Functional and structural optimization of the respiratory system of the bat *Tadarida brasiliensis* (Chiroptera, Molossidae): does airway geometry matter?

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Summary

We studied structure and function of the respiratory system in the bat *Tadarida brasiliensis* and compared it with those of two species of rodents, *Abrothrix andinus* and *A. olivaceus*. *Tadarida brasiliensis* had lower resting oxygen consumption, but higher maximum oxygen consumption and aerobic scope, than the rodents. The blood–gas barrier of the bat was thinner and its relative lung size was larger; however, alveolar surface density was similar among the three species. In consequence, *T. brasiliensis* has an oxygen diffusion capacity two or three times higher than that of the rodents. In *Tadarida brasiliensis* the characteristics of the lung were accompanied by geometrical changes in the proximal airway, such as high physical optimization as a consequence of small variations in the symmetry and the scaling ratio of the bronchial diameters. These may constitute an efficient way to save energy in respiratory mechanics and are the first report of airway adjustments to decrease entropy generation in bats.

Key words: bat, rodent, airway, lungs, diffusion capacity, optimization.

Introduction

The respiratory system is a good model for studying optimization from a functional perspective because it consists of linked structures with defined design parameters and an overall function that has a measurable upper limit, the maximum rate of oxygen consumption ($\dot{V}_{O_2max}$; Weibel, 1998). There have been several studies on the optimization of the respiratory system's airway from a physical perspective, i.e. minimum entropy production in the respiratory mechanics (Rohrer, 1915; Murray, 1926; Weibel and Gomez, 1962; Canals et al., 2002a), and recently a geometrical study of the bronchial tree (Canals et al., 2002b). The geometrical design of the airway must satisfy the twin requirements of good distribution of the inspired volume of air onto a two dimensional alveolar surface and good air conduction during respiration (Canals et al., 2004). The geometry and dimensions of branched structures that make up the airway are important factors in determining the efficiency of air distribution with minimal viscous dissipation (Mauroy et al., 2004). Large bifurcation angles in the bronchial tree can increase total lung volume, and at the same time induce the best distribution of terminal tips of the bronchial tree (Canals et al., 2004). In contrast, small bifurcation angles may be necessary to avoid turbulent flow,

when breathing rate is high (Kamiya et al., 1974). When the scaling ratio γ_d between the diameters of the daughter branches to that of the parent branch, is lower than an optimum $\gamma^* = \sqrt[3]{(1/2)} = 0.7937$, the resistance of the airway increases. If the scaling ratio is larger than γ^* dead space increases, requiring a larger tidal volume (Mauroy et al., 2004). The symmetry of the airway, taken as similar diameters (or lengths) of daughter branches at one bifurcation, may play an important role in the optimization of the airway. On the one hand, asymmetry may reduce the scaling ratio needed for a physical optimization of the airway γ^* (Mauroy et al., 2004). On the other hand the structural asymmetry of the airway may compensate any flow asymmetry due to inertial effects in symmetrical bronchial trees (Andrade et al., 1998).

Animals with high energetic requirements have physiological and structural compromise solutions for the required energetic efficiency, environmental demands and lifestyle constraints (Schmidt-Nielsen, 1997). Because flight is one of the most energetically expensive forms of locomotion, these compromises are particularly evident in flying animals. For example, birds and bats increase oxygen consumption 10- to 20-fold from rest to flight (Bartholomew et al., 1964) and

their mass-specific aerobic capacities are 2.5–3 times higher than those of non-flying mammals of the same size (Thomas, 1987). To satisfy these high oxygen demands, flying animals must optimize the structure of the respiratory tract (Maina, 2000a,b, 2002) and the cardiovascular system (Greenewalt, 1975; Johansen et al., 1987; Mathieu-Costello et al., 1992; Maina, 2000a,b).

Bat lungs are similar to those of non-flying mammals, but may be highly refined to operate at near maximum values. Maina (1998) identified the bats as having a 'narrow-based high-keyed strategy'. This strategy includes: (i) larger heart and cardiac output (Jürgens et al., 1981; Canals et al., 2005a), (ii) high hematocrit, high hemoglobin concentration and high blood oxygen transport capacity (Wolk and Bogdanowics, 1985) and (iii) optimization of respiratory structural parameters (Lechner, 1985; Maina, 1998, 2000a,b; Jürgens et al., 1981; Maina et al., 1991).

