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REVIEW

Physiological insight into the evolution of complex phenotypes: aerobic performance and the O_2 transport pathway of vertebrates

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ABSTRACT

Evolutionary physiology strives to understand how the function and integration of physiological systems influence the way in which organisms evolve. Studies of the O_2 transport pathway – the integrated physiological system that transports O_2 from the environment to mitochondria – are well suited to this endeavour. We consider the mechanistic underpinnings across the O_2 pathway for the evolution of aerobic capacity, focusing on studies of artificial selection and naturally selected divergence among wild populations of mammals and fish. We show that evolved changes in aerobic capacity do not require concerted changes across the O_2 pathway and can arise quickly from changes in one or a subset of pathway steps. Population divergence in aerobic capacity can be associated with the evolution of plasticity in response to environmental variation or activity. In some cases, initial evolutionary divergence of aerobic capacity arose exclusively from increased capacities for O_2 diffusion and/or utilization in active O_2-consuming tissues (muscle), which may often constitute first steps in adaptation. However, continued selection leading to greater divergence in aerobic capacity is often associated with increased capacities for circulatory and pulmonary O_2 transport. Increases in tissue O_2 diffusing capacity may augment the adaptive benefit of increasing circulatory O_2 transport owing to their interactive influence on tissue O_2 extraction. Theoretical modelling of the O_2 pathway suggests that O_2 pathway steps with a disproportionately large influence over aerobic capacity have been more likely to evolve, but more work is needed to appreciate the extent to which such physiological principles can predict evolutionary outcomes.

KEY WORDS: O_2 cascade, Symmorphosis, Physiological control concept, Exercise, Thermogenic capacity, High-altitude adaptation, Complex traits

Introduction

How does the way organisms work influence the way they evolve? This question lies at the heart of evolutionary physiology, a key goal of which is to understand the evolutionary origins and mechanistic underpinnings of variation in organismal performance and fitness (Feder et al., 2000; Garland and Carter, 1994; Husak, 2016). Understanding how integrated organismal functions evolve is key to appreciating the mechanisms of evolutionary change (Dalziel et al., 2009; Dean and Thornton, 2007), and great strides have been made in associating the evolution of complex organismal phenotypes with subordinate traits at the molecular, cellular and tissue levels of biological organization (Anholt and Mackay, 2018; Karageorgi et al., 2019; Natarajan et al., 2013; Nikinmaa and Waser, 2007). However, organismal function depends upon the integration of complex networks of tissues and organs into physiological systems, and there has been much less emphasis on understanding these systems-level mechanisms of evolutionary change (Garland et al., 2016; Mykles et al., 2010). Doing so may be especially important for establishing causal links between genotype, organismal phenotypes, performance and fitness, because physiological systems can have emergent properties that are hard to fully appreciate by only studying their component parts (Feldman and Del Negro, 2006; Macklem, 2008). Thus, the study of integrated physiological systems is required to understand the constraints and facilitations shaping evolutionary trajectories of traits at higher levels of biological organization (Losos, 2011; Walker, 2007).

The O_2 transport pathway (also called the O_2 cascade; see Glossary) of vertebrates is well suited to elucidating the systems-level mechanisms underlying how organismal function and performance evolve. The O_2 pathway moves O_2 from the environment to the mitochondria present throughout the body and is composed of a series of physiological steps – namely, ventilation (see Glossary), diffusion into the blood, circulation, diffusion into tissues and mitochondrial O_2 utilization (Fig. 1) (Weibel, 1984). O_2 flux through this pathway thus depends upon the functional integration of several organ systems, including respiratory, circulatory and musculoskeletal systems. The overall flux capacity of the O_2 pathway sets the ceiling for sustained aerobic performance, which is called aerobic capacity (see Glossary). Aerobic capacity can be measured in many species as an organism's maximal O_2 consumption ($\dot{V}_{O_2,max}$ or $\dot{M}_{O_2,max}$; see Glossary). Aerobic capacity is often measured and achieved during intense aerobic exercise, but in some cases $\dot{V}_{O_2,max}$ during thermogenesis can exceed exercise $\dot{V}_{O_2,max}$ and be a better reflection of aerobic capacity in small endotherms (Evans and Rose, 1988; Norin and Clark, 2016; Rezende et al., 2004a; Rosenmann and Morrison, 1974). Aerobic capacity is a performance trait that is well suited to evolutionary physiology studies, because it is influenced by both genetic and environmental (e.g. exercise training, acclimatization to challenging environments) factors (Schutte et al., 2016; Swallow et al., 1998) and is important for fitness in many taxa (Hayes and O'Connor, 1999; Plaut, 2001). Aerobic capacity is also a trait that can be readily dissected using the physiologists' toolkit, because maximal O_2 consumption and the capacities for flux through each of the steps in the O_2 pathway can be measured using various techniques.

Evolutionary physiology studies of the O_2 pathway hold great promise for answering key questions about how organisms evolve. Does the complexity of performance traits restrain the speed at which they evolve? What are the integrative mechanisms of adaptive evolution? To what extent do interacting and integrated traits (e.g. steps in the pathway) evolve in concert? Are changes in some traits

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Journal of Experimental Biology (2021) 224, jeb210849. doi:10.1242/jeb.210849

Glossary

Aerobic capacity
The maximal rate of O_2 consumption, which reflects the overall flux capacity of the O_2 transport pathway, expressed as the volume ($\dot{V}_{O_2,max}$) or mass ($\dot{M}_{O_2,max}$) of O_2 consumed over time. In endotherms, $\dot{V}_{O_2,max}$ is often measured during intense aerobic exercise or thermogenesis. In fish, $\dot{M}_{O_2,max}$ is often measured during prolonged intense swimming or just after exhaustive exercise.

Circulatory O_2 delivery
The rate of convective O_2 flow from the lungs/gills to the peripheral tissues. Equal to the product of cardiac output (the total flow rate of blood), blood haemoglobin content and the relative O_2 saturation of arterial blood.

Common-garden experiment
The comparison of genetically distinct taxa (populations, species, strains, etc.) in common environmental conditions, which is often used to distinguish genetic and environmental sources of variation in phenotype.

Diffusing capacity
The conductance (the reciprocal of resistance) for gas movement across a gas-exchange barrier (i.e. tissue barrier between alveolar air and blood in the lungs, between water and blood in the gills and between capillary blood and mitochondria at the peripheral tissues). It reflects the degree to which gases such as O_2 diffuse in response to a given partial pressure gradient. The rate of diffusion of a gas across a gas-exchange barrier is proportional to the product of diffusing capacity and the partial pressure gradient for that gas.

Ecotypes
Genetically differentiated populations that have adapted to some aspect of their local environments, but have not diverged to the point where reproductive isolation occurs.

O_2 transport pathway
The integrated physiological system that moves O_2 from the environment to the mitochondria within active O_2-consuming tissues. It is composed of a series of physiological steps – namely, ventilation, diffusion into the blood, circulation, diffusion into peripheral tissues and mitochondrial O_2 utilization.

Physiological epistasis
The phenomenon whereby phenotypic effects of genetic variation at one locus depend on genotypes at other loci. In the context of the O_2 transport pathway, the observation that effects of genetically based variation in one step in the pathway can be influenced by distinct genetically based variation in another step in the pathway represents physiological epistasis.

Phenotypic plasticity
Changes in an individual's phenotype in response to changes in the conditions experienced during the lifetime of the organism (e.g. environmental changes, sustained changes in activity). This broad term can refer to acclimatization (called acclimation when in a controlled laboratory environment) or developmental plasticity, and it encompasses both reversible and irreversible phenotypic changes.

