast to the results of Chappell and Snyder
193 (1984), we found that highland Hb variants increased Hb-O_2 affinity and improved arterial O_2
194 saturation in hypoxia, but they did *not* confer an associated increase in $\dot{V}O_2max$ in hypoxia.
195 Together, these findings suggest that increased Hb-O_2 affinity can help augment $\dot{V}O_2max$ in
196 hypoxia, but only in the highland genetic background. In other words, the adaptive benefit of
197 increased Hb-O_2 affinity may be contingent upon pre-existent changes in other traits in highland
198 mice.
199 We examined what other traits may determine the relative influence of Hb-O_2 affinity
200 using theoretical modelling of the O_2 pathway (Wearing et al., in press). Scrutinizing the
201 Johansen Plot in Fig. 2, the influence of increased Hb-O_2 affinity and the associated increase in
202 arterial O_2 saturation on $\dot{V}O_2max$ could become apparent at lower P_vO_2. This is because
203 increased Hb-O_2 affinity leads to a greater C_aO_2-C_vO_2 difference as venous O_2 content nears full
204 depletion. We therefore predicted that effects of Hb-O_2 affinity on $\dot{V}O_2max$ in hypoxia would be
205 revealed at higher levels of tissue O_2 extraction. We tested this prediction using mathematical
206 modelling of the O_2 pathway in our F_2 interpopulation hybrids, by examining the effects of
207 increasing tissue O_2 diffusing capacity (D_TO_2) on $\dot{V}O_2max$ in hypoxia. We found that as D_TO_2
208 was increased by up to 50%, there was a progressive increase in the advantage of highland Hb to

$\dot{V}O_2$max. Therefore, in deer mice adapting to high altitude, the adaptive benefit of increased Hb-O_2 affinity may have been contingent upon evolved increases in the capacity of active tissues to extract and consume O_2 from the blood. Indeed, tissue O_2 extraction at hypoxic $\dot{V}O_2$max is higher in highland deer mice than in lowland deer mice or white-footed mice (Tate et al., 2020), likely achieved at least in part from numerous evolved changes in muscle phenotype, including increases in capillarity, densities of oxidative muscle fibres, mitochondrial abundance, and respiratory capacities (Dawson et al., 2018; Lui et al., 2015; Mahalingam et al., 2020; Mahalingam et al., 2017; Scott et al., 2015; Scott et al., 2018). Our finding that the adaptive benefit of increased Hb-O_2 affinity may depend on other traits along the O_2 pathway would have been impossible to uncover without empirical and theoretical approaches to probe systems-level physiological function. This emphasizes the importance of considering the potential emergent properties of physiological systems and of placing findings on lower levels of biological organization into an integrative framework when investigating the evolution of complex performance traits.

Conclusions

Hierarchical reductionism provides a framework for understanding the evolution of complex performance traits such as aerobic capacity. Studies at lower levels of biological organization are extremely valuable in determining the mechanisms that underlie adaptive variation in complex performance phenotypes, but they need to be considered in the context of how they interact within integrated physiological systems. Changes that appear to result in straight-forward physiologically significant effects when observed at lower levels of biological organization may lead to interactions that result in complex emergent effects at higher levels of organization. Integrative physiological measurements, controlled breeding and genetic manipulation, and mathematical modelling of systems-level function can be used to appreciate such interactions within complex physiological systems. Hierarchical reductionism thus helps provide a richer and more nuanced understanding of the evolution of animal performance.