Little is known, however, about possible refinements or adjustments of the lungs' airways as related to high energetic requirements. Recent studies on the physical optimality of the airway of rats showed a gradual approach towards the physical optimum from proximal to distal zones, which could be explained by a transition from a central distributive zone to a physical domain along the successive bifurcations of the airway (Canals et al., 2004). Canals et al. (2004) developed a geometric pattern for the branching tree, and deduced useful relationships to estimate departures from both the physical and the geometrical optima of each bronchial bifurcation. They analyzed the bronchial tree of rabbits *Oryctolagus cuniculus*, rats *Rattus norvegicus*, a small rodent *Abrothrix olivaceus* and the bat *Tadarida brasiliensis*. They demonstrated that distances to optimum values decrease from proximal to distal zones of the bronchial tree, and that the distance to the physical optimum value is always lower than that to the geometrical optimum value.

In the present studies, we examine the structural and functional parameters that characterize the respiratory system of the bat *Tadarida brasiliensis*. We expected the respiratory system to respond to energetic demands as a complete system. Thus, we expected high maximum oxygen consumption alongside optimal design in both lungs and airways. To contrast the findings with those obtained using the same methodology in other animals with high energetic requirements, we compared the bat's structure with those of *Abrothrix olivaceus*, a common rodent of low altitude lands, and the high-Andean *Abrothrix andinus*.

Materials and methods
Animal models and sample size

Tadarida brasiliensis (Molossidae) Geoffroy Saint-Hilaire 1824 is a small bat that inhabits from sea level to 1000 m. This species usually colonizes urban buildings (Mann, 1978; Gantz and Martinez, 2000; Canals et al., 2002c). Its diet consists of small moths and beetles caught in flight in open areas. It is a streamlined bat with high wing loading and a large aspect ratio (Iriarte-Díaz et al., 2002) that facilitates high flight speeds. This species has been reported flying at an altitude of 3000 m during migration (Gantz and Martinez, 2000). *Abrothrix andinus* Philippi, 1858 is a high Andean-dwelling rodent that lives in sandy or rocky areas with shrub vegetation [3500 and 4600 m above sea level (masl)]. *Abrothrix olivaceus* Waterhouse, 1837 is found broadly in North and Central Chile where it dwells in grasslands, bushes, open forests and stony areas from sea level to 2500 masl. We studied 20 individuals of *T. brasiliensis* [body mass M_b=11.3±1.1 g (mean ± 1 S.D.)], 34 individuals of *A. olivaceus* (M_b=26.3±6.8g) and 10 individuals of *A. andinus* (M_b=25.5±3.2 g), in order to study their metabolism and bronchial tree. Three individuals of each species were selected for lung microscopy and another four or five for lung volume estimations. Animals were caught during spring and summer 2003 using Sherman traps or mist nets and immediately transported to the laboratory. Metabolic trials were performed within 24 h of capture. In all structural studies, the animals were euthanized by CO_2 exposure following the guidelines of the Ethical Committee of the Faculty of Sciences of the University of Chile.

Oxygen consumption

Resting and maximum metabolic rates were estimated by oxygen consumption using a modified closed-circuit automatic system, based on a manometric design of Morrison (Rosenmann and Morrison, 1974). Resting metabolic rate (RMR) was measured at 30°C, in post-absorptive resting animals. Maximal metabolic rate ($\dot{V}_{O_2max}$) was measured at 5°C in a He–O$_2$ (80–20%) atmosphere, following Rosenmann and Morrison's protocol. Metabolic scope MS was estimated as $\dot{V}_{O_2max}$–RMR. Each metabolic run was conducted for 2 h after an acclimation period of 15–30 min during the active circadian phase of each species. Body temperature was measured at the end of each measurement using an intra-rectal copper–constantan thermocouple. We measured MS, $\dot{V}_{O_2max}$ and RMR of 34 individuals of *A. olivaceus,* 9 *A. andinus* and 20 *T. brasiliensis*. Comparisons between species were analyzed with a one-way analysis of variance (ANOVA) followed by Tukey's multiple range tests.

Pulmonary structure

We removed the lungs of three individuals of each species (M_b=11.3±0.5 g, 26.3±2.0 g and 25.5±1.9 g for *T. brasiliensis*, *A. olivaceus* and *A. andinus,* respectively) and measured the thickness of the blood–gas barrier and the alveolar surface. We also estimated the lung's volume using the radiographic method (Canals et al., 2005b) in four or five other individuals of each species.

