

ATMOSPHERIC OXYGEN, GIANT PALEOZOIC INSECTS AND THE EVOLUTION OF AERIAL LOCOMOTOR PERFORMANCE

ROBERT DUDLEY*

Department of Zoology, University of Texas, Austin, TX 78712, USA and Smithsonian Tropical Research Institute, PO Box 2072, Balboa, Republic of Panama

*e-mail: r_dudley@utxvms.cc.utexas.edu

Accepted 28 October 1997; published on WWW 24 March 1998

Summary

Uniformitarian approaches to the evolution of terrestrial locomotor physiology and animal flight performance have generally presupposed the constancy of atmospheric composition. Recent geophysical data as well as theoretical models suggest that, to the contrary, both oxygen and carbon dioxide concentrations have changed dramatically during defining periods of metazoan evolution. Hyperoxia in the late Paleozoic atmosphere may have physiologically enhanced the initial evolution of tetrapod locomotor energetics; a concurrently hyperdense atmosphere would have augmented aerodynamic force production in early flying insects. Multiple historical origins of vertebrate flight also correlate temporally with geological periods of increased oxygen concentration and atmospheric density. Arthropod as well as amphibian gigantism appear to have been facilitated by a hyperoxic Carboniferous atmosphere

and were subsequently eliminated by a late Permian transition to hypoxia. For extant organisms, the transient, chronic and ontogenetic effects of exposure to hyperoxic gas mixtures are poorly understood relative to contemporary understanding of the physiology of oxygen deprivation. Experimentally, the biomechanical and physiological effects of hyperoxia on animal flight performance can be decoupled through the use of gas mixtures that vary in density and oxygen concentration. Such manipulations permit both paleophysiological simulation of ancestral locomotor performance and an analysis of maximal flight capacity in extant forms.

Key words: atmosphere, density, flight, gigantism, hyperoxia, insect, oxygen, Paleozoic.

Introduction

Changes in atmospheric gas composition impinge globally on animal and plant physiology. Much attention has recently been focused on anthropogenic forcing of atmospheric carbon dioxide, but the potential for historical fluctuations in both carbon dioxide and oxygen concentrations has rarely been contemplated by students of metazoan evolution. However, recent geophysical analyses indicate that Phanerozoic variation in both respiratory gases has been substantial; the implications of such changes are profound for the evolution of both arthropod and tetrapod physiology (Graham *et al.* 1995, 1997). This review discusses the geophysical evidence suggesting large-scale historical fluctuations in the earth's atmosphere and qualitatively outlines the underlying biotic and abiotic mechanisms. In addition to the potential role of hyperoxia in the evolution of animal flight and of gigantism in diffusion-limited forms, enhanced oxidative metabolism contributes to the accumulation of deleterious superoxide radicals and ultimately to animal senescence. Evolutionary responses to such senescent damage are poorly understood, but experimental hyperoxia provides a convenient means to select for increased metabolic expenditure as well as for adaptive responses to oxidant exposure. The study of Phanerozoic

changes in atmospheric oxygen levels thus provides an important historical context for the evaluation of diverse physiological adaptations in present-day organisms.

Geological records of atmospheric oxygen and carbon dioxide

The fixation of carbon dioxide and release of oxygen by plants have long been recognized as potential influences on the earth's atmosphere, but the potential impact of plant terrestrialization on atmospheric composition has only recently been realized (Berner, 1997). Mechanisms that have driven large-scale Phanerozoic fluctuations in gas content of the atmosphere derive from post-Silurian terrestrialization by plants together with changes in continental weathering, organic carbon deposition and biotic decomposition (Berner, 1990; Robinson, 1991; Visscher *et al.* 1996). In the late Paleozoic, carbon fixation in terrestrial plant ecosystems became decoupled from decomposition and release by carbon-reducing organisms, resulting in extensive deposition of coal and other carbonates. This disequilibrium between free and stored carbon production, together with extensive oxygen release by a diversifying

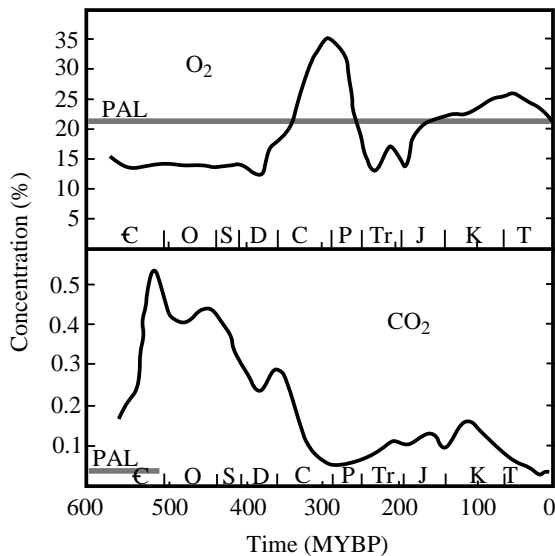


Fig. 1. Estimates of Phanerozoic oxygen and carbon dioxide atmospheric concentrations from Berner (1990, 1994) and Berner and Canfield (1989); see Berner (1997) for a summary of independent geochemical estimates of Proterozoic carbon dioxide concentrations. PAL, present atmospheric level (20.9% O₂; 0.036% CO₂); MYBP, million years before present; C, Cambrian; O, Ordovician; S, Silurian; D, Devonian; C, Carboniferous; P, Permian; Tr, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary.

arborescent flora, dramatically altered atmospheric carbon dioxide and oxygen concentrations (Fig. 1). Carbon dioxide levels through much of the Devonian and Carboniferous were probably much higher than the contemporary 0.036%, with an approximately tenfold reduction evident from the middle to late Paleozoic (Berner, 1990, 1994; Mora *et al.* 1996; Retallack, 1997). The late Paleozoic drawdown of this greenhouse gas was also probably associated with a substantial reduction in earth surface temperatures (Berner, 1994).