Ventilation
The convective flow of the environmental medium (air or water) at the gas-exchange barrier with the environment (lungs or gills). In mammals, ventilation is tidal because it moves air in and then out of lung alveoli. In fish, ventilation is unidirectional, with water flowing in one continuous direction across the gill lamellae.

more likely to constitute the first steps of adaptation? If so, is this because some traits have greater adaptive value and are more visible to selection (e.g. traits with high relative control over the organismal phenotype) or because they are less constrained in their response? Do evolved changes in some traits alter the adaptive value of changes in other traits? Does the evolution of organismal performance arise via similar underlying mechanisms across independent lineages and different vertebrate classes? These types of questions are difficult to answer using reductionism alone. Here, we will review some of the general physiological concepts and key studies of the O_2 pathway that have provided appreciable insight into these questions.

Physiological concepts underlying the evolution of aerobic performance and the O_2 transport pathway

The determinants of aerobic capacity in humans and other vertebrates have been subject to a long history of research, and previous suggestions for how different steps in the O_2 pathway contribute to variation in aerobic capacity generally fall on a spectrum between two extremes. On the one extreme, aerobic capacity has been suggested to be determined by a single rate-limiting step in the O_2 pathway, namely, circulatory O_2 delivery (see Glossary) (Bassett and Howley, 2000; Hillman et al., 2013). On the opposite extreme, aerobic capacity has been suggested by others to be determined by shared control, meaning that every step in the O_2 pathway is equally rate limiting (e.g. symmorphosis) (Weibel, et al., 1991). These extremes are captured in some proposed concepts on the determinants of variation in aerobic capacity, which we discuss in light of emerging evidence in the following sections.

Symmorphosis

'The structural design of the respiratory system should be matched to the functional requirements [for O_2 transport]… no more structure is formed and maintained than is required to satisfy functional needs' (Taylor and Weibel, 1981, p. 3).

Symmorphosis was an early concept aimed at explaining the mechanisms underlying variation in aerobic capacity. The original proposal by Taylor and Weibel was based on the idea that animals should be designed optimally (Taylor and Weibel, 1981; Weibel et al., 1991). The concept argued that the structural design of the O_2 transport pathway should be matched to functional demands, such that the structures involved in O_2 transport do not possess excess capacity beyond the physiological capacity of the fully integrated system (i.e. $\dot{V}_{O_2,max}$). A corollary to this idea is that each step in the O_2 pathway should have equivalent capacity and collectively set the upper limit to O_2 transport. The concept is based on the premise that it is wasteful to maintain structures in excess of the functional needs, and that natural selection should favour an optimized design.

To test this concept, Taylor, Weibel and others examined allometric variation across mammals (Taylor and Weibel, 1981; Weibel et al., 1981) and made comparisons between athletic and sedentary species (Taylor et al., 1987; Weibel et al., 1987). They observed a similar scaling relationship between exercise $\dot{V}_{O_2,max}$ and the absolute volume of mitochondria in active muscles (a key determinant of the capacity for mitochondrial O_2 utilization) across wild and domestic mammals spanning a wide range of body masses (Mathieu et al., 1981). Moreover, they observed comparable differences for each of these traits in comparisons between athletic and sedentary species of similar body mass (Hoppeler et al., 1987). They also found that both the capacity for O_2 diffusion from blood to the mitochondria in the muscle (estimated from the muscle's total volume of erythrocytes in capillaries) and the capacity for circulatory O_2 delivery (the product of maximal cardiac output and blood haemoglobin content) appeared to vary in a comparable way as exercise $\dot{V}_{O_2,max}$. However, similar relationships were not observed between exercise $\dot{V}_{O_2,max}$ and the capacity for O_2 diffusion from alveolar air to blood in the lungs (Gehr et al., 1981; Weibel et al., 1987). This led to their conclusion that symmorphosis is upheld for the internal compartments of the mammalian O_2 pathway (circulation, muscle O_2 diffusion and mitochondrial O_2 utilization), but not for the lung (Weibel et al., 1991).

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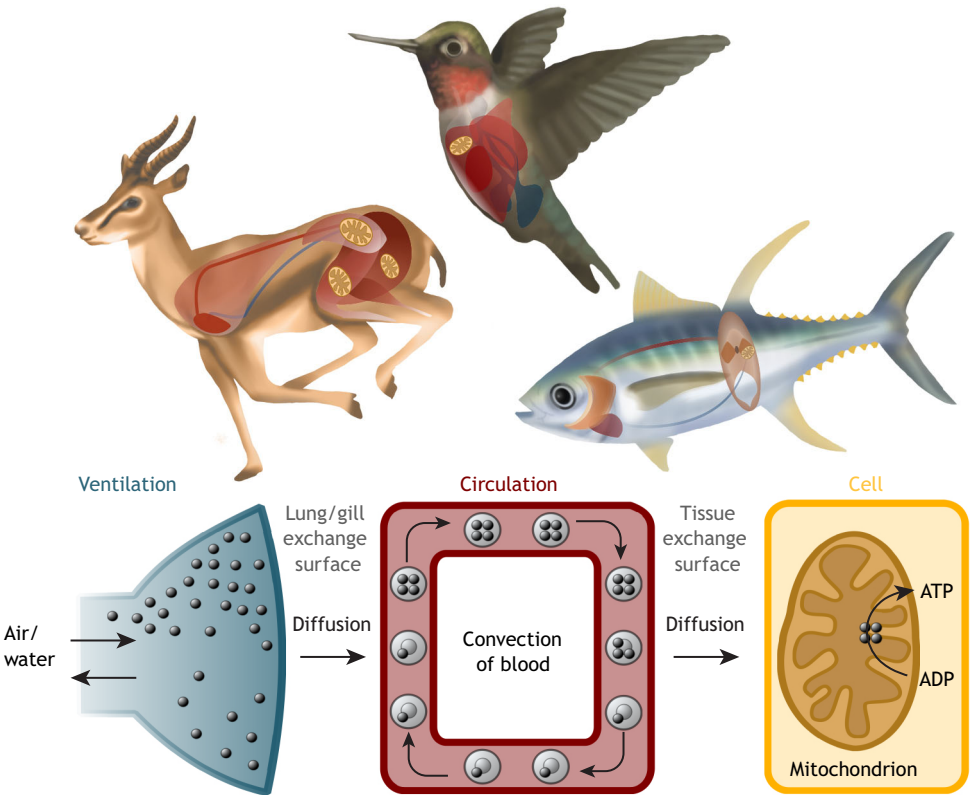

Fig. 1. Diagrammatic representation of the O_2 transport pathway. Vertebrates exhibit diverse gas exchange structures and circulatory plans, but in all vertebrate classes O_2 is transported from the surrounding medium (air or water) to mitochondria in the tissues along a pathway with several diffusive and convective steps: pulmonary (lung)/branchial (gill) ventilation, pulmonary/branchial O_2 diffusion, circulatory O_2 delivery, tissue O_2 diffusion and mitochondrial O_2 utilization during the process of oxidative phosphorylation.

The concept of symmorphosis received strong criticism from evolutionary physiologists. In a reanalysis of the allometric variation across mammals, in which interspecific variation about allometric equations was examined using residuals, associations between exercise $\dot{V}_{O_2,max}$ and various structural features of the O_2 pathway were not statistically significant (Garland and Huey, 1987). Symmorphosis proposed that capacities for O_2 flux are determined by structural design, but physiological factors can also contribute to the capacities that are realized *in vivo*. Several other theoretical arguments were also presented for why the O_2 pathway may not evolve optimally for O_2 transport (Dudley and Gans, 1991; Garland, 1998). Genetic or developmental constraints (Garland and Huey, 1987), stochastic evolutionary events (e.g. genetic drift) and competing forms of selection (e.g. sexual selection) can all lead to non-optimal outcomes (Brady et al., 2019). Furthermore, animals are not 'purpose built' for a single function (i.e. aerobic performance), as complex structures often serve multiple functions and may therefore be subject to design trade-offs between functions that act at cross purposes (Dudley and Gans, 1991). Indeed, the structures in the O_2 pathway are also employed in CO_2/pH regulation, water and ion homeostasis, and thermoregulation, such that responses to natural selection may balance these multiple competing demands (Dudley and Gans, 1991; Garland, 1998). The common view that emerged from these criticisms, as stated by Garland and Huey (1987, p. 1407), is that 'symmorphosis has heuristic value as a working hypothesis [but] it should not presently be considered an established principle'.