We followed Maina's methodology (Maina, 2002) to fixate lung tissues. We used sections of each lobule of both lungs to prepare 4–10 blocks, from which small pieces (1–2 mm thick) were obtained. These samples were fixed in glutaraldehyde (2.3% glutaraldehyde in phosphate buffer) for 2 h at 4°C, washed in buffer and postfixed with 1% osmium tetroxide, and then dehydrated in gradually increasing concentrations of

ethanol. Semithin sections (1 μm thick) were prepared and stained with 1% Toluidine Blue. Ultrathin sections (60–90 nm) were prepared and contrasted with lead citrate before examination by transmission electron microscopy (JEOL/JEM 100SX, Musashino, Tokyo, Japan)

The images were digitalized and studied using SCION IMAGE software (Frederick, MD, USA). The harmonic mean thickness of the blood–gas barrier (τ_h) was estimated by means of point-and-line counting in a grid, as suggested by Weibel (1970/71) and Maina (2002). Twelve measurements of τ_h in different parts of the lungs were obtained for each individual and the species effect on τ_h was analyzed by repeated-measures ANOVA. Alveolar surface density was estimated by measuring directly the alveolar perimeter in three fields of each lung section per individual in the optical microscope. We considered the ratio of alveolar perimeter to field area (R) as a good estimator of the alveolar surface density, following the principle of stereological methodology (Weibel, 1970/71; Weibel at al., 1981). Lung volume was estimated from radiographs of four individuals of *T. brasiliensis* (M_b=11.95±1.4 g), four individuals of *A. olivaceus* (M_b=26.0±4.3 g) and five individuals of *A. andinus* (M_b=25.4±3.0 g). On lung images, we traced a straight line (RL) between the costophrenic recesses, and measured: (i) the width of RL (W) as a lateral measurement; (ii) the height between RL and the top of the left (H_1) and the right (H_2) lung; and (iii) left (w_1) and right (w_2) lung width at the middle point of diaphragm domes. Recently, Canals et al. (2005b), showed that $Lv^*=0.496V_{RX}\approx1/2V_{RX}$ is a good estimator of lung volume in small mammals, where $V_{RX}=W[(H_1+H_2)/2]\times[(w_1+w_2)/2]$ represents the volume of a box containing the lungs. The parenchymal lung volume was estimated as $Lv_p=0.9Lv^*$ (Maina, 2000a,b). From these structural measurements the morphometric oxygen diffusion capacity was estimated using: $D_{tO_2}=\kappa(D_{sa}\times Lv_p)/\tau_h$, where D_{tO_2} is the oxygen diffusion capacity of the blood–gas barrier (tissue), κ is the Krogh's diffusion coefficient $\kappa=4.1\times10^{-8}$ cm^2 s^{-1} Pa^{-1} (Maina, 2002) and D_{sa} is the alveolar surface density.

Airway geometry

We initially dealt with all individuals of each species. Animals were euthanized and bronchographs were immediately performed, using an 18 g plastic catheter introduced into the trachea. Water-diluted barium sulfate was introduced into the airway followed by air, to displace the contrast medium filling to the thinnest bronchi. The procedure was performed using radioscopic visualization in the X-ray service of a public hospital in Chile (Hospital Del Salvador). Radiographs were taken at a distance of 1 m, at 100 mA, 0.04 s and between 24 and 34 kV. Bronchographs were digitalized in a standard format of 400 pixels from the clavicle plane to the distal bronchi. Bronchi order was assigned using Horsfield's system (Horsfield, 1990), which assigns bronchial order from distal to proximal zones. The distal (fine) airway has smaller order values than proximal ones. A bronchograph was

considered sufficient whenever more than 12 relative orders were recognized. The fractal dimensions of the bronchial images were computed by means of the box-counting method, implemented using BENOIT software (St Petersburg, FL, USA). We analyzed 15, 7 and 16 individuals of *A. olivaceus, A. andinus* and *T. brasiliensis,* respectively. Comparisons between the species were analyzed using a one-way ANOVA with Tukey multiple comparisons.

We selected the best radiographs for subsequent analyses, retaining only those in which the bronchial junctions were clearly recognizable. We classified a junction as distal if the relative Horsfield order of the thinnest bifurcation branch was lower than 3 and as proximal if its order was larger than 5. Three proximal and three distal junctions were selected to study the departures from optimality. To avoid underestimation due to rotations, the junctions selected from a particular level were those that did not overlap and had the largest bifurcation angle. Only 3, 2 and 4 individuals of *A. olivaceus, A. andinus* and *T. brasiliensis* satisfied these restrictive criteria and were therefore analyzed. The diameter, length and bifurcation angles (θ) of the parent and daughter branches of each bifurcation were measured from digitized images (Figs 1 and 2). The length and diameter scaling ratios (γ and γ_d) were estimated by dividing the average diameter of the daughter branches by that of the parent branch. Also, a diameter and length symmetry ratio (D_r and L_r, respectively) between the daughter branches were computed, always using
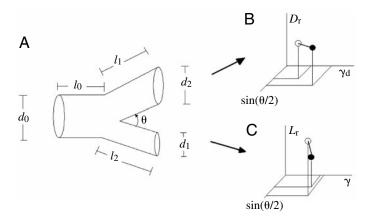