Concomitant with this reduction in carbon dioxide concentration, the oxygen concentration of the late Paleozoic atmosphere may have risen to as high as 35% (Berner and Canfield, 1989; see Fig. 1), a remarkable value compared with the 20.9% of the contemporary atmosphere. This elevation of oxygen partial pressure occurred against the background of a constant nitrogen partial pressure (Hart, 1978; Holland, 1984), yielding an increased total pressure of the atmosphere. Atmospheric oxygen concentrations are unlikely to have exceeded 35%, as this value represents an approximate threshold for spontaneous combustion of the biosphere (Watson *et al.* 1978; Kump, 1989). Both modelling (Berner and Canfield, 1989) and empirical (Makowski *et al.* 1989; Wignall and Twitchett, 1996; Isozaki, 1997) results demonstrate an oxygen crash from the high Upper Carboniferous levels to an early Triassic value as low as 15% (Fig. 1). Berner and Canfield (1989) indicate a subsequent, but apparently smaller, pulse in atmospheric oxygen concentrations starting in the mid Jurassic and continuing through the Cretaceous and much of the Tertiary (Fig. 1). Although not as pronounced as the late Paleozoic pulse

in oxygen, this latter increase represents a secondary but still significant Cretaceous and Tertiary elevation in atmospheric oxygen relative to the present-day level. Furthermore, aquatic levels of carbon dioxide and oxygen are coupled to the atmospheric partial pressures of these gases (Richards, 1965), suggesting widespread effects of the trends depicted in Fig. 1 across virtually all ecosystems. Biologists may well view with skepticism the accuracy of large-scale geophysical models in predicting climatic features of ancient ecosystems, but the qualitative mechanisms of carbon dioxide depletion and oxygen enhancement must apply in any model of plant terrestrialization during the Paleozoic. The precise magnitudes of these dramatic changes remain only broadly constrained, but a combination of biotic and abiotic factors was clearly sufficient to have driven major fluctuations in both carbon dioxide and oxygen concentrations of the atmosphere (Berner, 1997).

Variable oxygen partial pressures coupled with constant nitrogen partial pressure through the Phanerozoic further indicate that such physical features as air density, viscosity, diffusivity and heat conductivity varied substantially through the late Paleozoic and to a lesser extent during the Cretaceous and Tertiary (see Graham *et al.* 1995). Because Paleozoic hyperoxia involved an increase in oxygen concentration as well as in atmospheric density, this condition differs from that obtained experimentally in a hyperbaric chamber, within which oxygen concentration remains constant but total pressure increases. The use of the terms hypo- and hyperdensity therefore supplements the classical terminology of hypo- and hyperbaria, and is warranted to emphasize the potential decoupling of total pressure and air density. This approach also retains the use of hypo- and hyperoxia to describe oxygen partial pressure relative to normobaric values at the present atmospheric concentration of 20.9%.

Late Paleozoic gigantism and the end-Permian extinctions

A well-known yet poorly quantified feature of late Paleozoic terrestrial faunas is the widespread taxonomic distribution of animal gigantism (Briggs, 1985; Graham *et al.* 1995). Carboniferous gigantism was most evident among diverse lineages of flying insects (Carpenter, 1992), but was also present in additional arthropod taxa, such as millipedes and arthropleurids, and among the terrestrial labyrinthodont amphibians. The wingspans of the extinct dragonfly order Protodonata exceeded 70 cm in one species, whereas the wingspans of late Paleozoic Paleodictyoptera ranged from 0.9 to 43 cm (Shear and Kukalová-Peck, 1990; Carpenter, 1992). Some Carboniferous mayflies were characterized by wingspans of 8.5–45 cm (Kukalová-Peck, 1985). Giant Carboniferous hexapods are also found in the phylogenetically basal orders of wingless insects (Diplura and Thysanura; see Kukalová-Peck, 1987). Millipedes 1 m long, giant arthropleurids (an extinct arthropod class) and even giant arachnids round out the late Paleozoic cast of terrestrial arthropod giants (Briggs, 1985; Graham *et al.* 1995). Among terrestrial vertebrates, large amphibians reached body lengths of up to 2 m (Carroll, 1988).

These large amphibians were probably limited by the capacity for cutaneous respiration which, at least in contemporary urodeles, is known to restrict maximum body size through diffusive limitations (see Ultsch, 1974).

Few causal hypotheses have been advanced for Paleozoic gigantism, although predatory defense (Vermeij, 1987; Shear and Kukalová-Peck, 1990) as well as enhanced flight performance (Kukalová-Peck, 1978) are plausible ecological explanations. The suggestion that variable oxygen concentrations characterized Paleozoic times, however, implies a direct mechanism underlying increased body size in diffusion-limited forms (Graham *et al.* 1995). The most immediate physiological effect of an increased oxygen partial pressure is to increase diffusive flux in the tracheal system. Limits to insect body size imposed by tracheal diffusion (see Weis-Fogh, 1964) can therefore shift upwards as oxygen partial pressure increases (Graham *et al.* 1995); oxidative metabolism will be similarly enhanced. This straightforward hypothesis for late Paleozoic gigantism was suggested by Rutten (1966), Schidlowski (1971) and Tappan (1974), all of whom argued that the very large Carboniferous Protodonata would have required a hyperoxic atmosphere. However, Paleozoic gigantism through mitigation of aerobic diffusional constraints would be expected in a diversity of taxa supplemental to insects (Graham *et al.* 1995). Although hyperoxia does not exclude other potential mechanisms promoting gigantism (e.g. predator-prey interactions), a causal mechanism of diffusive enhancement would be imposed globally and would apply irrespective of taxonomic association. Not only hexapod insects but a diversity of arthropod classes as well as various amphibians displayed gigantism, consistent with hyperoxic relaxation of diffusion-limited respiration in these taxa. Although an increase in atmospheric pressure will decrease oxygen diffusivity within tracheal systems, given the dependence of gas diffusion coefficients on total pressure (Paganelli *et al.* 1975), the net effect of elevated oxygen levels on diffusive flux is nonetheless substantial. The elevation of oxygen partial pressure to an atmospheric concentration of 35%, when coupled with a constant nitrogen partial pressure, increases rates of oxygen diffusion by approximately 67%. This value probably represents the maximum increase during the Paleozoic oxygen pulse and would clearly have had a substantial impact on the function of respiratory systems.

