

## COMMENTARY

### Elevated performance: the unique physiology of birds that fly at high altitudes

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Accepted 9 May 2011

#### Summary

**Birds that fly at high altitudes must support vigorous exercise in oxygen-thin environments. Here I discuss the characteristics that help high fliers sustain the high rates of metabolism needed for flight at elevation. Many traits in the O<sub>2</sub> transport pathway distinguish birds in general from other vertebrates. These include enhanced gas-exchange efficiency in the lungs, maintenance of O<sub>2</sub> delivery and oxygenation in the brain during hypoxia, augmented O<sub>2</sub> diffusion capacity in peripheral tissues and a high aerobic capacity. These traits are not high-altitude adaptations, because they are also characteristic of lowland birds, but are nonetheless important for hypoxia tolerance and exercise capacity. However, unique specializations also appear to have arisen, presumably by high-altitude adaptation, at every step in the O<sub>2</sub> pathway of highland species. The distinctive features of high fliers include an enhanced hypoxic ventilatory response, an effective breathing pattern, larger lungs, haemoglobin with a higher O<sub>2</sub> affinity, further augmentation of O<sub>2</sub> diffusion capacity in the periphery and multiple alterations in the metabolic properties of cardiac and skeletal muscle. These unique specializations improve the uptake, circulation and efficient utilization of O<sub>2</sub> during high-altitude hypoxia. High-altitude birds also have larger wings than their lowland relatives to reduce the metabolic costs of staying aloft in low-density air. High fliers are therefore unique in many ways, but the relative roles of adaptation and plasticity (acclimatization) in high-altitude flight are still unclear. Disentangling these roles will be instrumental if we are to understand the physiological basis of altitudinal range limits and how they might shift in response to climate change.**

Key words: avian, bar-headed goose, oxygen cascade, respiratory physiology, pulmonary diffusion, capillary, mitochondria.

#### Introduction

High-altitude environments pose numerous challenges to animal life. The physical environment changes dramatically on ascent, with declines in oxygen availability, temperature, air density and humidity. Despite these challenges, many animals live successfully in the high mountains. Birds are particularly diverse in montane regions – many live at over 4000m above sea level and some surmount the world's highest mountain peaks during their migration (Fig. 1). Although some species are unique to high elevation, others are found across broad elevational gradients (McCracken et al., 2009b).

The decreases in total barometric pressure (hypobaria) and O<sub>2</sub> partial pressure (hypoxia) at high altitude are inescapable, unlike elevational declines in temperature and humidity, which can be buffered by local climatic variation. Hypobaria has unique consequences for flying animals, because the mechanical power output needed to sustain lift increases in thin air (Altshuler and Dudley, 2006). This amplifies the already high metabolic rates needed for flapping flight (Chai and Dudley, 1995) in an environment where the O<sub>2</sub> available to fuel metabolism is limited. According to Tucker, “some birds perform the strenuous activity of flapping flight at altitudes in excess of 6100m, an altitude at which resting, unacclimated man is in a state of incipient hypoxic collapse” (Tucker, 1968). How then can O<sub>2</sub> supply processes meet the high O<sub>2</sub> demands of flight at high altitudes? What unique physiological characteristics allow the highest-flying species (Fig. 1) to sustain the most metabolically costly form of vertebrate locomotion at elevations that can barely support life in many other animals?

In order to properly address these questions, one must consider the properties of the pathway that transports O<sub>2</sub> from the environment to the sites of O<sub>2</sub> demand throughout the body. This pathway is composed of a series of cascading physiological ‘steps’ (Fig. 2): (1) ventilation of the lungs with air; (2) diffusion of O<sub>2</sub> across the pulmonary gas-exchange surface, from the air to the blood; (3) circulation of O<sub>2</sub> throughout the body in the blood; (4) diffusion of O<sub>2</sub> from the blood to mitochondria in tissues (the pectoralis muscle is the primary site of O<sub>2</sub> consumption during flight); and (5) metabolic utilization of O<sub>2</sub> to generate ATP by oxidative phosphorylation. Although not a strict component of the O<sub>2</sub> transport cascade, properties of intracellular ATP turnover will also have important consequences for matching O<sub>2</sub> supply and O<sub>2</sub> demand. Not surprisingly, the answer to how birds fly at high altitudes lies, at least partly, in the characteristics of this pathway.

The objective of this Commentary is to review the importance of both ancestral and derived characteristics in the O<sub>2</sub> transport pathway of birds that fly at high altitudes. Many features of birds in general probably endowed high fliers with numerous exaptations (also known as pre-adaptations), but many uniquely derived and presumably adaptive traits also appear to be important for high-altitude flight.

#### The benefits of being avian

The hypoxia tolerance of birds has frequently been suggested to be greater than that of mammals. Although some ectothermic vertebrates are even more tolerant of hypoxia, birds possess a relatively high tolerance when considering the increase in