Fig. 1. (A) Scheme of a bronchial junction showing the variables measured and the method to compute geometrical (d_G) and physical (d_P) distances. The bifurcation angle is θ, d_0 and l_0 are diameter and length of the parent bronchi, respectively, and l_1, l_2, d_1 and d_2 are the length and diameters of the daughter branches. The sub-index '1' always indicates the lowest value. (B,C) From the measurements in A the length and diameter scaling ratios (γ and γ_d, respectively) and the length L_r and diameter D_r symmetry ratios were computed: $\gamma=[(l_1+l_2)/2]/l_0)$; $\gamma_d=[(d_1+d_2)/2)/d_0]$; $L_r=l_1/l_2$; $D_r=d_1/d_2$. (A,B) From these measurements one point in the physical domain and one point in the geometrical domain were obtained (open circles in B and C, respectively). Geometrical and physical Euclidean distances are represented by the lines between the open circle and the optimum points (solid circle) in each domain.

Table 1. *Oxygen consumption of* Abrothrix olivaceus, Abrothrix andinus *and the bat* Tadarida brasiliensis *at 30°C (resting) and at 5°C (maximum metabolic rate) in a He–O2 atmosphere**

	RMR (ml O_2 g^{-1} h^{-1})	$\dot{V}_{O2max}$ (ml O_2 g^{-1} h^{-1})	MS (ml O_2 g^{-1} h^{-1})
A. olivaceus	1.75±0.07 (34)	12.34±1.49 (23)	10.53±1.48 (23)
A. andinus	1.87±0.06 (9)	15.56±1.25 (9)	13.68±1.27 (9)
T. brasiliensis	1.04±0.07 (20)	22.25±0.82 (20)	20.14±0.72 (14)

*See text for details.

MS, metabolic scope ($\dot{V}_{O2max}$ – RMR).

Values are means ± S.D. (*N*).

the lower value in the numerator of the ratio, so that D_r and $L_r \leqslant 1$.

Recently Canals et al. (2004) proposed that $\theta=60°$ and $\gamma=\sqrt[3]{(1/2)}=0.7937$ should be expected for a geometrically optimized bronchial tree. From a physical perspective, in a symmetric bifurcation with laminar flow, $\theta=74.934°$ and $\gamma_d=\sqrt[3]{(1/2)}=0.7937$ is expected on the basis of minimum energy loss, minimum volume and pumping power principles (Kamiya et al., 1974; Zamir and Bigelow, 1984; Mauroy et al., 2004). Considering these bases, Canals et al. (2004) defined estimators of the physical and geometrical distances to the expected optima for each junction in the following way: *X*, *Y* and *Z* coordinates as $X=\sin(\theta/2)$ for both the physical and geometrical domains, $Y=\gamma$ for the geometrical and $Y=\gamma_d$ for the physical domains; and finally $Z=L_r$ for the geometrical and $Z=D_r$ for the physical domain, the latter estimating the symmetry of the bifurcation. With these definitions these authors expected a geometric optimum point $(X_0, Y_0, Z_0)=(\sin 30°, 0.7937, 1)=(0.5, 0.7937, 1)$ and a physical optimum point $(X_0, Y_0, Z_0)=(\sin 37.467°, 0.7937, 1)=(0.6083, 0.7937, 1)$; then by defining the departures from the optima as $dX=X–X_0$, $dY=Y–Y_0$, $dZ=Z–Z_0$ they proposed geometrical (d_G) and physical (d_p) distances to the optima as Euclidean distances in each domain as: $\sqrt{(dX^2+dY^2+dZ^2)}$ (Fig. 1).

The pair $\mathbf{D}=(d_G,d_p)$ was first considered as a single vector, and the species effect on that pair was analyzed by repeated-measures MANOVA. Then d_G, d_p, the bifurcation angle θ, the daughter–parent length (γ) and diameter (γ_d) scaling ratios, as well as the symmetry ratios D_r and L_r, were analyzed using repeated-measures two-way ANOVA with SNK multiple comparisons.