Also consistent with the hypothesis of diffusive constraints on oxidative metabolism is the winnowing of giant terrestrial arthropods parallel with the increasingly hypoxic conditions of the late Permian (Graham *et al.* 1995; see Fig. 1). Most characteristically, the various insect taxa that attained exceptionally large body sizes during the Carboniferous do not persist after the Permian (see Carpenter, 1992). The severe end-Permian extinctions of both terrestrial and marine taxa have been, in part, attributed to anoxic conditions (e.g. Berner, 1989; Gruszczynski *et al.* 1989; Hallam, 1991; Wignall and Hallam, 1992, 1993; Knoll *et al.* 1996), although a diversity of biotic and abiotic factors may have contributed synergistically to this effect (Erwin, 1992, 1994). The disappearance of giant

terrestrial arthropods with diffusion-limited respiratory systems is, however, consistent with the causal mechanism of atmospheric hypoxia restricting such taxa to progressively smaller body sizes (Graham *et al.* 1995). Similar conclusions apply to the giant semiaquatic and terrestrial amphibians of the late Paleozoic that became extinct by the end of the Permian. Further analysis of this hypothesis must rely on quantitative analyses of phyletic size change in relation to atmospheric levels of oxygen during the late Paleozoic, a task that will be substantially complicated by the paucity of complete fossil specimens from this period (see Carpenter, 1992). However, a secondary peak of insect gigantism (e.g. among the Ephemeroptera; R. Dudley, in preparation) appears to occur in the Cretaceous, when the atmosphere was also hyperoxic. Further paleontological description and analysis of arthropod gigantism are thus likely to be rewarding.

Physically variable atmospheres and the origin of animal flight

In addition to the direct physiological effects of variable oxygen concentrations, the physical consequences of differing atmospheric composition are both substantial in magnitude and diverse in character (Dudley and Chai, 1996). The physical effects of an increased air density would be most profound for the evolution of aerial locomotion; a greater density will result in increased force production by aerodynamic structures (Vogel, 1994) and possibly advantageous shifts of the Reynolds number. Harlé and Harlé (1911), in fact, proposed that giant Paleozoic insects would have required hyperdense air in order to fly. The end-Devonian to early Carboniferous origin of flight in insects (Wootton, 1990) correlates well with an increasing air density in the late Paleozoic (see Fig. 1). Enhanced aerodynamic force production by bodies and protowinglets of ancestral insects, together with higher Reynolds numbers favorable for lift generation (Vogel, 1994), would have facilitated evolution of both gliding and flapping flight in protopterygotes. The initial evolution of the intensely oxidative flight metabolism of insects would also have been facilitated by enhanced oxygen diffusion within the tracheal respiratory system. A hyperoxic atmosphere in the late Paleozoic would thus have contributed both biomechanically and physiologically to the evolution of aerial locomotor performance in animals. Secondary effects associated with changing atmospheric composition may also have contributed to insect flight evolution. For example, a reduction in earth surface temperatures associated with declining Paleozoic carbon dioxide concentrations might have increased the selective advantages of incipient thermoregulation in protopterygote winglets prior to fully developed aerodynamic functions (see Kingsolver and Koehl, 1985, 1994).

The three independent origins of vertebrate flapping flight may also have been facilitated by hyperdense and hyperoxic atmospheres (Table 1). The precise timing of flight evolution in birds, bats and pterosaurs remains indeterminate, but a likely late Jurassic appearance and Cretaceous diversification of birds

Table 1. *Evolution of animal flight and insect gigantism in relation to atmospheric oxygen and density*

Geological range	Atmospheric oxygen	Atmospheric density	Evolution of flapping flight	Insect gigantism
Pre-Carboniferous	Hypoxic	Hypodense	(No taxa)	(None)
Carboniferous to end-Permian	Hyperoxic	Hyperdense	Insects Pterosaurs (?)	Present
Triassic to early Jurassic	Hypoxic	Hypodense	(No taxa)	Absent
Mid-Jurassic to Cretaceous/Tertiary	Hyperoxic	Hyperdense	Birds Bats (?)	Present (e.g. Ephemeroptera)
End-Tertiary	Normoxic	Normodense	(No taxa)	Absent

The origin of flapping flight in insects is unclear but probably occurred in either the late Devonian or Lower Carboniferous (Wootton, 1990). Similarly, the origins of flight in bats and pterosaurs remain only broadly constrained in the fossil record (see text).

is temporally correlated with a time of increasing air density and oxygen availability (see Fig. 1). The widely debated flight abilities of *Archaeopteryx* (e.g. Ruben, 1991, 1993) may thus be placed in the broader context of increased oxygen concentration and air density in the early Cretaceous. The earliest known pterosaurs date from the Triassic and are consistent with Permian origins of flight, whereas pterosaur gigantism (e.g. *Quetzalcoatlus*) is confined to the Cretaceous (Wild, 1984; Wellnhofer, 1991). Most recently, the earliest fossils of microbats have been dated at 50 million years before the present (MYBP) and demonstrate seemingly modern morphology, whereas bat origins may be placed either within the early Paleocene or late Cretaceous (Jepsen, 1970; Altringham, 1996). One potential commonality among these three unrelated vertebrate taxa is origination and diversification during periods of elevated atmospheric oxygen partial pressure. Only a detailed fossil record for these taxa will unequivocally test this correlational hypothesis, but a non-uniformitarian perspective of atmospheric composition is minimally required as one (of numerous) alternative interpretations of vertebrate flight evolution. The aerodynamic effects of variable atmospheric composition cannot be determined for the extinct transitional forms of flying animals, but one approach to evaluating historical scenarios is to investigate the performance of extant organisms under postulated ancestral atmospheric conditions.