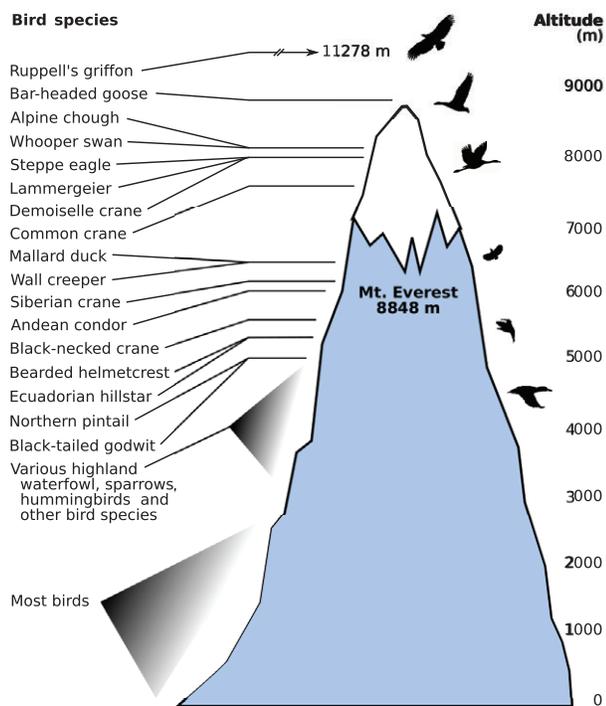


Fig. 1. Although most birds live and fly at relatively low altitudes, species from several avian orders live, migrate or occasionally ascend much higher. These include multiple species of raptor, waterfowl, crane, passerine, hummingbird and others. The highest flight altitudes reported from various sources in the literature are shown here (Eastwood and Rider, 1965; Swan, 1970; Faraci, 1986; Faraci, 1991; del Hoyo et al., 1999; Kanai et al., 2000; McCracken et al., 2009b).

metabolic demands associated with endothermy. Early work showed that lowland house sparrows (*Passer domesticus*) behaved normally, and could even fly for short periods in a wind tunnel, at a simulated altitude of 6100 m (Tucker, 1968). In contrast, mice were comatose and unable to maintain body temperature at the same simulated altitude. Comparisons of the few species for which tolerance (survival) data are available also support the suggestion that birds are more tolerant of hypoxia than mammals (Thomas et al., 1995). However, this issue has yet to be addressed with rigorous phylogenetic comparisons that incorporate species in both groups that are adapted to hypoxia.

The  $O_2$  transport pathway of birds has several distinctive characteristics that should support a greater capacity for vigorous activity and aerobic metabolism during hypoxia (Fig. 2). Increases in breathing (i.e. ventilation) are an important response of the respiratory system to hypoxia, and the magnitude of this response is dictated primarily by the partial pressures of  $O_2$  and  $CO_2$  and the pH of arterial blood (Scott and Milsom, 2009). The decline in arterial  $O_2$  tension (hypoxaemia) drives the increase in ventilation, whose secondary consequence is an amplification of  $CO_2$  loss to the environment. This causes hypocapnia (low partial pressure of  $CO_2$  in the blood), which reflexively inhibits breathing and causes an acid–base disturbance. It has been suggested that birds have a higher tolerance of hypocapnia than mammals (Scheid, 1990), which could arise from an ability to rapidly restore blood pH in the face of  $CO_2$  challenges (Dodd et al., 2007) and from the hypocapnic insensitivity of the brain vasculature (see below). The significance of this tolerance is that it would allow birds to breathe more before

depletion of  $CO_2$  in the blood impairs normal function, thus enhancing  $O_2$  transport to the gas-exchange surface.

The structure and function of the lungs is perhaps the best-known advantage of avian respiratory systems. The many distinctive features of bird lungs are the subject of an extensive literature that unfortunately can be dealt with only briefly here (Piiper and Scheid, 1972; Scheid, 1990; Maina, 2006). Air flows in one direction through the gas-exchange units of avian lungs (parabronchioles) and the arrangement of airway and vascular vessels creates a functionally cross-current gas exchanger (Fig. 3A). This differs substantially from the lungs of most other terrestrial vertebrates, in which gases flow in and out of terminal gas-exchange units (alveoli in mammals) such that capillary blood equilibrates with air having a uniform gas composition (uniform pool gas exchanger; Fig. 3B). The important consequence of this difference is that avian lungs can attain a superior efficiency for gas exchange in normoxia and moderate hypoxia (as explained in Fig. 3), although their advantage diminishes as hypoxia becomes severe (Scheid, 1990). The capacity for pulmonary  $O_2$  diffusion is also greater in birds because of the exceptional thinness and large surface area of the exchange tissue. Nevertheless, the diffusion barrier appears to be mechanically stronger in birds than in mammals, so pulmonary blood flow and pressure can increase without causing stress failure (West, 2009). Each of these distinctive features of avian lungs should improve  $O_2$  loading into the blood during hypoxia.

The capacity for delivering  $O_2$  throughout the body in the systemic circulation may be higher in birds than in other vertebrates. Birds have larger hearts and cardiac stroke volumes than mammals of similar body size (Grubb, 1983), suggesting that birds are capable of higher cardiac outputs. If this were indeed the case, birds would have an enhanced capacity for convective delivery of  $O_2$  in the blood during hypoxia. Cardiac output increases sevenfold to eightfold during flight (Peters et al., 2005) and threefold or more during hypoxia at rest (Black and Tenney, 1980), but maximum cardiac output has yet to be determined in birds, particularly during flight in hypoxia.

The distribution of blood flow throughout the body has consequences for hypoxia tolerance, and the mechanisms regulating this distribution are altered in birds compared with mammals. Hypoxaemia *per se* causes a preferential redistribution of  $O_2$  delivery towards sensitive tissues like the heart and brain and away from more tolerant tissues (e.g. intestines). However, increases in  $O_2$  delivery to the brain are offset in mammals at high altitudes because of the respiratory hypocapnia induced by increases in breathing. This causes a constriction of cerebral blood vessels that can completely abolish the hypoxaemic stimulation of cerebral blood flow. In contrast, the cerebral vessels of birds are insensitive to hypocapnia, such that blood flow is allowed to increase and  $O_2$  delivery is maintained (Faraci, 1991). This and possibly other distinctive features of the avian cerebral circulation (Bernstein et al., 1984) should improve brain oxygenation during hypoxia. Coupled with the inherently higher tolerance of avian neurons to low cellular  $O_2$  levels (Ludvigsen and Folkow, 2009), the central nervous system of birds appears to be well protected from cellular damage induced by a lack of  $O_2$ . Nevertheless, an intriguing question that has yet to be addressed is whether heightened blood flow increases intracranial pressure in birds, as frequently occurs in humans (Wilson et al., 2009). If so, birds may face the secondary challenge of avoiding or tolerating cerebral oedema and other neurological syndromes that can result from excessive intracranial pressure in mammals.