Results
Functional parameters

The RMR of *Tadarida brasiliensis* was lower than that of both *Abrothrix* species ($F_{2,60}=33.2$, $P\leqslant0.01$) whereas its maximum metabolic rate ($\dot{V}_{O2max}$) was higher ($F_{2,60}=17.3$,
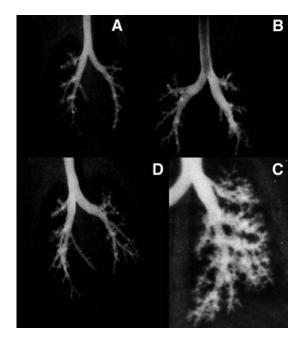

Fig. 2. (A-D) Different phases of the bronchial tree filling of *Tadarida brasiliensis* with contrast medium (barium sulfate).
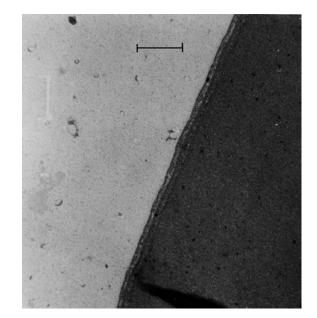

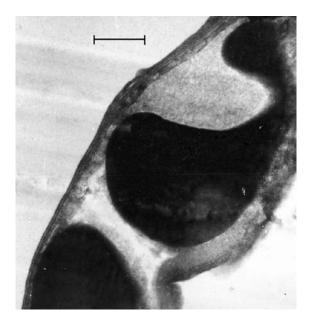Fig. 3. Electronic microscopic image (30 000×) of the air–blood barrier of *Abrothrix andinus*. Scale bar, 1 μm.

Fig. 4. Electronic microscopic image (12 000×) of the air–blood barrier of *Abrothrix olivaceus*. Scale bar, 1 μm.

Fig. 5. Electronic microscopic image (20 000×) of the air–blood barrier of *Tadarida brasiliensis*. Scale bar, 1 μm.

$P \ll 0.01$). Therefore *Tadarida brasiliensis* had a larger metabolic scope than the rodents (Table 1).

Structural lung parameters

The blood–gas barrier of *Tadarida brasiliensis* (*Tb*) was thinner than that of both *A. olivaceus* (*Ao*) and *A. andinus* (*Aa*) ($F_{2,6}=4.91$, $P=0.05$; *Tb–Ao*, $P=0.044$; *Tb–Aa*, $P=0.028$ and *Ao–Aa*, $P=0.75$ in Tukey multiple comparisons; Figs 3–5). The alveolar surface density of the bat was not significantly different than those of the rodents ($P>0.05$), but *T. brasiliensis* had a larger relative lung volume than rodents: 0.055 ± 0.011 ml g^{-1}, 0.043 ± 0.010 ml g^{-1} and 0.036 ± 0.006 ml g^{-1} for *T. brasiliensis*, *A. andinus* and *A. olivaceus*, respectively ($F_{2,10}=5.18$, $P=0.029$). The mass-specific alveolar surface was also higher in the bat (Table 2).
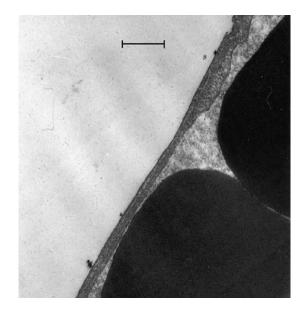
Geometrical and physical optimization of the airway

Comparing the airway radiographs of the three species, we found that all of them fill the lung's space to a similar extent. The fractal dimensions of the species were: *A. olivaceus*, 1.703 ± 0.021; *A. andinus*, 1.670 ± 0.012; and *T. brasiliensis*, 1.682 ± 0.012 ($F_{2,28}=0.734$; $P>0.05$). These were slightly higher than the values of 1.57 for *Rattus norvegicus*, 1.57–1.62 for *Oryctolagus cuniculus* and 1.57 for *Homo sapiens*, obtained using a somewhat different methodology by Canals et al. (1998).

The distances to the geometric and physical optima of the bronchial junctions decreased from the proximal zone to the distal zone [$F_{1,8}=26.7$, $P \ll 0.01$ for the geometrical distance; $F_{1,8}=58.69$, $P \ll 0.01$ for the physical distance; and $F_{2,7}=10.69$, $P<0.01$ for the vector $\mathbf{D}=(d_G,d_p)$ in the repeated-measures

Table 2. *Lung structural parameters of the rodents* Abrothrix olivaceus, Abrothrix andinus *and the bat* Tadarida brasiliensis