Neontological perspectives

The hyperdense conditions of the late Paleozoic were causally linked with hyperoxia, but the density and oxygen concentrations of experimental gas mixtures can be varied independently through combinations of oxygen and nitrogen with selected noble gases (Dudley and Chai, 1996; see Fig. 2). Unfortunately, hyperoxic manipulations have received little experimental attention relative to biomedically oriented studies of hypoxia. This deficiency is unfortunate because hyperoxic conditions are likely to have prevailed during much of early tetrapod diversification as well as during the multiple evolutionary origins of animal flight. The use of hyperoxia provides a classic test of

hypotheses for diffusive limitations in respiratory pathways; if enhanced oxygen availability does not yield increased rates of oxygen consumption, non-diffusive (i.e. convective and/or perfusive) limits to aerobic capacity are indicated. Much of the early literature in this area is apparently confounded methodologically (Welch, 1982), although whole-animal studies of exercising humans show that hyperoxia facilitates an increase in the maximal rate of oxygen consumption (Plet *et al.* 1992; Knight *et al.* 1993). Curiously, no experimental tests have been made to evaluate the effects of hyperoxia on the exercise metabolism of amphibians and reptiles.

An analysis of hyperoxic effects for a wide size range of amphibian taxa would also be informative to evaluate allometries of maximum rates of cutaneous and pulmonary respiration. However, neontological simulations of amphibian paleophysiology must necessarily consider carbon dioxide levels as well as oxygen availability. The wide range of carbon dioxide concentrations through the Paleozoic may have significantly influenced the evolution of vertebrate activity metabolism and of tetrapod endothermy (Graham *et al.* 1997). For example, hypercapnia can interact *via* respiratory acidosis with the metabolic effects of hypoxia (e.g. Kuhnen *et al.* 1987) and hyperoxia (e.g. Graham and Wilson, 1983). The induction of hypothermia by hypoxia in various vertebrates (Wood, 1991) suggests that a comparable exploration of the effects of hyperoxia on thermoregulation will be informative. Potential interactions between oxygen availability and hypercapnia during thermoregulatory responses are also relevant in the light of the covariance between these two gases during the Permian elaboration of tetrapod endothermy (Graham *et al.* 1997).

Animal flight poses a particularly interesting context for the study of maximal aerobic metabolism given that this locomotor mode demonstrates the physiological extremes of mass-specific oxygen uptake rates among vertebrate and invertebrate taxa. Given interests in high-altitude flight and the efficiency of oxygen extraction, the effects of hypoxia on avian respiratory physiology have been widely studied, albeit primarily in the context of resting metabolism (Faraci, 1991). However, a recent study found no hyperoxic enhancement of the maximum metabolic rates of hovering hummingbirds flying in hypodense

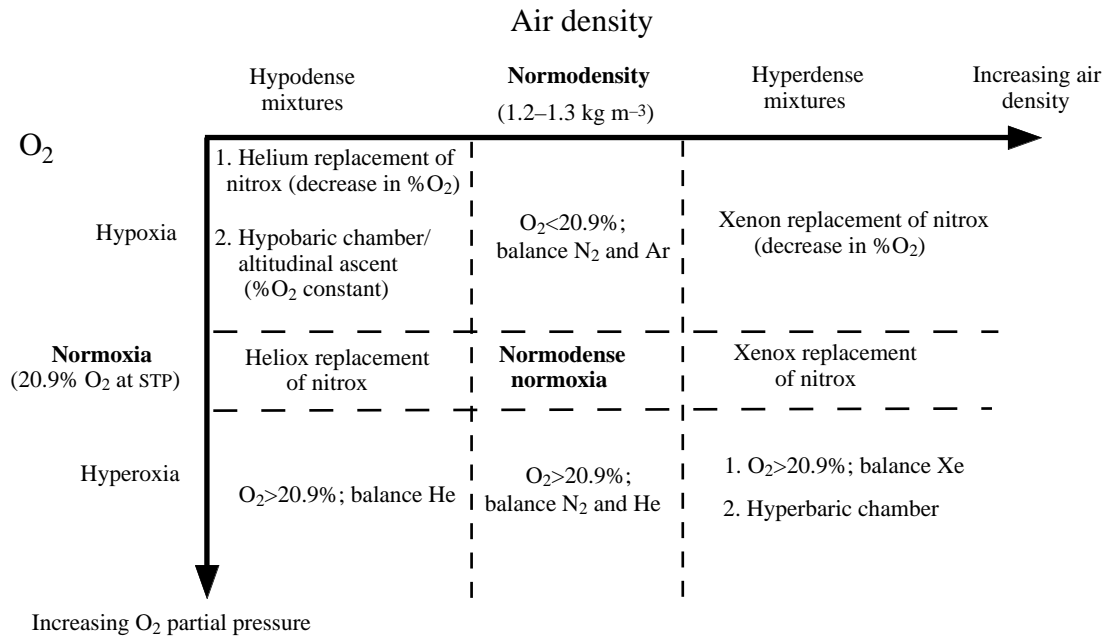


Fig. 2. Experimental methods for covarying oxygen partial pressure and air density of gas mixtures. Heliox (also known as helox), 20.9% $O_2/79.1\%$ He; nitrox, 20.9% $O_2/79.1\%$ N_2 ; xenox, 20.9% $O_2/79.1\%$ Xe. See Dudley and Chai (1996) for details.

air (Chai *et al.* 1996), suggesting non-diffusive limits to maximal flight performance. Flight is energetically more costly at lower air densities, and the use of helium to replace nitrogen is a simple and benign method of eliciting increased power production from flying animals (Dudley, 1995; Dudley and Chai, 1996). In hypodense and hypoxic gas mixtures achieved by replacing nitrox with pure helium (Fig. 2), hummingbird flight performance is clearly limited by oxygen availability (Chai and Dudley, 1996). Failure while hovering in such gas mixtures occurs at oxygen concentrations of 11–14%, corresponding to the oxygen partial pressure at an elevation of approximately 4000 m (Chai and Dudley, 1996).