Physiological control concept

An alternative concept to explain the underlying determinants of variation in aerobic capacity can be borrowed from metabolic control analysis of biochemical pathways (Fell and Thomas, 1995; Kacser et al., 1995). Similar to symmorphosis, this concept considers regulation of pathway flux to be determined by the controlling influences of all sites across a pathway, but unlike symmorphosis, control can be spread unevenly across sites. It is well recognized that fluxes through metabolic pathways are not determined entirely by small numbers of rate-limiting steps or bottlenecks, but rather by the combined influences of control by all steps (Fell and Thomas, 1995; Kacser et al., 1995). Some steps have a more controlling influence than others, such that each step in a pathway need not be equally rate limiting (Suarez and Moyes, 2012). We refer to this concept as the 'physiological control concept' when applied to physiological systems in general. The physiological control concept has been applied to the O_2 pathway in previous studies, such that each step in the pathway is assigned a 'flux control coefficient' that reflects its influence over aerobic capacity (di Prampero, 1985; Hochachka and Burelle, 2004; Jones, 1998; Scott and Milsom, 2009). Specifically, the control coefficient represents the fractional change in aerobic capacity (i.e. overall O_2 pathway flux) that results from a fractional change in capacity of a particular step, such that the control coefficients of all steps in the pathway will sum to 1. Control coefficients have usually been determined using mathematical modelling of the O_2 pathway, and although they have rarely been determined empirically, it is possible to manipulate the capacities of some steps in the O_2 pathway *in vivo* and measure the effect on aerobic capacity. For example, maximal cardiac output has been manipulated using atrial pacing in rats (Gonzalez et al., 1998), and blood haemoglobin content has been manipulated by transfusion or pharmacological treatments in various species (Gallaugher et al., 1995; Schuler et al., 2010).

The physiological control concept has considerable advantages over symmorphosis, because it does not apply any underlying

assumptions about how changes in aerobic capacity can be achieved. It can be used to examine the general question of whether subordinate traits (e.g. steps in the O_2 pathway) with disproportionate influence over complex organismal phenotypes (e.g. aerobic capacity) are more likely to contribute to evolutionary change. Furthermore, the fact that control coefficients are constrained to sum to a value of 1 realistically models how interactions between steps in the pathway can lead to physiological epistasis (see Glossary) (Cheverud and Routman, 1995; Dykhuizen and Dean, 2009). For example, evolved increases in capacity at one step in the O_2 pathway should reduce its relative influence over aerobic capacity, and thus increase the relative influence of other steps. Prolonged periods of selection on aerobic capacity could thereby lead to shifts over time in which steps of the O_2 pathway have the greatest influence over aerobic capacity and thus evolve under selection (Dykhuizen and Dean, 2009). By this rationale, responses to selection on aerobic capacity could also vary between lineages or within a population and over evolutionary time, contingent upon the nature of physiological control in the taxa under selection at a particular time. The physiological control concept provides a framework in which to examine these possibilities.

Case studies on the evolution of aerobic capacity and the O_2 transport pathway

We will now discuss three case studies that provide deeper insight into the evolution of aerobic capacity and the O_2 pathway. Broad-scale comparisons of distantly related species, such as those discussed above, have shown that variation across several steps in the O_2 pathway can underlie evolutionary variation in aerobic capacity. However, comparisons between species that are already well diverged in multiple aspects of physiology, morphology and behaviour have a limited ability to answer the questions we posed at the outset about the evolution of complex phenotypes. Such questions are better addressed by studies in which the selective pressures driving the evolution of aerobic capacity are clear and the responses to selection can be measured on shorter time scales, such as in artificial selection experiments or in comparisons between populations differentiating under natural selection (Garland and Adolph, 1991; Swallow et al., 2009). Aerobic capacity is plastic and can respond to environmental variation and training (Norin and Metcalfe, 2019; Storz et al., 2010b), so it is also essential to distinguish evolved variation from phenotypic change that is not genetically encoded (Garland and Adolph, 1991). These features are accounted for in the case studies below, in which the evolution of aerobic capacity was examined in response to artificial selection and between natural populations.

We start with two case studies in mammals, and for the sake of brevity must neglect some other exceptional work on the evolution of aerobic capacity. This includes studies of artificial selection in house mice and bank voles (Kolb et al., 2010; Sadowska et al., 2008, 2009, 1998), in which some of the underlying determinants of evolved changes in aerobic capacity have been uncovered (Jaromin et al., 2016; Lipowska et al., 2019; Rezende et al., 2006b; Syme et al., 2005; Templeman et al., 2012; Wong et al., 2009), but the entire O_2 pathway has yet to be examined. We sought to add taxonomic breadth with our third case study on the evolution of swimming performance in post-glacial fishes, in which *in vivo* measurements of the entire O_2 pathway are also unavailable but proxy measures have provided valuable insight into O_2 pathway evolution. Our broad consideration of the entire O_2 pathway also requires that we consider mechanistic aspects of each step in the pathway somewhat briefly, including the potential constraints and trade-offs that may influence their function and evolution, and we direct readers to other excellent publications for this information (e.g. Berberi and Careau, 2019; Burggren et al., 2014; Evans et al., 2005; Garland, 2014; Hsia et al., 2016; Storz, 2018; Wilson and James, 2004). Nevertheless, we hope the case studies below illustrate the richer and more nuanced appreciation of the evolution of aerobic capacity that can be gained from artificial selection experiments and from comparative studies of microevolutionary variation among natural populations.

Artificial selection for endurance exercise capacity in rats

An instructive case study on the evolution of aerobic capacity comes from divergent selection experiments for endurance running capacity in rats (Koch and Britton, 2001; Koch and Britton, 2019). Selection was based on total distance run in a standardized running test with incremental increases in speed until exhaustion. Starting with an outbred founder population (which could run 355 m on average), high-capacity runners (HCR) and low-capacity runners (LCR) were selected and used as breeders to create HCR and LCR lines. Selection continued in each generation for the best and worst runners within the HCR and LCR lines, respectively, and led to progressive divergence in running capacity that were driven largely (but not exclusively) by increases in the HCR line. By 28 generations of selection, the distance to exhaustion increased to ∼2000 m in the HCR line and decreased to ∼250 m in the LCR line, in association with differences in exercise-induced $\dot{V}_{O_2,max}$ (Koch and Britton, 2019). The systems-level determinants of this evolutionary divergence in $\dot{V}_{O_2,max}$ was determined at generations 7 and 15, for which the results are summarized in Fig. 2.

$\dot{V}_{O_2,max}$ was 12% higher in HCR than in LCR among rats studied at generation 7 (Henderson et al., 2002). This difference in $\dot{V}_{O_2,max}$ existed in conjunction with 33% higher O_2 diffusing capacity (see Glossary) of peripheral tissues (D_{T,O_2}) in HCR compared with LCR, which increased O_2 extraction from the blood, but there were no differences in circulatory O_2 delivery (Q_{O_2}), O_2 diffusing capacity of the lungs (D_{L,O_2}) or alveolar ventilation (V_A) (Henderson et al., 2002). The increased D_{T,O_2} in HCR rats was associated with increased capillarity in the gastrocnemius, an important locomotor muscle in the hindlimb, along with increased activity of oxidative enzymes (citrate synthase, hydroxyacyl-CoA dehydrogenase), suggesting that the capacity for mitochondrial O_2 utilization in the muscle was also enhanced (Howlett et al., 2003).