	Abrothrix olivaceus	*Abrothrix andinus*	*Tadarida brasiliensis*
M_b (g) ($N=3$)	26.3±2.00	25.48±1.93	11.25±0.50
τ_h (μm)	0.303±0.037	0.345±0.057	0.230±0.086
D_{sa} (cm^{-1})	589.37±45.26	791.8±229.68	690.28±156.96
Lv_p (cm^3) ($N=4/5$)	0.846±0.28	0.972±0.12	0.585±0.09
AA (cm^2)	498.60	769.62	403.81
AA/M_b (cm^2 g^{-1})	18.95	30.20	35.89
ADFc (cm^{-2})	(19.45±2.68)×10^6	(22.95±5.75)×10^6	(30.01±4.18)×10^6
D_{tO_2} (ml O$_2$ s^{-1} Pa^{-1})	0.0000674	0.0000914	0.0000719
D_{tO_2}/M_b (ml O$_2$ s^{-1} Pa^{-1} g^{-1})	2.565×10^{-6}	3.589×10^{-6}	6.398×10^{-6}

M_b, body mass; τ_h, harmonic mean thickness of the blood-gas barrier (tissue); D_{sa}, area density of the alveolar surface; Lv_p, lung volume (parenchyma); AA, alveolar surface ($=D_{sa}\times Lv_p$); AA/M_b, mass-specific alveolar surface; ADFc, anatomic diffusion factor (D_{sa}/τ_h); D_{tO_2}, morphometric oxygen diffusion capacity (see text); D_{tO_2}/M_b, mass-specific diffusion capacity (tissue).

The estimated values are presented as a single value and the measured values as means ± S.D.

Table 3. *Geometrical and physical distances to an optimal bronchial junction in proximal and distal zones of the bronchial tree of* Abrothrix olivaceus, Abrothrix andinus *and the bat* Tadarida brasiliensis

	d_G			d_p		
Species	Proximal	Distal	Average	Proximal	Distal	Average
Abrothrix olivaceus (N=3)	0.600±0.063	0.423±0.113	0.511±0.126	0.510±0.143	0.233±0.056	0.371±0.180
Abrothrix andinus (N=2)	0.578±0.063	0.378±0.003	0.479±0.117	0.468±0.007	0.249±0.059	0.358±0.130
Tadarida brasiliensis (N=4)	0.535±0.097	0.382±0.072	0.449±0.119	0.300±0.057	0.283±0.049	0.292±0.052
Average	0.560±0.081	0.382±0.727	0.471±0.118	0.388±0.127	0.263±0.053	0.326±0.114

d_G, geometrical and d_p, physical distance to optimal value.

Table 4. *Bifurcation angles (θ) in proximal and distal zones of the bronchial tree of* Abrothrix olivaceus, Abrothrix andinus *and the bat* Tadarida brasiliensis

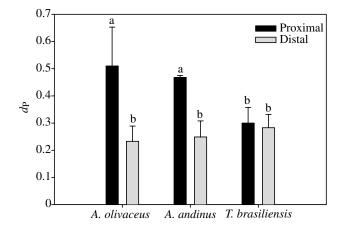|	θ		
Species	Proximal zone	Distal zone	Average
Abrothrix olivaceus	59.6±25.8	76.0±18.3	68.2±23.1
Abrothrix andinus	58.9±4.7	78.7±14.1	69.7±14.7
Tadarida brasiliensis	56.2±11.3	70.9±18.9	63.5±17.1
Average	57.4±15.3	73.5±17.9	65.7±18.4

Fig. 6. Distance from the physical optimum (d_p) for bifurcations in the proximal and distal zone of the airway of the rodents *Abrothrix andinus*, *A. olivaceus* and the bat *Tadarida brasiliensis*. Different letters indicate significant differences, from SNK multiple comparison test, at α=0.05 level.

MANOVA]. We did not find interspecific effects in scalar or vector distances in the studied species [$F_{2,8}$=1.1, P=0.378; $F_{2,8}$=1.97, P=0.211 and $F_{4,14}$=2.49, P=0.09 for d_G, d_p and the vector $\mathbf{D}=(d_G,d_p)$ respectively; Table 3]. However, we found a significant interaction between species and zone factors on the physical distance ($F_{2,8}$=17.47, P≪0.01). This interaction was the result of a smaller physical distance in the bat than in the rodents in the proximal zone. In contrast, in the distal zone, the distances of all species were very similar (Fig. 6).

Geometrical distances depend on the bifurcation angle θ, the ratio between the length of the daughter and the parent branches (γ) and on the ratio between the lengths of daughter branches (L_r). Physical distances are determined by the bifurcation angle θ, the ratio between the diameter of the daughter and the parent branches (γ_d) and the ratio between diameters of the daughter branches (D_r). When we explored the behavior of these components of both distances we did not find interspecific differences ($F_{2,14}$=0.21, P>0.05), but the

bifurcation angle increased from proximal to distal zone ($F_{1,14}$=16.13, P≪0.01; Table 4). Neither of the geometrical components γ and L_r differed among species ($F_{2,8}$=0.99 and $F_{2,8}$=0.49, P>0.05 for γ and L_r, respectively) but both increased from proximal to distal zones ($F_{1,8}$=9.3, P<0.05 and $F_{1,8}$=15.0, P<0.01) (Tables 5 and 6).