This remarkable performance demonstrates a lower bound to oxygen utilization by hummingbird flight muscle, but potential upper bounds to flight metabolism remain unclear. The use of hypodense but normoxic gas mixtures suggests, however, that biomechanical rather than physiological constraints exist for the maximum flight capacity of hovering hummingbirds. Ruby-throated hummingbirds flying in heliox (21% $O_2/79\%$ He) fail to sustain hovering at air densities slightly less than half of those of normobaric air; the oxygen concentration is unchanged in this manipulation, although the density corresponds to an altitude of approximately 6000 m (Chai and Dudley, 1995). Hummingbirds fail in hovering when the angular extent of wing motions on either side (the stroke amplitude) is near 180° ; interference from the opposite wing appears to limit aerodynamic force production and ultimately hovering ability. The failure of hyperoxia to enhance hovering performance in this taxon is therefore not surprising.

Altitudinal gradients involve changes in both oxygen partial pressure and air density (see Fig. 2). Flight performance at reduced air density has not been well studied in insects, even

though high-altitude distributions are commonplace in many insects. Insects demonstrate a remarkable ability to fly at low air densities under both hypoxic (e.g. Galun and Fraenkel, 1961) and normoxic (e.g. Dudley, 1995) conditions. The allometric dependence of flight performance at low density has not yet been completely determined, although limited data suggest that smaller animals can fly at lower densities and attain higher levels of muscle power output (Dudley and Chai, 1996). Hummingbirds are an ideal taxon in which to evaluate further questions of physiological and biomechanical adaptation to hypoxic and hypodense air. Hummingbird species diversity is greatest over the altitudinal range 1500–2500 m, and some of the larger species are, somewhat paradoxically, most common at high altitudes. For example, the 20–22 g giant hummingbird (*Patagona gigas*) is resident at elevations up to 4000 m (Ortiz-Crespo, 1974); this species would be an ideal candidate for focal studies of flight biomechanics and physiology. Moreover, patterns of intra- and interspecific variation in bird wing length across altitudinal gradients suggest that wing length is increased to offset the higher induced power costs at low air densities (see Dudley and Chai, 1996). The recent use of load-lifting methods to assay maximum performance in hummingbirds (Chai *et al.* 1997), when combined with hypodense manipulations of hovering flight, promises to be of great utility in analyses of inter- and intraspecific adaptation across altitudinal gradients.

For insects, future experimental manipulations might include total density reduction under both normoxic and hyperoxic conditions to determine whether increased oxygen availability augments maximum metabolic rate. Allometric and phylogenetic comparisons among volant insects and vertebrates are also warranted. Sphingid moths match

hummingbirds for body mass and hovering ability; a study of these two taxa under conditions of maximal oxygen consumption would enable direct comparisons of design limits to tracheal and pulmonary respiration. To date, no hyperoxic manipulations have been implemented on flying insects near maximal performance, although the metabolic rate of hovering honeybees is independent of oxygen partial pressure under normobaria (Joos *et al.* 1997). The use of hyperdense and hyperbaric mixtures (Fig. 2) may also reveal biomechanical compensation and energetic responses not well-documented in flying animals. For example, hyperbaric but normoxic gas mixtures reduce the metabolic costs of hovering flight in honeybees (Withers, 1981), consistent with reduced aerodynamic costs of flight at higher air densities.

Moreover, the ontogenetic responsiveness of the tracheal system of insect larvae to both hypo- and hyperoxia (Loudon, 1988, 1989; Greenberg and Ar, 1996) suggests that an experimental evaluation of the flight performance of adult pterygotes grown under conditions of variable oxygen availability would be informative for paleobiological reconstructions. Ontogenetic and chronic adult exposure of bats and birds to hyperoxia might also reveal compensatory responses in growth and flight physiology. For example, the growth of avian embryos is enhanced under hyperoxia in some, but not all, species (Temple and Metcalfe, 1970; Metcalfe *et al.* 1981; Williams and Swift, 1988), and adult birds chronically exposed to hypobaria exhibit acclimation of blood variables (McGrath, 1971; Pionetti and Bouverot, 1977). The ninefold set of discrete combinatorial interactions between oxygen availability and air density (Fig. 2) thus provides ample experimental opportunity to evaluate the phenotypic plasticity of flight performance in relation to variable atmospheric composition.

Whereas selection for enhanced aerobic performance is generally viewed as a positive evolutionary outcome by locomotor physiologists, the indirect consequences of aerobic metabolism may pose specific biochemical challenges. For example, metabolically generated oxidants, and particularly superoxide radicals, are an important proximate cause of accumulated biochemical damage and ultimately of senescence (Shigenaga *et al.* 1994; Sohal and Weindruch, 1996). The primary biochemical response to such damage is the presence of enzymes, including catalases, peroxidases and superoxide dismutases, that specifically mitigate the deleterious presence of superoxide radicals (Fridovich, 1978; Packer, 1995; Beckman and Ames, 1997). That this response is under genetic control and potentially subject to the powerful effects of natural selection has been elegantly demonstrated using transgenic methods – overexpression of catalase and superoxide dismutase in *Drosophila melanogaster* reduces oxidative damage and significantly extends lifespan (Orr and Sohal, 1994). If the proximate controls of longevity depend substantially on such mitigation of oxidative damage, then the evolutionary regulation of lifespan probably reflects variable selective regimes acting on the expression of these enzymes. Indeed, selection for enhanced lifespan in *Drosophila* also increases superoxide dismutase activity (Tyler *et al.* 1993).

Given the enhanced longevity of flying animals (e.g. Pomeroy, 1990), coupled with the greatly increased rate of activity metabolism associated with flight, a combined evolutionary and biochemical analysis of senescence in volant forms will be directly relevant to the evolution of longevity.

The use of gas mixtures of variable composition permits detailed experimental protocols to be applied to this important issue. For example, the imposition of normobaric hyperoxia is known to increase oxidative damage and to reduce lifespan in *Drosophila melanogaster* (Sohal *et al.* 1993; Baret *et al.* 1994). Longevity in hypo- and hyperdense flight media can thus be compared under hyperoxic conditions to evaluate directly the deleterious oxidative effects of total metabolic expenditure, permitting energetic expenditures during flight to be decoupled experimentally from ambient oxygen exposure. Selection schemes for increased flight performance (e.g. Weber, 1996) can also be effected at variable oxygen levels and at variable air densities, permitting metabolic expenditure to be decoupled from hyperoxic effects. The use of *Drosophila* permits a parallel analysis of concomitant selection for enhanced superoxide dismutase expression and/or effectiveness. Such selection experiments would potentially link mechanistic interpretations of senescence with evolutionary theories of aging (e.g. Partridge and Barton, 1993).