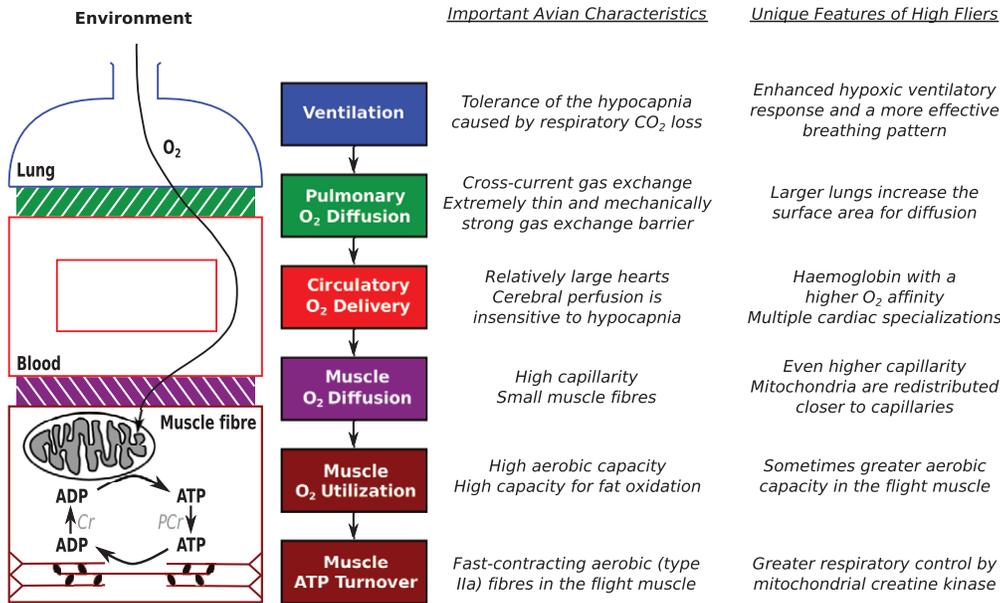


Fig. 2. The transport of O<sub>2</sub> occurs along several steps of a cascading physiological pathway from atmospheric air to the mitochondria in tissue cells (e.g. muscle fibres). The effectiveness of this pathway at transporting O<sub>2</sub> during hypoxia is imperative for flight at high altitudes, which depends upon several distinctive characteristics of birds in general and many unique features that have evolved in high fliers. The properties of O<sub>2</sub> utilization and ATP turnover in the flight muscle are also important to consider in high fliers, such as how ATP equivalents are moved between sites of ATP supply and demand [which can occur via phosphocreatine (PCr) by virtue of the creatine kinase shuttle; see text]. Cr, creatine.

The capacity for O<sub>2</sub> to diffuse from the blood into the tissues is higher in birds compared with mammals and other vertebrates. The best evidence for this difference is the systematically higher ratio of capillary surface area to muscle-fibre surface area in the flight muscle of birds compared with the locomotory muscles of mammals (Mathieu-Costello, 1990). At least two factors account for this difference: (1) the tight mesh of capillaries surrounding avian muscle fibres, due to a high degree of branching between longitudinal vessels, and (2) the smaller aerobic fibres of birds compared with similar-sized mammals (Mathieu-Costello, 1990). The heart and brain also have higher densities of capillaries in birds compared to mammals (Faraci, 1991). Diffusion of O<sub>2</sub> from the blood to the mitochondria in various tissues should therefore be higher in birds than in other vertebrates during hypoxaemia.

Although these distinctive characteristics of birds should enhance hypoxia tolerance by improving the overall capacity for O<sub>2</sub> transport, being avian is not in itself sufficient for flight at high altitudes. The flight muscle of birds has a very high aerobic capacity, by virtue of fast-contracting oxidative fibres (type IIa) that have abundant mitochondria (Mathieu-Costello, 1990; Scott et al., 2009b), and the high rates of metabolism during flight are supported primarily by lipid fuels (Weber, 2009). Lipid oxidation is essential for supporting long-duration flight, but it amplifies the amount of O<sub>2</sub> required to produce a given amount of ATP when compared with carbohydrate oxidation. The metabolic demands of flight are further intensified at high altitudes by hypobaria, which requires that birds flap harder to produce lift (Chai and Dudley, 1995). The implication of these factors is that high-altitude flight requires very high rates of O<sub>2</sub> transport when very little O<sub>2</sub> is available. This is clearly not possible for most lowland birds – many species cannot tolerate severe hypoxia (Black and Tenney, 1980) and some fly long distances to avoid high-elevation barriers during their migration (Irwin and Irwin, 2005). What then are the uniquely derived attributes that differentiate the high fliers?