$\dot{V}_{O_2,max}$ was 49% higher in HCR than in LCR among rats studied at generation 15 (Gonzalez et al., 2006). This continued divergence in $\dot{V}_{O_2,max}$ was associated with further divergence in D_{T,O_2}, now 56% greater in HCR than in LCR, along with a maintenance or expansion of metabolic differences in the gastrocnemius (Gonzalez et al., 2006; Howlett et al., 2009). However, the continued divergence in $\dot{V}_{O_2,max}$ was also associated with differences in circulatory O_2 delivery, lung function and pulmonary ventilation. Cardiac output and Q_{O_2} were 42% greater in HCR than in LCR, in association with increased stroke volume and heart mass (Gonzalez et al., 2006). V_A and D_{L,O_2} were 78% and 21% higher in HCR than in LCR, respectively, in association with larger mass-specific lung volume (Kirkton et al., 2009).

These results in rats provide insight into the temporal sequence of changes that underlie evolved divergence in aerobic performance (Fig. 2). The early divergence in $\dot{V}_{O_2,max}$ could be entirely explained by increases in the capacities for O_2 diffusion and mitochondrial O_2 utilization in locomotor muscle, constituting the first steps in the response to selection. The continued divergence in $\dot{V}_{O_2,max}$ was

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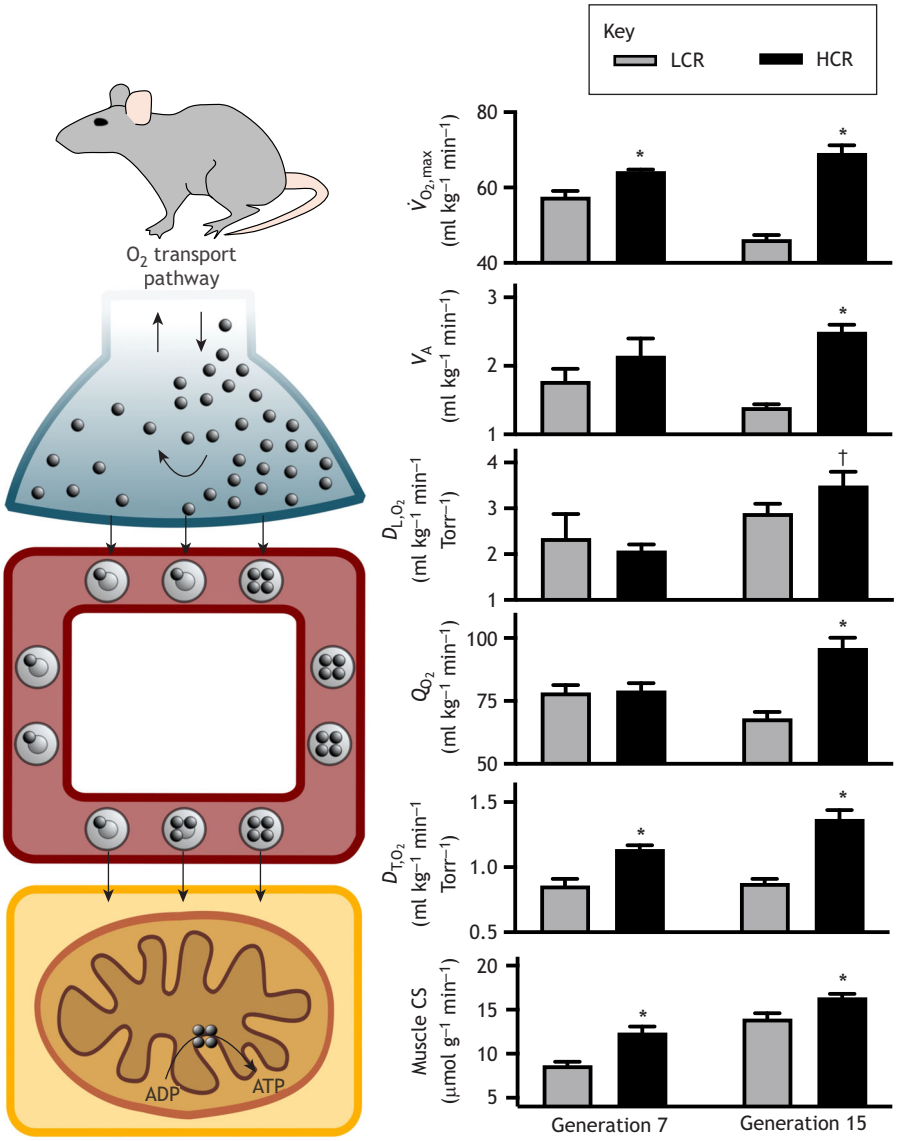

Fig. 2. Evolved changes in exercise $\dot{V}_{O_2,max}$ in response to divergent selection for aerobic running capacity in rats were underlaid by changes in only a subset of steps in the O_2 transport pathway, dependent upon the duration of selection. Lines of high-capacity runners (HCR) and low-capacity runners (LCR) were compared after 7 and 15 generations of selection. Functional measurements across the O_2 pathway were made at exercise $\dot{V}_{O_2,max}$: V_A, alveolar ventilation; D_{L,O_2}, O_2 diffusing capacity of the lungs; Q_{O_2}, circulatory O_2 delivery (the product of cardiac output and arterial O_2 content); D_{T,O_2}, O_2 diffusing capacity of the peripheral tissues; Muscle CS, the activity of citrate synthase in the gastrocnemius, an important locomotor muscle in the hindlimb. * and †, P<0.05 and P=0.07 in comparisons between lines within a generation. Data are means±s.e.m. from several source publications (Gonzalez et al., 2006; Henderson et al., 2002; Howlett et al., 2003, 2009; Kirkton et al., 2009), where additional details can be found.

subsequently associated with evolved changes at every step of the O_2 pathway. As stated by Gonzalez et al. (2006, p. 1293), 'this supports the idea that a continuing enhancement of the capacity of skeletal muscle to transport and utilize O_2 requires improvement in the rate of delivery of O_2 to the tissues'. By extension, continued increases in D_{T,O_2} should have diminishing value as the tissues approach full extraction of O_2 from the blood, and only by augmenting circulatory O_2 delivery can $\dot{V}_{O_2,max}$ continue to increase. It is also possible that the functional benefit of evolved changes in cardiorespiratory physiology may be contingent upon D_{T,O_2}, such that increasing Q_{O_2} only has functional benefit after the initial increase in D_{T,O_2}. This possibility is further explored in the next case study.

Evolution of thermogenic capacity in deer mice native to high altitudes

Deer mice (*Peromyscus maniculatus*) that have adapted to the cold and hypoxic environment at high altitude provide another instructive case study on the evolution of aerobic capacity. Deer mice inhabit a wide altitudinal range, from near sea level to over 4300 m elevation in the Rocky Mountains (Natarajan et al., 2015; Snyder et al., 1982). Thermogenic $\dot{V}_{O_2,max}$ can equal or exceed values of exercise $\dot{V}_{O_2,max}$ in this species (Chappell and Hammond, 2004; Hayes and Chappell, 1986), and there is evidence that higher thermogenic $\dot{V}_{O_2,max}$ improves survival over harsh winters at high altitude (Hayes and O'Connor, 1999). High-altitude populations of deer mice have responded to this selection pressure by evolving increased $\dot{V}_{O_2,max}$ in hypoxia (Cheviron et al., 2013; Lui et al., 2015). Furthermore, thermogenic $\dot{V}_{O_2,max}$ can increase in response to hypoxia and/or cold exposure during adulthood and in early development in deer mice (Chappell and Hammond, 2004; Chappell et al., 2007; Hayes and Chappell, 1986; Rezende et al., 2004b; Robertson and McClelland, 2021), which provides a useful opportunity to examine how the evolution of plasticity can contribute to population divergence in aerobic capacity.