Conclusions

The adequacy of geophysical information concerning atmospheric composition is intrinsic to paleorespiratory analysis. A substantial reduction of atmospheric carbon dioxide levels through the Paleozoic coupled with a late Paleozoic oxygen pulse seem likely, given the congruence of global climate models and specific isotopic results, yet further analyses are warranted, particularly for terrestrial as distinct from marine facies. Correlational analyses of trends in body size evolution should extend beyond documentation of the occurrence of gigantism to include a detailed analysis of phyletic size change in relation to postulated environmental parameters. The present-day physiological simulations of ancestral respiratory patterns supplement paleontological reconstructions and, together with experiments involving ontogenetic exposure to hyperoxia and specific selection regimes, may provide insight into the plasticity of respiratory design as well as the nature of evolutionary responses to oxidative damage. Experimental manipulations of air density and oxygen concentration can be used to evaluate the biomechanics of maximal flight performance and can specifically place physiological performance within the ecological framework of adaptation across altitudinal gradients. A diversity of physiological analyses relevant to both past and present-day adaptation are enabled by the simple recognition that atmospheric composition has varied substantially through geological time.

I thank Doug Altshuler, Peng Chai, Carl Gans, Dmitry Grodnitsky, Jon Harrison, Austen Riggs and Steve Roberts for comments on the manuscript, and the NSF (IBN-9601089) for financial support.

References

- ALTRINGHAM, J. D. (1996). *Bats: Biology and Behaviour*. Oxford: Oxford University Press.
- BARET, P., FOUARGE, A., BULLENS, P. AND LINTS, F. (1994). Life-span of *Drosophila melanogaster* in highly oxygenated atmospheres. *Mech. Ageing Dev.* **76**, 25–31.
- BECKMAN, K. B. AND AMES, B. N. (1997). Oxidants, antioxidants and aging. In *Oxidative Stress and the Molecular Biology of Antioxidant Defenses* (ed. J. G. Scandalios), pp. 201–246. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
- BERNER, R. A. (1989). Drying, O₂ and mass extinction. *Nature* **340**, 603–604.
- BERNER, R. A. (1990). Atmospheric carbon dioxide levels over Phanerozoic time. *Science* **249**, 1382–1386.
- BERNER, R. A. (1994). GEOCARB II: a revised model of atmospheric CO₂ over Phanerozoic time. *Am. J. Sci.* **294**, 56–91.
- BERNER, R. A. (1997). The rise of plants and their effect on weathering and atmospheric CO₂. *Science* **276**, 544–546.
- BERNER, R. A. AND CANFIELD, D. E. (1989). A new model for atmospheric oxygen over Phanerozoic time. *Am. J. Sci.* **289**, 333–361.
- BRIGGS, D. E. G. (1985). Gigantism in Palaeozoic arthropods. *Spec. Pap. Palaeontol.* **33**, 157.
- CARPENTER, F. M. (1992). *Treatise on Invertebrate Paleontology*, Part R, *Arthropoda* 4, vols 3, 4 (*Hexapoda*). Lawrence: University of Kansas Press.
- CARROLL, R. L. (1988). *Vertebrate Paleontology and Evolution*. New York: W. H. Freeman and Co.
- CHAI, P., CHEN, J. S. C. AND DUDLEY, R. (1997). Transient hovering performance of hummingbirds under conditions of maximal loading. *J. exp. Biol.* **200**, 921–929.
- CHAI, P. AND DUDLEY, R. (1995). Limits to vertebrate locomotor energetics suggested by hummingbirds hovering in heliox. *Nature* **377**, 722–725.
- CHAI, P. AND DUDLEY, R. (1996). Limits to flight energetics of hummingbirds hovering in hypodense and hypoxic gas mixtures. *J. exp. Biol.* **199**, 2285–2295.
- CHAI, P., HARRYKISSOON, R. AND DUDLEY, R. (1996). Hummingbird hovering performance in hyperoxic heliox: effects of body mass and sex. *J. exp. Biol.* **199**, 2745–2755.
- DUDLEY, R. (1995). Extraordinary flight performance of orchid bees (Apidae: Euglossini) hovering in heliox (80% He/20% O₂). *J. exp. Biol.* **198**, 1065–1070.
- DUDLEY, R. AND CHAI, P. (1996). Animal flight mechanics in physically variable gas mixtures. *J. exp. Biol.* **199**, 1881–1885.
- ERWIN, D. H. (1992). *The Great Paleozoic Crisis: Life and Death in the Permian*. New York: Columbia University Press.