The physical components γ_d and D_r were similar among species ($F_{2,8}$=1.12 and $F_{2,8}$=1.57, P>0.05 for γ_d and D_r, respectively), but both increased from proximal to distal zones ($F_{2,8}$=4.2, P<0.05 and $F_{2,8}$=23.6, P<0.01). Furthermore, D_r

Table 5. *Geometric (length) scaling ratio (γ) and physical (diameter) scaling ratio (γ_d) in proximal and distal zones of the bronchial tree of* Abrothrix olivaceus, Abrothrix andinus *and the bat* Tadarida brasiliensis

	γ			γ_d		
Species	Proximal	Distal	Average	Proximal	Distal	Average
Abrothrix olivaceus	0.624±0.325	0.801±0.031	0.713±0.228	0.689±0.062	0.752±0.035	0.721±0.057
Abrothrix andinus	0.624±0.049	0.832±0.243	0.737±0.180	0.656±0.114	0.716±0.019	0.686±0.075
Tadarida brasiliensis	0.484±0.106	0.767±0.112	0.625±0.181	0.718±0.035	0.727±0.021	0.722±0.028
Average	0.551±0.182	0.788±0.115	0.669±0.191	0.698±0.057	0.732±0.026	0.715±0.046

Table 6. *Length (L$_r$) and diameter (D$_r$) symmetry ratios in proximal and distal zones of the bronchial tree of* Abrothrix olivaceus, Abrothrix andinus *and the bat* Tadarida brasiliensis
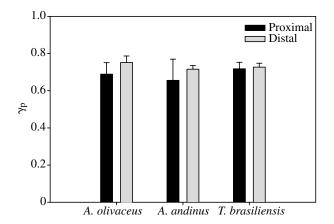
Species	L_r			D_r		
	Proximal	Distal	Average	Proximal	Distal	Average
Abrothrix olivaceus	0.625±0.033	0.701±0.121	0.663±0.090	0.585±0.152	0.824±0.033	0.705±0.164
Abrothrix andinus	0.472±0.015	0.819±0.126	0.646±0.214	0.606±0.076	0.796±0.081	0.701±0.127
Tadarida brasiliensis	0.629±0.091	0.748±0.091	0.689±0.106	0.767±0.069	0.779±0.056	0.773±0.060
Average	0.600±0.091	0.748±0.101	0.674±0.121	0.688±0.125	0.795±0.054	0.741±0.109

Fig. 7. Scaling ratio (γ_d) for bifurcations in the proximal and distal zone of the airway of the rodents *Abrothrix andinus*, *A. olivaceus* and the bat *Tadarida brasiliensis*.

showed a species×zone interaction effect due to larger D_r values in the proximal zone of *T. brasiliensis* ($F_{2,8}$=7.25, P<0.05). Although a similar pattern could be observed for γ_p, the species–zone interaction was not significant (Fig. 7).

Discussion
Functional parameters

The $\dot{V}_{O_2max}$ of *T. brasiliensis* was in the same range as those of small insectivores (see Sparti, 1992) and similar to that of Glossophagine bats (M_b=7–28 g) during short hovering events, which ranges from 20.5 ml O$_2$ g^{-1} h^{-1} to 27.3 ml O$_2$ g^{-1} h^{-1} (Winter et al., 1998; Voigt and Winter, 1999). The $\dot{V}_{O_2max}$ of *T. brasiliensis* was lower than that expected from the allometric relationship $\dot{V}_{O_2max}$=186.91$M_b^{-0.3}$ (M_b in kg and $\dot{V}_{O_2max}$ in ml O$_2$ min^{-1} kg^{-1}) proposed by Thomas (1987; 43.1 ml O$_2$ g^{-1} h^{-1}), but is 179.3% of the value predicted by the allometric relationship $\dot{V}_{O_2max}$=28.05$M_b^{-0.337}$ (M_b in g and $\dot{V}_{O_2max}$ in ml O$_2$ g^{-1} h^{-1}) for rodents (Rezende et al., 2004). Thomas (1987) estimated that metabolic rates of flying mammals are twice as high as those of running mammals. However, our data agree with Harrison and Roberts (2000), who suggested that small bats have aerobic capacities that are only about 50% higher than those of running mammals.