Common-garden experiments (see Glossary) are a powerful tool for uncovering the genetic and environmental contributions to population differentiation (Garland and Adolph, 1991), and have been used to uncover the roles of evolutionary adaptation and phenotypic plasticity (see Glossary) to the superior aerobic capacity of high-altitude mice. High-altitude deer mice, low-altitude deer mice and white-footed mice (*P. leucopus*; a species that is

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exclusively found at low altitude) have been bred in captivity and chronically exposed as adults to hypoxia and/or cold in a full factorial design. Measurements of thermogenic $\dot{V}_{O_2,max}$ in hypoxia suggest that natural selection has augmented aerobic capacity in high-altitude populations by accentuating the plastic response to chronic hypoxia, without affecting the plastic response to chronic cold (Tate et al., 2020). This evolved increase in $\dot{V}_{O_2,max}$ is summarized in Fig. 3 for comparisons between high-altitude deer mice and low-altitude white-footed mice (which we often refer to as highlanders and lowlanders, respectively, for simplicity below). Chronic hypoxia increased $\dot{V}_{O_2,max}$ by ∼50% in high-altitude deer mice but had negligible effects in white-footed mice, such that $\dot{V}_{O_2,max}$ was ∼70% greater in high-altitude mice than in low-altitude mice (Fig. 3). Low-altitude deer mice are more responsive to chronic hypoxia than white-footed mice, but are still far less responsive than their high-altitude counterparts (Tate et al., 2017, 2020).

Concurrent cardiorespiratory measurements suggested that this accentuated plasticity in $\dot{V}_{O_2,max}$ was not associated with corresponding variation across the entire O_2 pathway (Fig. 3). For lung O_2 transport, the combined effects of total ventilation and pulmonary O_2 extraction (E_{L,O_2}; the percentage of inspired O_2 that is extracted from each breath) appeared to contribute to the variation in $\dot{V}_{O_2,max}$. More specifically, population differences in total ventilation and E_{L,O_2} were offsetting in normoxia, but not in chronic hypoxia, where the population difference in E_{L,O_2} was associated with the difference in $\dot{V}_{O_2,max}$. The greater overall E_{L,O_2} in highlanders in both environments is likely a result of evolved changes in lung structure that should augment O_2 diffusing capacity (West et al., 2021). Circulatory O_2 transport (Q_{O_2}) exhibited a similar pattern of variation to that of $\dot{V}_{O_2,max}$, resulting from a fixed increase in arterial O_2 saturation (∼6–7% saturation units) and a greater increase in cardiac output in response to chronic hypoxia in highlanders compared with lowlanders (Tate et al., 2020). Tissue O_2 extraction (E_{T,O_2}) was greater in highlanders overall, but this particular trait varied little in highlanders in response to chronic hypoxia. Similar patterns of variation were exhibited by citrate synthase activity and various other phenotypes of the gastrocnemius muscle, including capillarity, respiratory capacity, fibre-type composition and mitochondrial volume density (Lui et al., 2015; Mahalingam et al., 2020, 2017; Scott et al., 2015). These findings suggest that the superior aerobic capacity of highlanders in chronic hypoxia is attributable to the evolution of accentuated plasticity in the capacity of some steps in the O_2 pathway (Q_{O_2}), in conjunction with evolved changes in the trait means for the capacity of other steps in the O_2 pathway (D_{L,O_2}, D_{T,O_2} and mitochondrial O_2 utilization of skeletal muscle). Therefore, there can be an uncoupling of the variation in $\dot{V}_{O_2,max}$ and the variation in its underlying determinants across the O_2 pathway.

A key question that arises from the above findings is the extent to which the effect of variation at each step in the O_2 pathway on $\dot{V}_{O_2,max}$ is dependent on other steps in the O_2 pathway. Insights into this question come from studies of inter-population hybrids of deer mouse populations from high and low altitudes that have sought to examine the effects of genetically encoded variation in the affinity of haemoglobin (Hb) for O_2 on $\dot{V}_{O_2,max}$ (Chappell and Snyder, 1984; Wearing et al., 2021). High-altitude deer mice have evolved increased Hb–O_2 affinity as a result of amino acid substitutions in the α- and β-chain subunits of the $\alpha_2\beta_2$ Hb tetramer, which is believed to help safeguard arterial O_2 saturation and thus circulatory O_2 transport in hypoxia (Ivy et al., 2020; Natarajan et al., 2013; Snyder et al., 1982; Storz et al., 2010a, 2009, 2007). The systems-level effects of Hb–O_2 affinity cannot be studied by comparing high-altitude with low-altitude mice, because many O_2 pathway traits co-vary across populations. Instead, controlled breeding approaches to create inter-population hybrids provide an

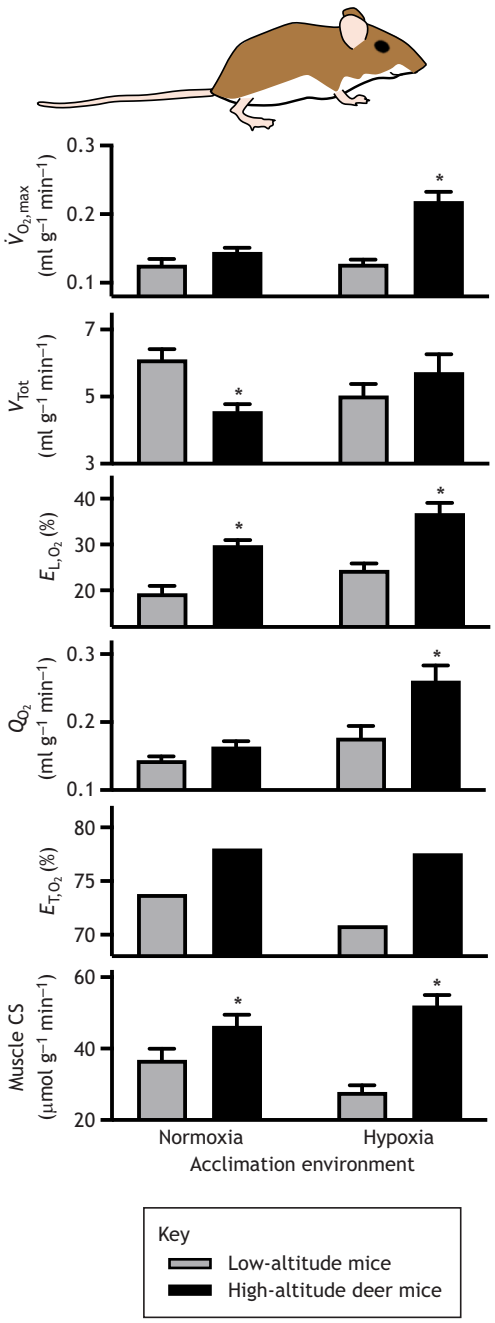

Fig. 3. Effects of high-altitude adaptation in deer mice on thermogenic $\dot{V}_{O_2,max}$ in hypoxia arose from evolved increases in the plastic response to chronic hypoxia, and were underpinned by differences in only a subset of steps in the O_2 transport pathway. High-altitude deer mice and low-altitude mice were raised in common conditions at sea level and then acclimated as adults to either normoxia or hypoxia. Functional measurements across the O_2 pathway were made at thermogenic $\dot{V}_{O_2,max}$: V_{Tot}, total ventilation; E_{L,O_2}, pulmonary O_2 extraction; Q_{O2}, circulatory O_2 delivery (the product of cardiac output, blood haemoglobin content and arterial O_2 saturation); E_{T,O_2}, tissue O_2 extraction (only average values are available); Muscle CS, the activity of citrate synthase in the gastrocnemius, an important thermogenic muscle in the hindlimb. *P<0.05 in comparisons between populations within an acclimation environment. Data are means±s.e.m. from several source publications (Mahalingam et al., 2020; Tate et al., 2020), where additional details can be found.

opportunity to study the effects of variation in single genes or phenotypes on a common genetic background. In one study, the effect of Hb–O_2 affinity on $\dot{V}_{O_2,max}$ was studied by backcrossing different α-globin genotypes into a highland genetic background (Chappell and Snyder, 1984). That study found that mice with highland α-globins had increased Hb–O_2 affinity and higher $\dot{V}_{O_2,max}$ in hypoxia than mice with lowland α-globins. In another study, the effect of Hb–O_2 affinity was studied on an admixed genetic background, using an F2 intercross breeding design to randomize associations between allelic variants of α- and β-globins (Wearing et al., 2021). Wearing et al. (2021) found that mice with highland Hb genotypes had increased Hb–O_2 affinity and improved arterial O_2 saturation in hypoxia (which likely augmented circulatory O_2 delivery because there was no systematic effect of Hb genotype on heart rate or blood haemoglobin content), but in this case there was no associated improvement in $\dot{V}_{O_2,max}$. A key difference between these studies is that the former study examined the effect of Hb–O_2 affinity in mice that otherwise had the physiological characteristics of highlanders, whereas the latter study examined the effect of Hb–O_2 affinity in mice that were less similar to highlanders. Therefore, increased circulatory O_2 delivery may only improve aerobic capacity in mice that already have some other key physiological characteristics of the high-altitude population.