- ERWIN, D. H. (1994). The *Permo-Triassic* extinction. *Nature* **367**, 231–236.
- FARACI, F. M. (1991). Adaptations to hypoxia in birds: how to fly high. *A. Rev. Physiol.* **53**, 59–70.
- FRIDOVICH, I. (1978). The biology of oxygen radicals. *Science* **201**, 875–880.
- GALUN, R. AND FRAENKEL, G. (1961). The effect of low atmospheric pressure on adult *Aedes aegyptii* and on housefly pupae. *J. Insect Physiol.* **7**, 161–176.
- GRAHAM, J. B., AGUILAR, N., DUDLEY, R. AND GANS, C. (1997). The late Paleozoic atmosphere and the ecological and evolutionary physiology of tetrapods. In *Amniote Origins: Completing the Transition to Land* (ed. S. S. Sumida and K. L. M. Martin), pp. 141–167. New York: Academic Press.
- GRAHAM, J. B., DUDLEY, R., AGUILAR, N. AND GANS, C. (1995). Implications of the late Palaeozoic oxygen pulse for physiology and evolution. *Nature* **375**, 117–120.
- GRAHAM, T. E. AND WILSON, B. A. (1983). Effects of hypercapnia and hyperoxia on metabolism during exercise. *Med. Sci. Sports Exerc.* **15**, 514–519.
- GREENBERG, S. AND AR, A. (1996). Effects of chronic hypoxia, normoxia and hyperoxia on larval development in the beetle *Tenebrio molitor*. **42**, 991–996.
- GRUSZCZYŃSKI, M., HALAS, S., HOFFMAN, A. AND MAKOWSKI, K. (1989). A brachiopod calcite record of the oceanic carbon and oxygen isotopic shifts at the Permo/Triassic boundary. *Nature* **337**, 64–68.
- HALLAM, A. (1991). Why was there a delayed radiation after the end-Paleozoic extinctions? *Hist. Biol.* **5**, 257–262.
- HARLÉ, É. AND HARLÉ, A. (1911). Le vol de grands reptiles et insectes disparus semble indiquer une pression atmosphérique levée. *Bull. Soc. Geol. Fr. 4 Ser.* **11**, 118–121.
- HART, M. H. (1978). The evolution of the atmosphere of the earth. *Icarus* **33**, 23–39.
- HOLLAND, H. D. (1984). *The Chemical Evolution of the Atmosphere and Oceans*. Princeton: Princeton University Press.
- ISOZAKI, Y. (1997). Permo-Triassic boundary superanoxia and stratified superocean: records from lost deep sea. *Science* **276**, 235–238.
- JEPSSEN, G. L. (1970). Bat origins and evolution. In *Biology of Bats*, vol. 1 (ed. W. A. Wimsatt), pp. 1–64. London: Academic Press.
- JOOS, B., LIGHTON, J. R. B., HARRISON, J. F., SUAREZ, R. K. AND ROBERTS, S. P. (1997). Effects of ambient oxygen tension on flight performance, metabolism and water loss of the honeybee. *Physiol. Zool.* **70**, 167–174.
- KINGSOLVER, J. G. AND KOEHL, M. A. R. (1985). Aerodynamics, thermoregulation and the evolution of insect wings: differential scaling and evolutionary change. *Evolution* **39**, 488–504.
- KINGSOLVER, J. G. AND KOEHL, M. A. R. (1994). Selective factors in the evolution of insect wings. *A. Rev. Ent.* **39**, 425–451.
- KNIGHT, D. R., SCHAFFARTZIK, W., POOLE, D. C., HOGAN, M. C., BEBOUT, D. E. AND WAGNER, P. D. (1993). Effects of hyperoxia on maximal leg O₂ supply and utilization in men. *J. appl. Physiol.* **75**, 2586–2594.
- KNOLL, A. H., BAMBACH, R. K., CANFIELD, D. E. AND GROTZINGER, J. P. (1996). Comparative earth history and Late Permian mass extinction. *Science* **273**, 452–457.
- KUHNEN, G., WLOCH, B. AND WUNNENBERG, W. (1987). Effects of acute hypoxia and/or hypercapnia on body temperatures and cold induced thermogenesis in the golden hamster. *J. therm. Biol.* **12**, 103–107.
- KUKALOVÁ-PECK, J. (1978). Origin and evolution of insect wings and their relation to metamorphosis, as documented by the fossil record. *J. Morph.* **156**, 53–126.
- KUKALOVÁ-PECK, J. (1985). Ephemeroïd wing venation based upon new gigantic Carboniferous mayflies and basic morphology, phylogeny and metamorphosis of pterygote insects (Insecta, Ephemeroïd). *Can. J. Zool.* **63**, 933–955.
- KUKALOVÁ-PECK, J. (1987). New Carboniferous Diplura, Monura and Thysanura, the hexapod ground plan and the role of thoracic lobes in the origin of wings (Insecta). *Can. J. Zool.* **65**, 2327–2345.
- KUMP, L. R. (1989). Chemical stability of the atmosphere and ocean. *Global planet. Change* **1**, 123–126.
- LOUDON, C. (1988). Development of *Tenebrio molitor* in low oxygen levels. *J. Insect Physiol.* **34**, 97–103.