**The unique attributes of high fliers**

The physiology of birds that fly at high altitudes differs in many ways from that of lowland birds. The basis for this conclusion comes largely from studying the bar-headed goose [*Anser indicus*

(Latham 1790)], a species that can tolerate severe hypoxia [~21 Torr or ~2.8 kPa (1 Torr=133 Pa), equivalent to 12000 m elevation] (Black and Tenney, 1980) and has been seen flying over the Himalayas at nearly 9000 m elevation during its migration between South and Central Asia (Swan, 1970) (Fig. 1). Studies of bar-headed geese have revealed many important insights into the physiological basis for high-altitude flight and, when coupled with comparative phylogenetic approaches, its evolutionary origins. My discussion of the unique attributes of high fliers will focus largely – out of necessity – on this species, but will also highlight work in other species when possible. Most of the previous work looking for inherent differences between high- and low-altitude birds compared animals in a common environment at sea level. This will be the case in the following discussion unless otherwise stated.

It is useful to begin this discussion by outlining the most influential steps in the O<sub>2</sub> transport pathway during exercise in hypoxia. We have assessed this issue in waterfowl using theoretical modeling to calculate the physiological control coefficient for each step in the pathway (Fig. 4) (Scott and Milsom, 2006; Scott and Milsom, 2009). This approach allows physiological traits to be altered individually so that their influence on the whole O<sub>2</sub> pathway can be assessed without compensatory changes in other traits. A physiological trait with a larger control coefficient will have a greater influence on flux through the pathway, so an increase in the capacity of this trait will have a greater overall benefit. Interestingly, the proportion of control vested in each step was dependent on the inspired O<sub>2</sub> (Fig. 4). At sea level (inspired O<sub>2</sub> tensions ~150 Torr) and in moderate hypoxia (~90 Torr, equivalent to ~4500 m elevation), circulatory O<sub>2</sub> delivery capacity (which incorporates both maximum cardiac output and blood haemoglobin concentration) and the capacity for O<sub>2</sub> diffusion in the muscle retained most of the control over pathway flux (Fig. 4). In contrast, ventilation and the capacity for O<sub>2</sub> diffusion in the lungs became much more influential in severe hypoxia (~40 Torr, roughly 9000 m), whereas muscle diffusion remained important and circulatory O<sub>2</sub> delivery capacity became less so (Fig. 4). These results suggest that every step in the O<sub>2</sub> transport pathway can be influential and that the relative benefit of each step changes with altitude.

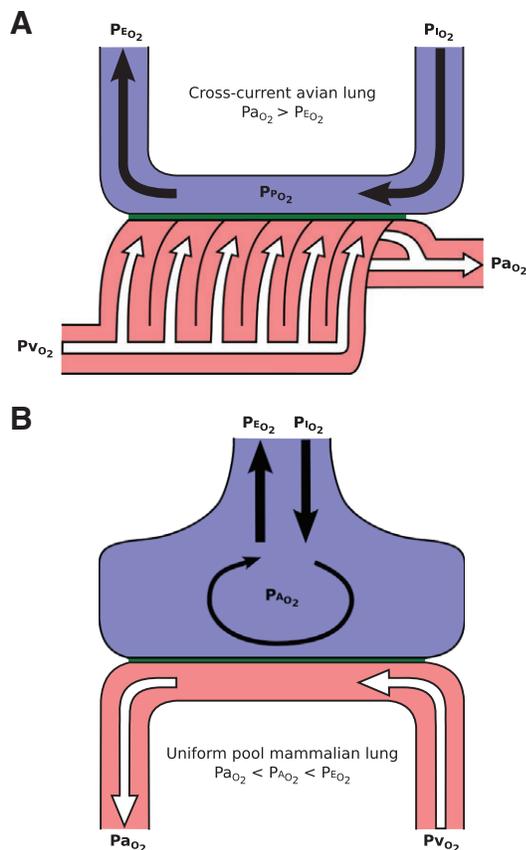


Fig. 3. Schematics of (A) the cross-current model of gas exchange in the avian lung and (B) the uniform pool model of gas exchange in the lungs of mammals and most other terrestrial vertebrates. In the cross-current model, inspired air flows through rigid parabronchioles that are oriented perpendicular to blood capillaries. The partial pressure of  $O_2$  ( $P_{O_2}$ ) in the parabronchioles ( $P_{PO_2}$ ) declines along their length as  $O_2$  diffuses into the blood, such that capillaries leaving the exchanger near the entrance of airflow (right side of figure) take up more  $O_2$  than capillaries leaving near the exit (left side). The contents of all capillaries mix to dictate the  $P_{O_2}$  of arterial blood ( $P_{AO_2}$ ), which can have a higher  $P_{O_2}$  than expired air ( $P_{EO_2}$ ). In the uniform pool model, gas flows in and out of terminal alveoli. Capillary blood flowing past these alveoli extract  $O_2$ , such that capillary  $P_{O_2}$  rises and alveolar  $P_{O_2}$  ( $P_{AO_2}$ ) declines uniformly to less than the  $P_{O_2}$  of gas that entered the alveoli. Arterial blood leaving the lungs has a  $P_{O_2}$  that is at best equal to  $P_{AO_2}$  (but is generally slightly less), which is less than the average  $P_{EO_2}$ . The cross-current model is therefore considered to be more efficient at gas exchange than the uniform pool model (Piiper and Scheid, 1972).  $P_{IO_2}$ , inspired  $P_{O_2}$ ;  $P_{VO_2}$ ,  $P_{O_2}$  of venous blood.