The physiological characteristics underlying these divergent findings were examined by means of sensitivity analyses performed using theoretical modelling of the O_2 pathway, and the results showed that the influence of Hb–O_2 affinity and circulatory O_2 delivery on $\dot{V}_{O_2,max}$ were highly dependent on D_{T,O_2} (Wearing et al., 2021). This analysis made use of Fick diffusion equations and other established equations describing O_2 flux through the O_2 pathway, as previously described in detail (Wagner, 1996), in which equations were first solved using empirical data followed by subsequent determination of the effects of varying D_{T,O_2} and/or Hb–O_2 affinity (alone or in combination) on $\dot{V}_{O_2,max}$. Increasing Hb–O_2 affinity only augmented $\dot{V}_{O_2,max}$ at higher values of D_{T,O_2}, and the influence of Hb–O_2 affinity was particularly strong at values of D_{T,O_2} that were sufficient to deplete venous O_2 levels for the lower Hb–O_2 affinity. These findings indicated that evolved increases in Hb–O_2 affinity should only contribute to enhancing $\dot{V}_{O_2,max}$ in hypoxia if the O_2 diffusing capacity of thermogenic tissues (i.e. skeletal muscle and/or brown adipose tissue) is high enough to take advantage of an increased rate of O_2 delivery. Therefore, in high-altitude deer mice, the benefit of increasing Hb–O_2 affinity may have been contingent upon evolved increases in the capacity to extract O_2 from the blood by virtue of increases in capillarity and respiratory capacity in the skeletal muscle (Lui et al., 2015; Mahalingam et al., 2017). Such changes in the muscle may have been particularly important in deer mice, because the increased Hb–O_2 affinity in high-altitude populations is not accompanied by an enhanced Bohr effect to augment O_2 unloading (Jensen et al., 2016).

These studies of high-altitude deer mice illustrate important features about the adaptive evolution of aerobic capacity in response to natural selection. Firstly, adaptive increases in $\dot{V}_{O_2,max}$ can arise from evolved increases in phenotypic plasticity (i.e. the magnitude of the response to chronic hypoxia; top panel of Fig. 3). Secondly, evolved increases in $\dot{V}_{O_2,max}$ can emerge from differences in only a subset of steps in the O_2 pathway (Fig. 3). Thirdly, the influence of a focal trait on $\dot{V}_{O_2,max}$ may often be contingent upon the capacity of others, suggesting that the adaptive value of many traits can be critically dependent on the functional integration between steps of the O_2 pathway. However, the potential for the adaptive value of some traits to be contingent upon other integrated traits suggests that responses to selection may vary between lineages based on the physiological characteristics of their ancestor. The subject of whether convergent mechanisms underlie the evolution of $\dot{V}_{O_2,max}$ is discussed in greater detail in the next case study.

Evolution of swimming performance in post-glacial fishes

Swimming activity and aerobic capacity have evolved in many populations of northern fishes that colonized lakes and rivers after the retreat of the Pleistocene glaciers ∼10,000–60,000 years ago (Dalziel et al., 2015, 2012b; Rogers et al., 2002; Taylor, 1999). Prolonged swimming capacity has undergone independent evolved changes in multiple populations of some species of post-glacial fishes, allowing for tests of convergence in the mechanisms underlying O_2 pathway evolution. Here, we review two natural systems in which swimming capacity has repeatedly evolved: the evolution of a more actively swimming, 'dwarf' limnetic ecotype (see Glossary) from a 'normal' benthic ancestor in lake whitefish (*Coregonus clupeaformis*) (Rogers et al., 2002) and the evolution of nonmigratory, stream-resident ecotypes from migratory ancestors in threespine stickleback (*Gasterosteus aculeatus*) (Dalziel et al., 2012b). Although *in vivo* measurements of O_2 pathway function have not been conducted, insights into the evolution of the O_2 pathway have been made based on proxy measures collected using histological and biochemical approaches (Fig. 4).

In lake whitefish, actively swimming limnetic ecotypes exist in a number of lakes in eastern North America, and are thought to have evolved from an ancestor resembling current-day benthic ecotypes (Bernatchez et al., 2010). The ancestors of current-day ecotypes began to diverge ∼60,000 years ago and then came back into contact ∼10,000–12,000 years ago, when resource competition and ecological opportunities led to continued genomic and phenotypic divergence (Bernatchez et al., 2010). This included the evolution of higher swimming activity in limnetic fish (Rogers et al., 2002), accompanied by selection on genes involved in aerobic metabolism and by extensive differences in metabolic gene expression in the swimming muscle compared with benthic fish (Derome et al., 2006; Evans and Bernatchez, 2012; Hébert et al., 2013; Jeukens and Bernatchez, 2012; Renaut et al., 2011).

In an initial study, Dalziel et al. (2015) compared a single well-diverged benthic–limnetic ecotype pair, using fish that were bred and reared in the laboratory. Limnetics had higher values for traits associated with O_2 utilization by skeletal muscle, including the density and mitochondrial enzyme activity of red oxidative muscle fibres (the main fibre type powering aerobic swimming) as well as the activity of electron transport system (ETS) enzymes in isolated muscle mitochondria. Surprisingly, this more oxidative muscle phenotype was not associated with increased capillary density, a key trait influencing O_2 diffusing capacity. Limnetics also had 17% larger heart ventricles (when expressed relative to body mass), which is expected to have increased maximal cardiac output and circulatory O_2 delivery. In contrast, there were no differences between ecotypes in the total number of gill lamellae (Laporte et al., 2016) and limnetics had slightly lower hematocrit (Dalziel et al., 2015). Together, these data suggest that capacities for muscle O_2 utilization and circulatory O_2 transport underlie increased swimming performance of limnetic whitefish, with no current evidence for evolved changes at other steps in the O_2 pathway. Interestingly, comparisons between these ecotype pairs after swim training suggested that the increased ventricle mass in limnetics might have evolved via genetic assimilation (Dalziel et al., 2015), a situation in which formerly plastic traits evolve to become constitutively expressed (Ehrenreich and Pfennig, 2016).