- LOUDON, C. (1989). Tracheal hypertrophy in mealworms: design and plasticity in oxygen supply systems. *J. exp. Biol.* **147**, 217–235.
- MAKOWSKI, K., GRUSZCZYŃSKI, M., HOFFMAN, A. AND HALAS, S. (1989). Oceanic stable isotope composition and a scenario for the Permo-Triassic crisis. *Hist. Biol.* **2**, 289–309.
- MCGRATH, J. J. (1971). Acclimation response of pigeons to simulated high altitude. *J. appl. Physiol.* **31**, 274–276.
- METCALFE, J., MCCUTCHEON, I. E., FRANCISCO, D. L., METZENBERG, A. B. AND WELCH, J. E. (1981). Oxygen availability and growth of the chick embryo. *Respir. Physiol.* **46**, 81–88.
- MORA, C. I., DRIESE, S. G. AND COLARUSSO, L. A. (1996). Middle to late Paleozoic atmospheric CO₂ levels from soil carbonate and organic matter. *Science* **271**, 1105–1107.
- ORR, W. C. AND SOHAL, R. S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* **263**, 1128–1130.
- ORTIZ-CRESPO, F. I. (1974). The Giant Hummingbird *Patagona gigas* in Ecuador. *Ibis* **116**, 347–359.
- PACKER, L. (1995). Oxidative stress, antioxidants, aging and disease. In *Oxidative Stress and Aging* (ed. R. G. Cutler, L. Packer, J. Bertram and A. Mori), pp. 1–14. Basel: Birkhäuser Verlag.
- PAGANELLI, C. V., AR, A., RAHN, H. AND WANGENSTEEN, O. D. (1975). Diffusion in the gas phase: the effects of ambient pressure and gas composition. *Respir. Physiol.* **25**, 247–258.
- PARTRIDGE, L. AND BARTON, N. H. (1993). Optimality, mutation and the evolution of ageing. *Nature* **362**, 305–311.
- PIONETTI, J.-M. AND BOUVEROT, P. (1977). Effects of acclimation to altitude on oxygen affinity and organic phosphate concentrations in pigeon blood. *Life Sci.* **20**, 1207–1212.
- PLET, J., PEDERSEN, P. K., JENSEN, F. B. AND HANSEN, J. K. (1992). Increased working capacity with hyperoxia in humans. *Eur. J. appl. Physiol.* **65**, 171–177.
- POMEROY, D. (1990). Why fly? The possible benefits for lower mortality. *Biol. J. Linn. Soc.* **40**, 53–65.
- RETALLACK, G. J. (1997). Early forest soils and their role in Devonian global change. *Science* **276**, 583–585.
- RICHARDS, F. A. (1965). Dissolved gases other than carbon dioxide. In *Chemical Oceanography*, vol. 1 (ed. J. P. Riley and G. Skirrow), pp. 197–225. London: Academic Press.
- ROBINSON, J. M. (1991). Phanerozoic atmospheric reconstructions: a terrestrial perspective. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **97**, 51–62.
- RUBEN, J. (1991). Reptilian physiology and the flight capacity of *Archaeopteryx*. *Evolution* **45**, 1–17.
- RUBEN, J. (1993). Powered flight in *Archaeopteryx*: response to Speakman. *Evolution* **47**, 935–938.
- RUTTEN, M. G. (1966). Geologic data on atmospheric history. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2**, 47–57.
- SCHIDLowski, M. (1971). Probleme der atmosphärischen Evolution im Präkambrium. *Geol. Rundsch.* **60**, 1351–1384.
- SHEAR, W. A. AND KUKALOVÁ-PECK, J. (1990). The ecology of Paleozoic terrestrial arthropods: the fossil evidence. *Can. J. Zool.* **68**, 1807–1834.
- SHIGENAGA, M. K., HAGEN, T. M. AND AMES, B. G. (1994). Oxidative damage and mitochondrial decay in aging. *Proc. natn. Acad. Sci. U.S.A.* **91**, 10771–10778.
- SOHAL, R. S., AGARWAL, S., DUBEY, A. AND ORR, W. C. (1993). Protein oxidative damage is associated with life expectancy of houseflies. *Proc. natn. Acad. Sci. U.S.A.* **90**, 7255–7259.
- SOHAL, R. S. AND WEINDRUCH, R. (1996). Oxidative stress, caloric restriction and aging. *Science* **273**, 59–63.
- TAPPAN, H. (1974). Molecular oxygen and evolution. In *Molecular Oxygen in Biology* (ed. O. Hayaishi), pp. 81–135. Amsterdam: North Holland.
- TEMPLE, G. F. AND METCALFE, J. (1970). The effects of increased incubator oxygen tension on capillary development in the chick chorioallantois. *Condor* **90**, 187–192.
- TYLER, R. H., BRAR, H., SINGH, M., LATORRE, A., GRAVES, J. L., MUELLER, L. D., ROSE, M. R. AND AYALA, F. J. (1993). The effect of superoxide dismutase alleles on aging in *Drosophila*. *Genetica* **91**, 143–149.
- ULTSCH, G. R. (1974). Gas exchange and metabolism in the Sirenidae (Amphibia, Caudata). I. Oxygen consumption of submerged sirenids as a function of body size and respiratory surface area. *Comp. Biochem. Physiol.* **47A**, 485–498.
- VERMEIJ, G. J. (1987). *Evolution and Escalation*. Princeton: Princeton University Press.
- VISSCHER, H., BRINKHUIS, H., DILCHER, D. L., ELSIK, W. C., ESHET, Y., LOOY, C. V., RAMPINO, M. R. AND TRAVERSE, A. (1996). The terminal Paleozoic fungal event: Evidence of terrestrial ecosystem destabilization and collapse. *Proc. natn. Acad. Sci. U.S.A.* **93**, 2155–2158.
- VOGEL, S. (1994). *Life in Moving Fluids: The Physical Biology of Flow*. Princeton: Princeton University Press.
- WATSON, A., LOVELOCK, J. E. AND MARGULIS, L. (1978). Methanogenesis, fires and regulation of atmospheric oxygen. *Biosystems* **10**, 293–298.
- WEBER, K. E. (1996). Large genetic change at small fitness cost in large populations of *Drosophila melanogaster* selected for wind tunnel flight: rethinking fitness surfaces. *Genetics* **144**, 205–213.
- WEIS-FOGH, T. (1964). Diffusion in insect wing muscle, the most active tissue known. *J. exp. Biol.* **41**, 229–256.
- WELCH, H. G. (1982). Hyperoxia and human performance: a brief review. *Med. Sci. Sports Exerc.* **14**, 253–262.
- WELLNHOFER, P. (1991). *The Illustrated Encyclopedia of Pterosaurs*. London: Salamander Books Ltd.
- WIGNALL, P. B. AND HALLAM, A. (1992). Anoxia as a cause of the Permian/Triassic mass extinction: facies evidence from northern Italy and the western United States. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **93**, 21–46.
- WIGNALL, P. B. AND HALLAM, A. (1993). Griesbachian (Earliest Triassic) palaeoenvironmental changes in the Salt Range, Pakistan and southeast China and their bearing on the Permo-Triassic mass extinction. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **102**, 215–237.
- WIGNALL, P. B. AND TWITCHETT, R. J. (1996). Oceanic anoxia and the End Permian mass extinction. *Science* **272**, 1155–1158.
- WILD, R. (1984). Flugsaurier aus der Obertrias von Italien. *Naturwissenschaften* **71**, 1–11.
- WILLIAMS, J. B. AND SWIFT, K. (1988). Oxygen consumption and growth of Northern Bobwhite embryos under normoxic and hyperoxic conditions. *Condor* **90**, 187–192.
- WITHERS, P. C. (1981). The effects of ambient air pressure on oxygen consumption of resting and hovering honeybees. *J. comp. Physiol.* **B 141**, 433–437.
- WOOD, S. C. (1991). Interactions between hypoxia and hypothermia. *A. Rev. Physiol.* **53**, 71–85.
- WOOTTON, R. J. (1990). Major insect radiations. In *Major Evolutionary Radiations* (ed. P. D. Taylor and G. P. Larwood), pp. 187–208. Oxford: Clarendon Press.