High capacities at several steps in the  $O_2$  transport pathway have been shown to distinguish high-flying birds from their lowland cousins (Fig. 2), confirming the theoretical predictions. The first step of this pathway, ventilation, appears to be enhanced in high-altitude birds to improve  $O_2$  uptake into the respiratory system. Bar-headed geese breathe more than low-altitude waterfowl when exposed to severe hypoxia (inspired  $O_2$  tensions  $\sim 23$ – $35$  Torr or  $\sim 3.1$ – $4.7$  kPa) (Black and Tenney, 1980; Scott and Milsom, 2007) and the magnitude of their ventilatory response is greater than in any other bird species yet studied (Scott and Milsom, 2009). Bar-headed geese also breathe with a more effective breathing pattern, taking much deeper breaths (i.e. higher tidal volumes) than low-altitude birds during hypoxia. There are at least two mechanistic causes for these differences: (1) ventilatory insensitivity to

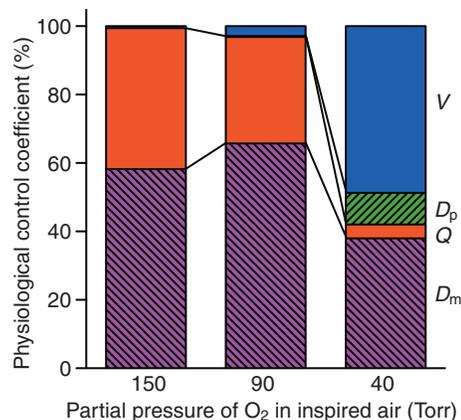


Fig. 4. Physiological control analysis of flux through the  $O_2$  transport pathway in waterfowl. The influence of the respiratory system (ventilation,  $V$ , and the capacity for pulmonary  $O_2$  diffusion,  $D_p$ ) on  $O_2$  transport increases and that of circulatory  $O_2$  delivery capacity ( $Q$ ; the product of maximum cardiac output and  $4 \times$  blood haemoglobin concentration) declines as hypoxia becomes more severe. The capacity for  $O_2$  diffusion in the muscle ( $D_m$ ) has a large influence on  $O_2$  transport at all partial pressures of inspired  $O_2$ . Control coefficients were calculated using theoretical modelling of the respiratory system with a haemoglobin  $P_{50}$  that is typical of highland birds (25 Torr or 3.3 kPa), and are defined as the fractional change in  $O_2$  transport rate divided by the fractional change of any given step in the  $O_2$  transport pathway. Expressed as a percentage, the control coefficients for all steps in the pathway will sum to 100. Modified from Scott and Milsom (Scott and Milsom, 2006; Scott and Milsom, 2009).

respiratory hypocapnia and (2) a blunting of the metabolic-depression response to hypoxia (Scott and Milsom, 2007; Scott et al., 2008). These differences increase the amount and partial pressure of  $O_2$  that ventilates the pulmonary gas-exchange surface during hypoxia. Bar-headed geese also have enlarged lungs (Scott et al., 2011), as do numerous other highland species sampled at high altitudes (Carey and Morton, 1976), which should enhance the second step of the  $O_2$  transport pathway by increasing the area of the gas-exchange surface. The respiratory system of high-altitude birds therefore seems capable of loading more  $O_2$  into the blood during hypoxia than that of lowland birds.

The circulatory delivery of  $O_2$  throughout the body is also enhanced in high-altitude birds. The most pervasive mechanism for sustaining the circulation of  $O_2$  in hypoxia is an alteration in the  $O_2$ -binding properties of haemoglobin in the blood. Numerous high-altitude birds, such as the bar-headed goose (Fig. 5), Andean goose (*Chloephaga melanoptera*) (Black and Tenney, 1980), Tibetan chicken (*Gallus gallus*) (Gou et al., 2007) and Ruppell's griffon (*Gyps rueppellii*) (Weber et al., 1988), are known to possess haemoglobins with an increased  $O_2$  affinity. This can dramatically increase  $O_2$  delivery and pulmonary  $O_2$  loading in hypoxia by increasing the saturation of haemoglobin (and thus the  $O_2$  content of the blood) at a given  $O_2$  partial pressure (Fig. 5A), and can, in doing so, greatly improve flux through the  $O_2$  transport pathway (Scott and Milsom, 2006). The genetic and structural bases for haemoglobin adaptation to high altitude have been resolved in many species. For example, the bar-headed goose possesses a major ( $HbA$ ) and minor ( $HbD$ ) form of haemoglobin, whose  $\alpha$  subunits contain four ( $\alpha^A$ ) (Fig. 5B) and two ( $\alpha^D$ ) uniquely derived amino-acid substitutions, respectively (McCracken et al., 2010). One of the substitutions in  $\alpha^A$  (Pro-119 to Ala) (green in Fig. 5B) is thought to cause a large increase in  $O_2$  affinity (Jessen et al., 1991) by altering the interaction between