Journal of Experimental Biology (2021) 224, jeb210849. doi:10.1242/jeb.210849

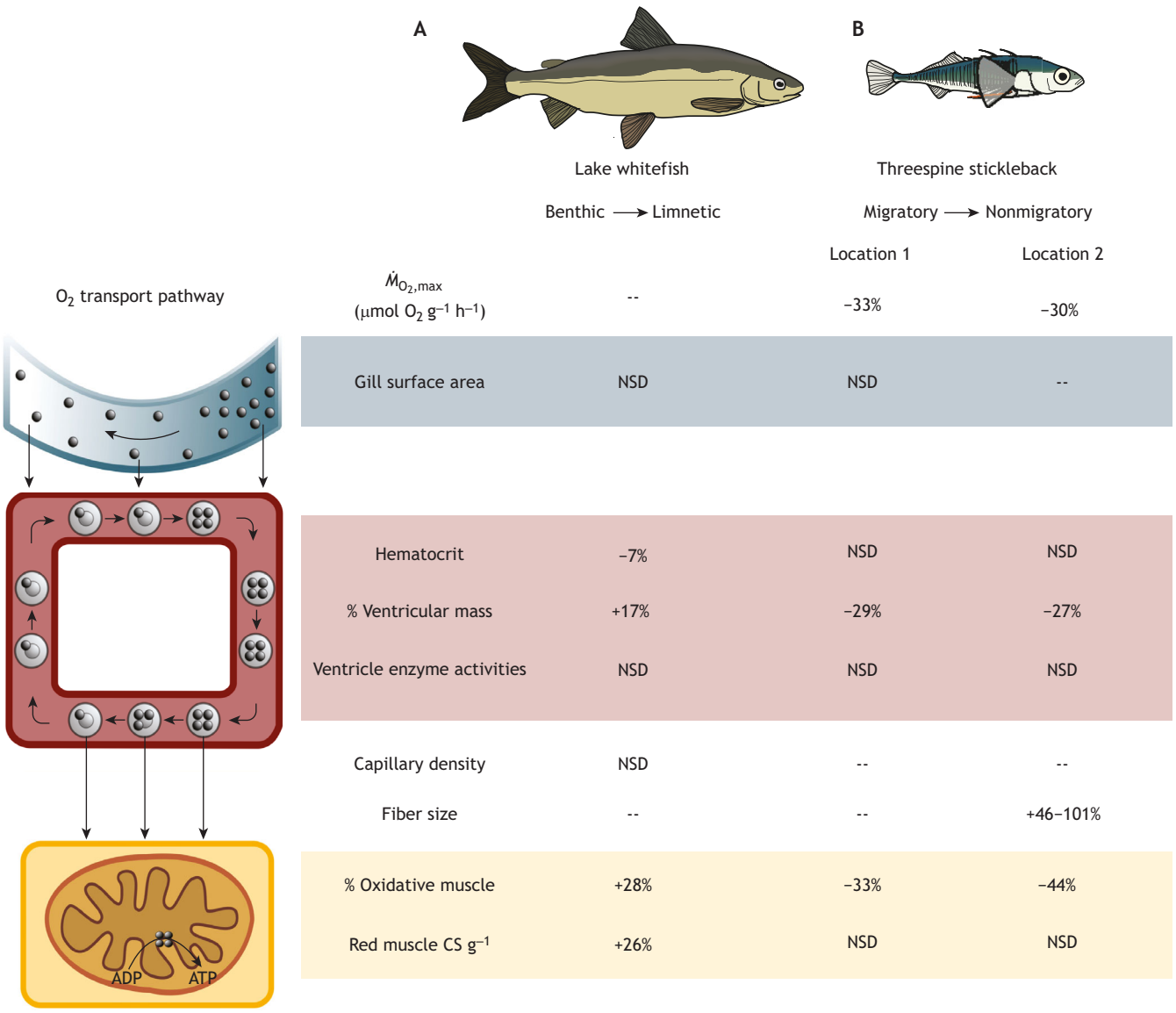

Fig. 4. Swimming capacity has evolved rapidly in post-glacial fishes, often involving a common subset of steps in the O_2 pathway across independent lineages. Percent changes are presented relative to the putative ancestral-like ecotype. (A) Changes in proxy traits at steps in the O_2 pathway as limnetic ecotypes of lake whitefish (*Coregonus clupeaformis*) evolved from a less-active benthic ecotype. Data presented are from laboratory-reared juveniles (Dalziel et al., 2015; Laporte et al., 2016). (B) Change in proxy traits as nonmigratory ecotypes of threespine stickleback (*Gasterosteus aculeatus*) evolved from a migratory ancestor with a higher aerobic capacity. Data are presented for laboratory-reared fish from two migratory–nonmigratory ecotype pairs, with percent change in the nonmigratory ecotype for each population relative to the sympatric migratory ecotype (Dalziel et al., 2012a,b). CS g^{-1}, citrate synthase activity per gram tissue; NSD, no significant difference; - -, unmeasured trait.

Given the challenges associated with inferring adaptation based on comparisons of only two taxa (Garland and Adolph, 1994), subsequent comparisons were made between ecotypes across several wild-caught ecotype pairs, allowing for tests of convergence during the evolution of the O_2 pathway (Dalziel et al., 2017). Current benthic–limnetic ecotype pairs exist along a gradient of ecological speciation, in which the genetic divergence between some pairs is much greater than between some others owing to differences in the strength of divergent selection and homogenizing gene flow among lakes (Gagnaire et al., 2013; Renaut et al., 2011). Therefore, this speciation gradient can also shed insight into possible temporal steps by which aerobic capacity has evolved. Limnetics from all ecotype pairs had a greater abundance of red muscle than benthics. However, only limnetics from the two most genetically divergent ecotype pairs had larger ventricles, whereas higher muscle mitochondrial enzyme activities were only found in limnetics from the most divergent ecotype pair. These data suggest that there is some convergence in the mechanistic response of the O_2 pathway to selection, particularly for steps that may be first to evolve during selection for prolonged swimming capacity (i.e. changes in red muscle abundance), but others may only evolve during the latter stages of divergence.

The post-glacial evolution of reduced aerobic capacity has occurred in threespine stickleback, in which nonmigratory stream-resident ecotypes have a lower capacity for prolonged swimming and $\dot{M}_{O_2,max}$ than modern-day migratory ecotypes that are thought to represent the ancestral condition (Dalziel et al., 2012b; Morozov

et al., 2018; Taylor and McPhail, 1986; Tudorache et al., 2007). Studies on laboratory-bred fish from two sets of migratory–nonmigratory ecotype pairs found that the evolved reduction in $\dot{M}_{O_2,max}$ was associated with reduced relative masses of the pectoral muscle (sticklebacks are labriform swimmers that use the pectoral fin for swimming) and ventricle, with no compensatory change in mitochondrial enzyme content per gram tissue in either skeletal or cardiac muscles (Dalziel et al., 2012a). Surprisingly, gill surface area did not evolve in either population of nonmigratory ecotype, contrary to expectations that the combination of selection for reductions in passive ion loss in freshwater and relaxed selection for a high $\dot{M}_{O_2,max}$ would lead to reductions in gill surface area (Gonzalez, 2011).

The relative importance of individual traits on the evolution of $\dot{M}_{O_2,max}$ in stickleback was further studied using F2 interpopulation hybrids, in which genetic linkage among loci was reduced to allow focal traits to be studied in a randomized genetic background (Dalziel and Schulte, 2012). Critical swimming speed (U_{crit}) was used as a proxy for $\dot{M}_{O_2,max}$, based on the strong correlation between these traits (Dalziel et al., 2012b). The mass and citrate synthase activity of the pectoral muscle, as well as ventricle mass, collectively explained ∼18% of the variation in U_{crit}, supporting the hypothesis that decreases in these traits are causally related to the evolutionary reductions in $\dot{M}_{O_2,max}$. Furthermore, there was a significant interactive effect of these traits on U_{crit}, such that the influence of one focal trait (e.g. ventricle mass) was greater in fish that had higher values of another focal trait (e.g. pectoral muscle mass) (Dalziel et al., 2012b). However, much of the variation in U_{crit} across F2 sticklebacks remained unexplained, arguing that unmeasured traits also contribute to evolutionary variation in U_{crit}.