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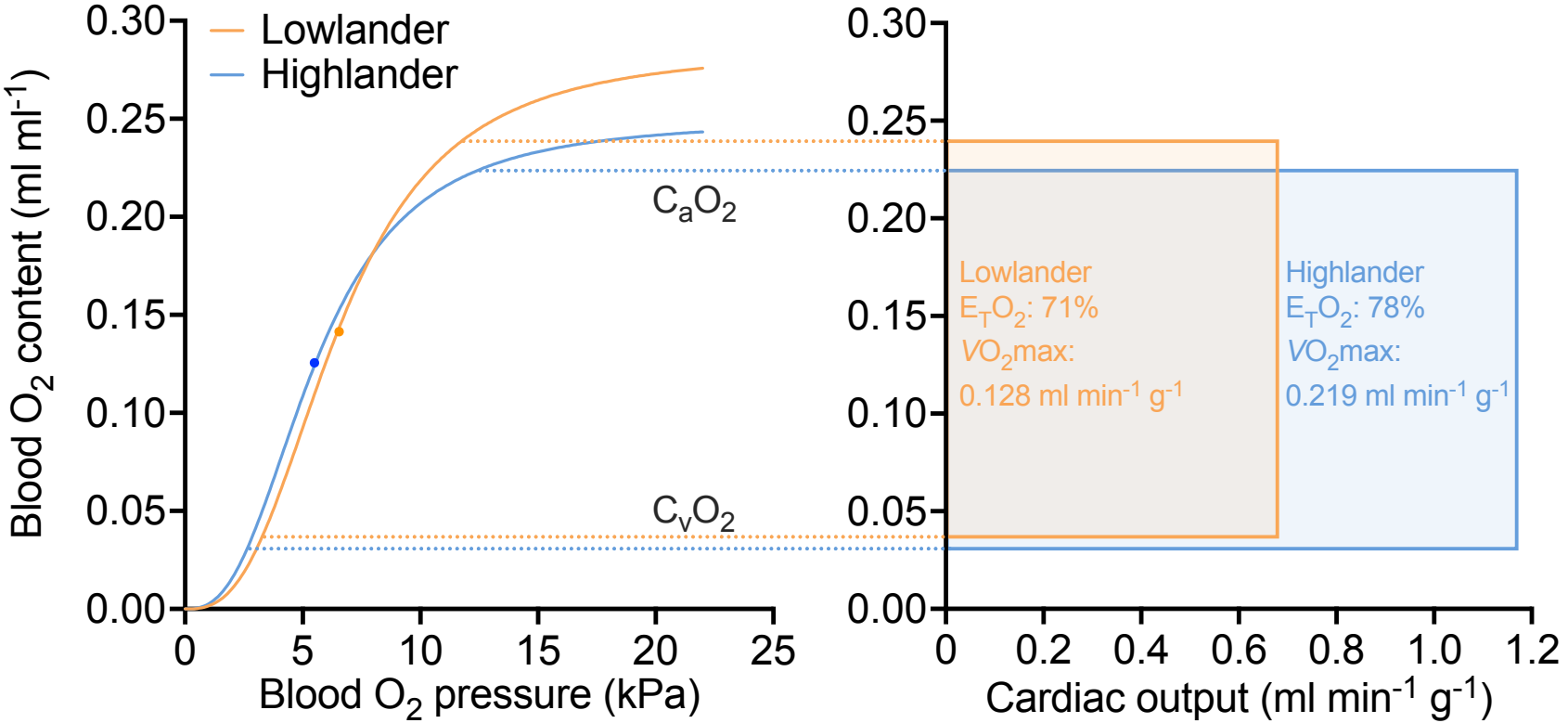
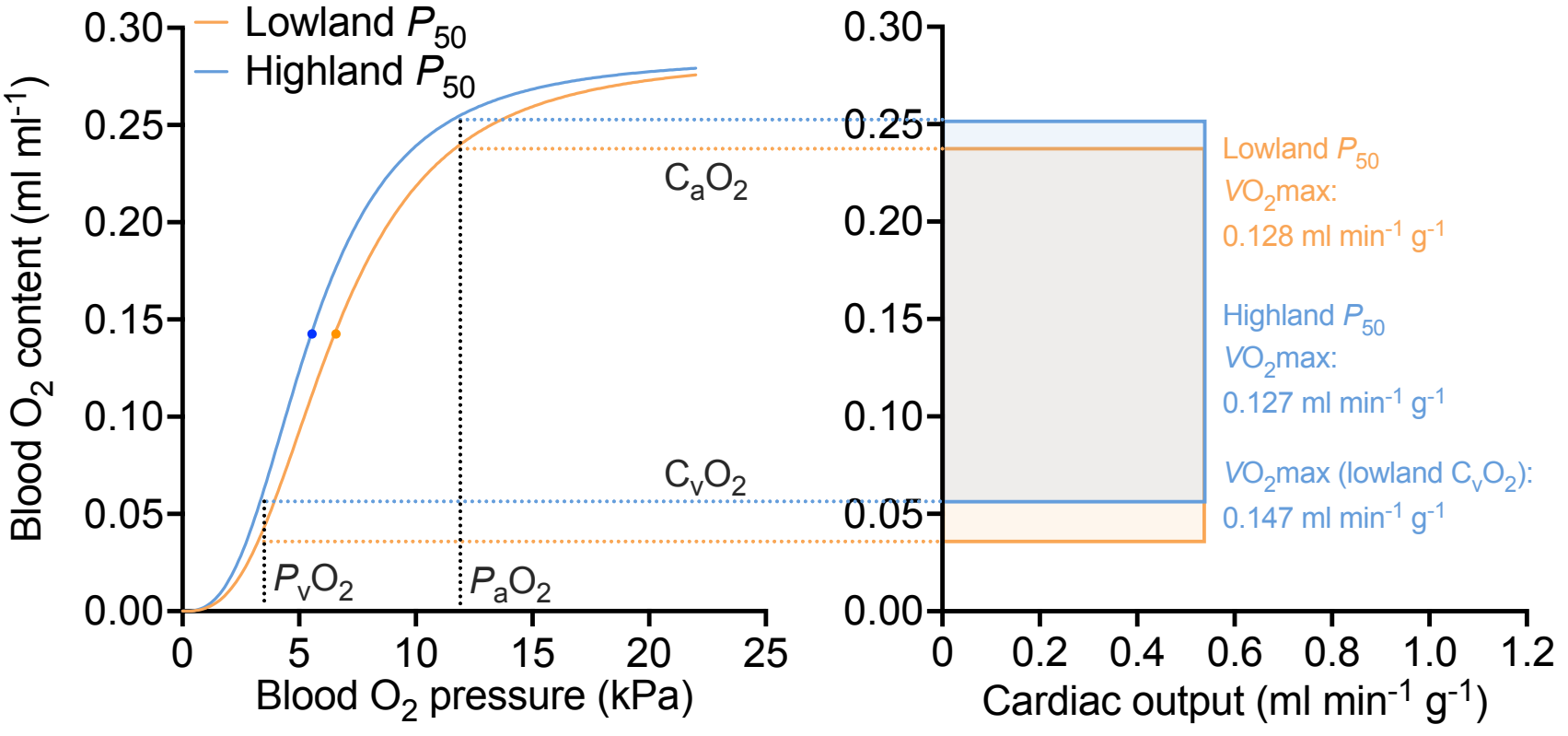
Figure legends