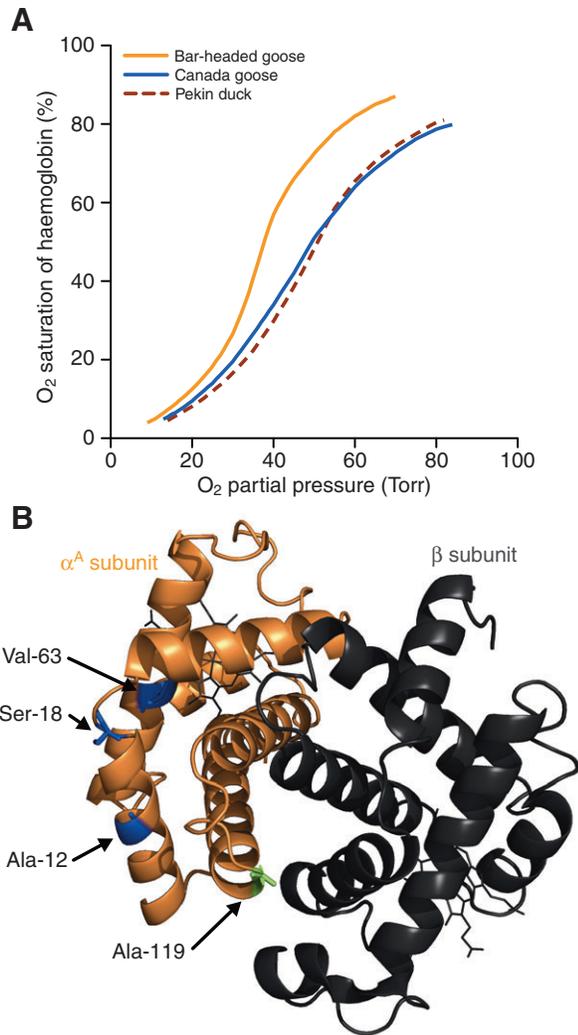


Fig. 5. High-altitude adaptations in the haemoglobin (Hb) of bar-headed geese. (A) The  $O_2$  affinity of bar-headed goose Hb is higher than that of lowland waterfowl, as reflected by a leftward shift in the  $O_2$  equilibrium curve of blood (measured at a pH of 7.3). Redrawn from Black and Tenney (Black and Tenney, 1980). 1 Torr=133 Pa. (B) The  $\alpha^A$  subunit of bar-headed goose Hb contains four uniquely derived amino-acid substitutions (blue and green). Ala-119 (green) has a large influence on  $O_2$  binding because it alters the interaction between  $\alpha$  and  $\beta$  subunits. For simplicity, only one out of two  $\alpha$  and  $\beta$  subunits that compose the complete Hb tetramer are shown. This cartoon was drawn in Pymol from the previously published structure of oxygenated Hb (Zhang et al., 1996) (Protein Data Bank ID, 1A4F).

$\alpha$  and  $\beta$  subunits and destabilizing the deoxygenated state of the protein (Zhang et al., 1996). Parallel genetic changes can sometimes arise in the haemoglobin of different highland species (e.g. Andean waterfowl) (McCracken et al., 2009b). Highland haemoglobin genotypes can even be maintained when gene flow from low altitudes is high, presumably because they are strongly favoured by natural selection (McCracken et al., 2009a).

The circulation of  $O_2$  may also be sustained in hypoxia by specializations in the heart that safeguard cardiac output. Bar-headed geese have a higher density of capillaries in the left ventricle of the heart (Fig. 6A), which should help maintain the  $O_2$  tension in cardiac myocytes and thus preserve function when hypoxaemia

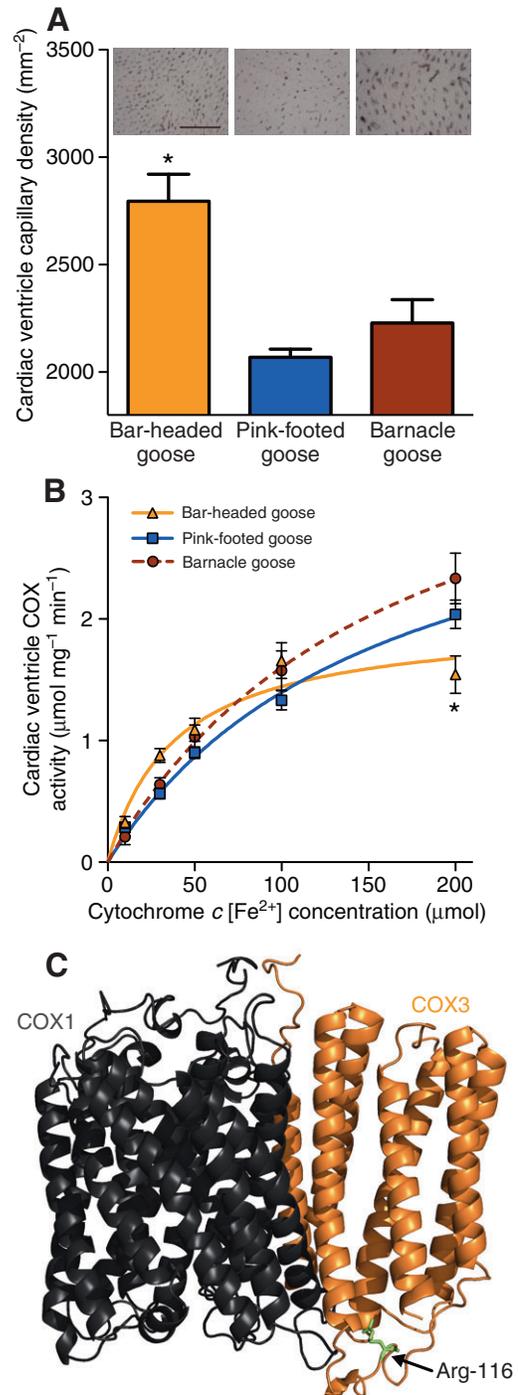


Fig. 6. Cardiac adaptations to high altitude in bar-headed geese. (A) Capillary density is enhanced in the hearts (left ventricle) of bar-headed geese compared with low-altitude geese. Insets are representative images of capillary staining in bar-headed geese (left), pink-footed geese (centre) and barnacle geese (right). Scale bar, 100  $\mu$ m. (B) Cytochrome *c* oxidase (COX) from the hearts of bar-headed geese has a different maximal activity (lower  $V_{max}$ ) and substrate kinetics (lower  $K_m$  for cytochrome *c* [ $Fe^{2+}$ ], cytochrome *c* in its reduced state) than COX from the two species of low-altitude geese. Asterisk represents a significant difference from both low-altitude species. (C) COX subunit 3 (COX3) of bar-headed geese contains a single amino acid mutation at a site that is otherwise conserved across all vertebrates (Trp-116 to Arg) and is predicted by structural modeling to alter the interaction between COX3 and COX1. Modified from Scott et al. (Scott et al., 2011).