Overall, these studies in natural populations of fish have shown that aerobic capacity can evolve quite rapidly (∼10,000 to 60,000 years) and often in conjunction with the evolution of traits related to O_2 utilization by swimming muscles and circulatory O_2 transport. The evolution of aerobic performance is also associated with changes in circulatory O_2 transport in sockeye salmon (*Oncorhynchus nerka*). In this species, wild-caught upriver populations with longer and more difficult migrations have higher aerobic scope and higher maximal cardiac output than coastal spawning populations with a less rigorous migration (Eliason et al., 2011). In general, work to date has found little evidence that evolved changes in gill morphology underlie the evolution of $\dot{M}_{O_2,max}$ between closely related ecotypes and populations (Dalziel et al., 2015, 2012a; Eliason et al., 2013; Laporte et al., 2016). This may be because gills are a counter-current gas exchanger that has the potential to be more effective than lungs at extracting O_2 (Piiper and Scheid, 1972) and gills can respond rapidly to changes in O_2 demand with plastic changes in surface area (Gilmour and Perry, 2018; Sollid et al., 2003), such that evolved changes in branchial O_2 diffusing capacity may not be necessary for early divergence in $\dot{M}_{O_2,max}$. Alternatively, the gills are also an organ with many other functions (osmoregulation, pH regulation, etc.), which may constrain its response to selection for changes in $\dot{M}_{O_2,max}$ (Gonzalez, 2011). An important caveat is that proxy measures have often been used to evaluate the importance of different steps in the O_2 pathway in fish evolutionary studies. Detailed *in vivo* measurements of O_2 pathway function at $\dot{M}_{O_2,max}$ in divergent ecotypes or strains, preferably reared in a common environment, are needed to further examine this possibility. Such measurements would also provide insight into whether the unique aspects of the O_2 pathway in fish compared with other vertebrates might affect the relative flux control or functional trade-offs at specific O_2 pathway steps (Eliason and Stecyk, 2020; Harter and Brauner, 2017; Malte, 2011; Moyes and Le Moine, 2011; Nikinmaa et al., 2019; Rummer and Brauner, 2020; Thorarensen, 2011).

Emergent patterns for the evolution of aerobic capacity and the O_2 transport pathway

The case studies above provide insight into key questions about how organisms evolve. Firstly, despite the complex functional integration of several organs and tissues required to support aerobic capacity, evolutionary responses to selection can be swift. Artificial selection for endurance running capacity in rats shows that significant evolutionary changes in exercise $\dot{V}_{O_2,max}$ can occur within 10 generations (Henderson et al., 2002), consistent with results of selection for voluntary wheel-running behaviour in mice (Rezende et al., 2006a; Swallow et al., 1998). The evolution of aerobic capacity in natural populations is not expected to occur so rapidly, because the strength of selection is likely lower than what is experienced during artificial selection, and there may be greater constraints owing to potential trade-offs and conflicting selective pressures. Nevertheless, the evolution of $\dot{M}_{O_2,max}$ in wild post-glacial fishes has occurred on a relatively short geological time span, showing that complex, integrated performance traits can evolve 'quickly' in nature (Dalziel et al., 2012a,b). In some cases, adaptive divergence is associated with the evolution of plasticity of aerobic capacity, such as the amplified response to chronic hypoxia observed in high-altitude deer mice (Tate et al., 2017, 2020). Future work comparing more recently diverged ecotypes should help hone in on how quickly aerobic capacity can evolve, and how this pace compares with the sometimes rapid pace of morphological adaptation (e.g. changes in plate morphology in <50 years in stickleback) (Lescak et al., 2015).

The case studies also provide insight into the integrative mechanisms underlying the evolution of aerobic capacity and offer general perspectives on the adaptive evolution of complex traits. In all cases, evolved changes in aerobic capacity did not require concerted changes across the entire O_2 pathway, supporting previous findings and arguments that symmorphosis is not a general principle underlying the evolution of aerobic capacity. The early evolution of aerobic capacity could arise from increases in the capacity for O_2 diffusion and utilization in active peripheral tissues, without any changes in pulmonary or circulatory O_2 transport (Henderson et al., 2002). Theoretical modelling of the O_2 pathway based on the physiological control concept has suggested that tissue O_2 diffusing capacity has a disproportionately large influence over aerobic capacity across multiple species (Scott and Milsom, 2006; Wagner, 1996), indicating that changes in active peripheral tissues may often constitute the first steps of adaptation because they have significant adaptive value and are more visible to selection. However, the adaptive value of initial changes in active peripheral tissues will diminish as blood O_2 extraction rises and venous O_2 content approaches zero. Responses to continued selection on aerobic capacity should then necessitate increased capacities for pulmonary and circulatory O_2 transport (Gonzalez et al., 2006; Kirkton et al., 2009). Indeed, results in deer mice suggested that increases in tissue O_2 diffusing capacity may increase the functional significance of changes in circulatory traits (Wearing et al., 2021). Similarly, results in stickleback suggested that ventricle mass had a larger influence on swimming capacity in fish with larger pectoral muscles and higher citrate synthase activity (Dalziel and Schulte, 2012). Therefore, modest changes in aerobic capacity can be achieved by disproportionate changes in capacity for only a subset of steps in the O_2 pathway, but larger changes likely require broader

changes across the entire O_2 pathway. This probably explains why the pattern of symmorphosis is apparent in some inter-specific comparisons of highly diverged species (Weibel et al., 1991), but not for comparisons between less diverged taxa that exhibit more modest differences in aerobic capacity.

The emergent theme across all of the above case studies, along with other research, is that the evolution of aerobic capacity does involve some common underlying mechanisms. Evolutionary changes in the capacities for O_2 diffusion and/or utilization in active muscles have been observed across many studies in mammals, including those discussed above (Templeman et al., 2012; Wong et al., 2009). Evolved changes in active muscles were also seen in some fishes, suggesting that muscle O_2 diffusion or utilization may be a common first target during the evolution of aerobic capacity in many vertebrates. These findings could suggest that the value of increasing these muscle traits never falls to zero, potentially because evolved increases in capacity for circulatory O_2 delivery always arise before peripheral tissues become capable of near-full extraction of O_2 from the blood. The importance of venous blood for O_2 supply to the spongy myocardium may also limit the advantage of increasing tissue O_2 extraction in some teleost fish (Steffensen and Farrell, 1998). Evolved changes in capacities for pulmonary/branchial O_2 transport were more idiosyncratic, and the importance of such changes may vary across independent lineages and differ between vertebrate classes. Additional work in other vertebrate groups is needed to better appreciate these issues, but there are few studies of aerobic capacity with sufficiently detailed *in vivo* measurements of O_2 pathway function in other vertebrates (Butler et al., 1977).

A key issue to consider is whether physiological principles could have predicted the observed mechanisms underlying the evolution of aerobic capacity. Theoretical modelling based on the physiological control concept can help identify steps in the pathway with the largest influence on aerobic capacity, and thus predict which might be the most likely to evolve. Therefore, the previous findings from such modelling that tissue O_2 diffusing capacity often has a large influence on aerobic capacity (Scott and Milsom, 2006; Wagner, 1996) provides a theoretical basis on which the evolved changes in muscle phenotypes could have been predicted. Modelling based on the physiological control concept has yet to be carried out in any of the case studies discussed above, but such approaches have the potential to uncover how changes in the relative influence of different steps in the O_2 pathway on aerobic capacity might have corresponded to their subsequent evolution.

Conclusions and perspectives

Studies on the evolution of aerobic capacity have provided general insight into the mechanisms underlying the evolution of complex phenotypes. Understanding the evolution of physiological traits in natural populations is a challenging endeavour, and evolutionary physiologists attempting to link physiology to performance and fitness may fear that efforts to do so might 'suffer the fate of implosion from complexity' (Kingsolver and Huey, 2003). We argue that a constructive path forward for understanding the evolution of aerobic capacity is to use *a priori* analyses based on the physiological control concept (using theoretical modelling, alongside empirical approaches when possible) to identify steps in the O_2 pathway that have the greatest influence, and then determine empirically whether these traits underlie evolved variation in aerobic capacity in natural populations or in response to artificial selection. This will help better appreciate the extent to which physiological principles can predict evolutionary outcomes. It will also help identify traits that may be constrained in their response to selection owing to developmental constraints or trade-offs (Brakefield and Roskam, 2006; Losos, 2011; Maynard Smith et al., 1985; Roff and Fairbairn, 2007; Uller et al., 2018; Walker, 2007). We hope that a greater integration of physiological principles into evolutionary thinking can help shed appreciable insight into the fundamental question of how the way organisms work influences the way they evolve.

Acknowledgements

The authors would like to thank Samantha Lau for creating illustrations used in Figs 1, 2 and 4.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants to G.R.S. (RGPIN-2018-05707) and A.C.D. (RGPIN-2016-04303). G.R.S. is supported by the Canada Research Chairs Program.

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