Figure 1. Johansen plot illustrating the contributions of traits underlying circulatory O_2 delivery and tissue O_2 extraction on the evolved increase in $\dot{V}O_2max$ in hypoxia in high-altitude deer mice. Data are for high-altitude deer mice and low-altitude white-footed mice acclimated to chronic hypoxia. The circle on each O_2 equilibrium curve in the left panel represents the Hb-O_2 affinity (P_{50}) measured in erythrocytes of highlanders (P_{50} of 5.5 kPa) and lowlanders (P_{50} of 6.5 kPa). The data used to generate these figures were obtained or calculated from data reported by Ivy et al. (2020) and Tate et al. (2020). C_aO_2, arterial O_2 content; C_vO_2, venous O_2 content; E_TO_2, tissue O_2 extraction. See text for additional details.

Figure 2. Johansen plot illustrating the potential effects of haemoglobin-O_2 affinity on $\dot{V}O_2max$ in hypoxia in deer mice. The O_2 equilibrium curves in the left panel were generated using the Hb-O_2 affinity (P_{50}) measured in erythrocytes of high-altitude deer mice (P_{50} of 5.5 kPa) and low-altitude white-footed mice (P_{50} of 6.5 kPa) and the blood haemoglobin content of lowlanders in chronic hypoxia, and each P_{50} is represented by a circle. The panel on the right shows the $\dot{V}O_2max$ (represented by the areas of the boxes) that would result for each P_{50} for similar lowland values of cardiac output and O_2 tension in arterial blood (P_aO_2) and venous blood (P_vO_2). It also illustrates the greater $\dot{V}O_2max$ that would result for the highland P_{50} at the venous O_2 content achieved in lowlanders. C_aO_2, arterial O_2 content; C_vO_2, venous O_2 content. The data used to generate these figures were obtained or calculated from data reported by Ivy et al. (2020) and Tate et al. (2020). See text for additional details.


Lowlander
Highlander
Blood O_2 content (ml ml^{-1})
0.30
0.25
0.20
0.15
0.10
0.05
0.00
C_aO_2
C_vO_2
0
5
10
15
20
25
Blood O_2 pressure (kPa)
Lowlander
E_TO_2: 71%
$\dot{V}O_2$max:
0.128 ml min^{-1} g^{-1}
Highlander
E_TO_2: 78%
$\dot{V}O_2$max:
0.219 ml min^{-1} g^{-1}
0
0.2
0.4
0.6
0.8
1.0
1.2
Cardiac output (ml min^{-1} g^{-1})


Lowland P_{50}
Highland P_{50}
Blood O_2 content (ml ml^{-1})
0.30
0.25
0.20
0.15
0.10
0.05
0.00
C_aO_2
C_vO_2
P_vO_2
P_aO_2
0
5
10
15
20
25
Blood O_2 pressure (kPa)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Lowland P_{50}
VO_2max:
0.128 ml min^{-1} g^{-1}
Highland P_{50}
VO_2max:
0.127 ml min^{-1} g^{-1}
VO_2max (lowland C_vO_2):
0.147 ml min^{-1} g^{-1}
0
0.2
0.4
0.6
0.8
1.0
1.2
Cardiac output (ml min^{-1} g^{-1})