occurs at high altitudes (Scott et al., 2011). Cellular function could also be challenged if the production of reactive O<sub>2</sub> species increases at high altitudes, as occurs in some lowland animals when declining O<sub>2</sub> levels at cytochrome *c* oxidase (COX, the enzyme that consumes O<sub>2</sub> in oxidative phosphorylation) shift the electron transport chain of mitochondria towards a more reduced state (akin to a buildup of electrons) (Aon et al., 2010). However, COX from bar-headed goose hearts has a higher affinity for its substrate (cytochrome *c* in its reduced state) (Fig. 6B), which could allow the electron transport chain to operate in a less reduced state and thus minimize oxidative damage by reactive O<sub>2</sub> species (Scott et al., 2011). A possible cause of this difference is a single mutation in subunit 3 of the COX protein, which occurs at a site that is otherwise conserved across vertebrates (Trp-116 to Arg) (green in Fig. 6C) and appears to alter inter-subunit interactions (Scott et al., 2011). These (and likely other) unique specializations may explain how bar-headed geese maintain arterial blood pressure and increase cardiac power output to deeper levels of hypoxia than Pekin ducks (G.R.S. and W. K. Milsom, unpublished). Cardiac specializations in high-altitude birds may have a transcriptional basis, based on a comparison of cardiac gene expression in late-stage embryos of Tibetan chickens and lowland breeds (Li and Zhao, 2009): embryonic hypoxia altered the expression of over 70 transcripts in all chickens, but an additional 12 genes (involved in energy metabolism, signal transduction, transcriptional regulation, cell proliferation, contraction and protein folding) were differentially expressed in only the highland Tibetan breed. Overall, these findings lend some credence to a previous suggestion that the hypoxaemia tolerance of the heart has a strong influence on the ability to fly at high altitudes (Scheid, 1990).

The capacity for O<sub>2</sub> to diffuse from the blood to mitochondria in the flight muscle is also enhanced in high-altitude birds. Andean coot (*Fulica americana peruviana*) populations that reside and were sampled at high altitudes had a higher capillarity and a smaller fibre size in the flight muscle than populations residing at low altitudes (León-Velarde et al., 1993). Because there were no differences in muscle aerobic capacity between coot populations, the increase in O<sub>2</sub> diffusing capacity should serve to improve O<sub>2</sub> transport in hypoxia rather than to match differences in cellular O<sub>2</sub> demands. Similar differences exist between bar-headed geese and lowland waterfowl from a common environment at sea level (Scott et al., 2009b). Mitochondria are also redistributed closer to capillaries in the aerobic fibres of bar-headed geese (Scott et al., 2009b), which reduces intracellular O<sub>2</sub> diffusion distances. These various mechanisms for improving the diffusion capacity for O<sub>2</sub> in the flight muscle should help sustain mitochondrial O<sub>2</sub> supply when hypoxaemia occurs at high altitudes.

In addition to improvements in the capacity to transport O<sub>2</sub> during hypoxia, various features of metabolic O<sub>2</sub> utilization and ATP turnover are altered in the flight muscle of high-altitude birds. This does not generally include changes in the inherent metabolic capacity of individual muscle fibres, based on observations in bar-headed geese of the abundance and respiratory capacities of mitochondria as well as the activities of metabolic enzymes (Scott et al., 2009b; Scott et al., 2009a). However, inherently higher aerobic capacities can exist for the whole muscle by virtue of increases in the proportional abundance of aerobic fibres (Scott et al., 2009b). Furthermore, the metabolic capacity of individual fibres can sometimes (Mathieu-Costello et al., 1998), but not always (León-Velarde et al., 1993), increase after high-altitude acclimatization. Increases in aerobic capacity, and the associated increases in overall mitochondrial abundance, could be important

for counterbalancing the inhibitory effects of low O<sub>2</sub> levels on the respiration of individual mitochondria [this strategy is discussed in Hochachka (Hochachka, 1985)]. Mitochondrial ATP production is also more strongly regulated by creatine kinase in bar-headed geese than in low-altitude waterfowl (Scott et al., 2009a) and the expression of mitochondrial creatine kinase is upregulated by hypoxia in Tibetan chickens (Li and Zhao, 2009). A potential consequence of these alterations is that energy supply and demand in the muscle is better coupled *via* the creatine kinase shuttle, a system important for moving ATP equivalents around the cell [this system is described in Andrienko et al. (Andrienko et al., 2003)]. An interesting possibility is that bar-headed geese developed a more active shuttle to compensate for the redistribution of mitochondria, which moved these organelles closer to capillaries but further from the contractile elements that constitute the major sites of ATP demand in the flight muscle.

### Can flapping flight be sustained above the high peaks?

It has been suggested that the iconic migration of bar-headed geese, which takes some individuals of this species over the highest peaks in the Himalayas, is impossible without vertical wind assistance (Butler, 2010). This suggestion was based on the observation that captive bar-headed geese forced to run on a treadmill do not perform as well in hypoxia (inspired O<sub>2</sub> tension ~50 Torr or ~6.7 kPa) as in normoxia (Fedde et al., 1989). However, it was clearly not an impairment of the cardiorespiratory system at supplying O<sub>2</sub> that impaired running performance in this study, as ventilation and cardiac output were both well below what can be sustained by this species during severe hypoxia at rest (Black and Tenney, 1980; Scott and Milsom, 2007). The more parsimonious explanation is that the leg muscles cannot sustain high activity during hypoxaemia, which is not terribly surprising given that this tissue is inactive when bar-headed geese fly at high altitudes. Nevertheless, the possibility that some of the highest-flying birds depend on wind assistance is intriguing and warrants examination with empirical data.

Most birds migrate below 4000 m elevation and, when possible, may alter flight altitude to take advantage of favourable wind, temperature, humidity or pressure (Liechti et al., 2000; Dokter et al., 2011). It is unclear to what extent this strategy is employed by high-altitude birds, but some evidence suggests that favourable conditions are not requisite for flying high. For example, demoiselle cranes (*Anthropoides virgo*) that were tracked on their southward migration between central and southern Asia flew over the Himalayas at 5000–6000 m elevation into a headwind (Kanai et al., 2000). Bar-headed geese have been tracked at 5000–7750 m elevation while crossing the Himalayan peaks in a single non-stop flight (Köppen et al., 2010; Hawkes et al., 2011) (although personal accounts have verified that at least some individuals of this species can fly over 1000 m higher; Fig. 1). We have found that bar-headed geese climbing the southern Himalayan face actually avoid flying in the afternoons when upslope tailwinds could reduce the metabolic requirements of flight, and prefer instead to fly in the stable and colder conditions overnight and early morning when there is a slight downdraft (Hawkes et al., 2011). These data suggest that active flight is indeed possible without wind assistance up to at least 6000 m elevation. A definitive answer to whether flapping flight can be sustained above the highest peaks awaits physiological and biomechanical data for birds flying at even higher altitudes.

### Conclusions and perspectives

The ability of birds to fly at high altitudes is critically dependent on the effective transport of O<sub>2</sub> from hypoxic air to all of the tissues

## Glossary

Hypobaria	Reduced barometric pressure in the environment
Hypocapnia	Reduced CO <sub>2</sub> content in arterial blood
Hypoxaemia	Reduced O <sub>2</sub> content in arterial blood
Hypoxia	Reduced partial pressure of O <sub>2</sub> in the environment

## Acknowledgements

I would like to thank L. A. Hawkes, J. U. Meir, W. K. Milsom and J. F. Storz for useful comments on a previous version of this manuscript. This work was supported by a NSERC Postdoctoral Fellowship.

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- of the body. Part of this effectiveness comes from many characteristics that distinguish the O<sub>2</sub> transport pathway of all birds in general from that of other vertebrates. Although not truly adaptive for high-altitude flight, these characteristics were undoubtedly an important basis upon which high-altitude adaptation could proceed. As it did so, unique specializations appear to have arisen at every step of the O<sub>2</sub> transport pathway of high fliers to facilitate their impressive exercise performance. However, it is not yet certain whether the numerous examples above are sufficient to entirely explain high-altitude flight.
- One area we know relatively little about is the relative roles of genetic adaptation *versus* phenotypic plasticity in the ability of birds to fly at high altitudes. Most of the previous work aimed at revealing the unique attributes of high fliers compared birds in a common environment at sea level. These studies were a useful first step in elucidating inherent and heritable differences, but it is probable that acclimatization to high-altitude hypoxia also shapes the physiology and flight capacity of highland residents (Cheviron et al., 2008). This could also be true of elevational migrants that spend time staging higher than their native altitudes before they cross high mountain ranges (e.g. bar-headed geese). However, not all hypoxia responses are beneficial – some are in fact maladaptive (Storz et al., 2010) – so future work is needed to understand the intricacies of how adaptation and plasticity interact in high-flying birds.
- We know less about the uniquely derived specializations for coping with low barometric pressure, cold and dry air than we do about those for coping with hypoxia. Birds that are adapted to high altitudes have larger wings to help offset the detrimental effects of low air density on lift generation (Feinsinger et al., 1979; Lee et al., 2008). This reduces the power output required to fly at elevation, but it does not completely eliminate the need for highland birds to flap harder (i.e. with larger wing stroke amplitudes) at elevation than lowland birds at sea level (Altshuler and Dudley, 2003). Highland birds may also have a higher capacity for increasing their resting metabolism as a means of generating heat in the cold (Lindsay et al., 2009); however, it is unclear whether thermogenic adaptations are even necessary for dealing with cold during flight, because a lot of heat is already being generated by the active flight muscles (Torre-Bueno, 1976; Ward et al., 1999). Water loss during flight is high enough to constrain flight duration at sea level (Engel et al., 2006) and is therefore expected to be a major issue at elevation, but it is unclear whether unique water-saving strategies have evolved in high-flying birds. Therefore, we still have much to learn about the unique physiology of the high fliers.
- Climate change is projected to have a large effect on avian communities (Gasner et al., 2010). Species distributions are expected to move to higher elevations as their historical climate envelopes (defined by temperature and humidity) shift upslope, which is forecasted to have particularly catastrophic effects on the abundance of current highland species. An implicit assumption of this prediction is that altitudinal clines in variables that will be less affected by climate change (i.e. hypoxia and low barometric pressure) will not limit the upward movement of lowland populations. This assumption appears valid for barometric pressure, based on a study that combined climate envelope modeling with an analysis of how altitude affects flight biomechanics (Buermann et al., 2011), but the same may not be true for hypoxia. Disentangling the relative influences of these variables, through a combination of integrative mechanistic studies in the laboratory and ecophysiological studies in the field, is key to understanding the potential effects of global change on avian physiology and ecology.
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