Structural lung parameters
The mass-specific alveolar surface was similar to that of several bats such as *Phyllostomus hastatus* (37.2 cm^2 g^{-1}), *Cheiromeles torquatus* (33.3 cm^2 g^{-1}) and *Cynopterus brachyotes* (30.0 cm^2 g^{-1}; Maina et al., 1991), but lower than in other small bat species whose mass-specific alveolar surfaces ranged from 50–63.2 cm^2 g^{-1} (Maina et al., 1991, 2000a,b).

The harmonic mean thickness of the tissue barrier was 0.230 µm, similar to those of other bats, which range from 0.1204 µm in *Phyllostomus hastatus* to 0.3033 in *Pteropus poliocephalus* (Maina and King, 1984). The estimated mass-specific diffusion capacity (tissue), D_{tO_2}, of *T. brasiliensis,* 6.98×10^{-6} ml O$_2$ s^{-1} Pa^{-1} g^{-1}, is within the range 1.07×10^{-6}–23.4×10^{-6} ml O$_2$ s^{-1} Pa^{-1} g^{-1} reported for bats weighing between 5.1 g and 456.03 g (Maina and King, 1984; Maina et al., 1991).

Tadarida brasiliensis showed a mass-specific D_{tO_2} from 2–3 times higher than those of the studied rodents. From data of Maina et al. (1991) and Maina (2000b), we observed that D_{LO_2} is about 1/10 of D_{tO_2} (D_{LO_2}/D_{tO_2} ratio=0.092±0.070 in birds and 0.100±0.031 in bats). Assuming this value, we may compare our rodent data with those of Gehr et al. (1981) for several mammals. Our rodents had a mass-specific D_{tO_2} that was slightly lower than those estimated for *Mus musculus* (42 g; 5.07×10^{-6} ml O$_2$ s^{-1} Pa^{-1} g^{-1}), *Mus wagneri* (13 g; 7.46×10^{-6} ml O$_2$ s^{-1} Pa^{-1} g^{-1}) and *Rattus rattus* (140 g; 4.85×10^{-6} ml O$_2$ s^{-1} Pa^{-1} g^{-1}). Both maximum metabolic rates and the structurally derived D_{tO_2} showed an increase from low values in *A. olivaceus*, medium values in *A. andinus* to high values in the bat *T. brasiliensis*. This may be correlated with the energetic requirements of the habitats and life styles of these species. *A. olivaceus* inhabits grasslands, bushes, open forests and stony areas from sea level up to 2500 masl, while *A. andinus* usually dwells in high-Andean zones between 3500 and 4600 masl. *T. brasiliensis* is a flying mammal that usually flies long distances and at high altitude (Gantz and Martinez, 2000; Kunz and Fenton, 2003).

Geometrical and physical optimization of the airway
Breathing work depends on the resistance of all components of the respiratory systems to respiratory movements and airflow. Among them, the main component of the total respiratory resistance is the resistance generated within the airway (80% in humans). This resistance is generated primarily in the proximal airway because of its reduced sectional area relative to that of the distal airways. For example, in humans the airways with bronchial diameters less than 2 mm are only

responsible for 20% of the airway resistance. Thus, the geometry of the proximal airway is a key factor in determining the work needed for breathing. Another relevant factor is breathing frequency. The increments of breathing frequency are paralleled by a decrease in elastic work, but mainly by increments in the work to overcome the turbulent flow (turbulent work) and the viscous resistance (viscous work) that lead an increased total work (West, 2000).

Geometrically, the airways of the studied species approached the optimum from proximal to distal, sacrificing the angle, but varying the symmetry and improving γ. From a physical perspective, the optimum was approached from proximal to distal, improving the symmetry, bifurcation angle and the scaling ratio, γ_d. However, the airway of bats departed from this pattern, maintaining a better γ_d in the proximal zone. In a previous report, Canals et al. (2004) found a decrease in both geometrical and physical distances to the optimum from proximal to distal zones in the airway of several mammals. The reported distances for rabbits and rats are in the range of the reported values in the present study. For example, average values in *Oryctolagus cuniculus* and *Rattus norvegicus* were d_p=0.374 and d_p=0.417, respectively.

In *T. brasiliensis* a decrease was evident in the geometrical distance but not in the physical distance. This latter was always close to the physical optimum. The diameter ratio, γ_d, was close to, but lower than the expected $\gamma=\sqrt[3]{(1/2)}$=0.7937 in both proximal and distal zones. The bifurcation angle, however, varied from 52.2° to 70.9° from proximal to distal airway zones, respectively. The first value was near the geometrical optimum angle (60°). This could be associated with ascendancy of geometrical principles in the distribution of the proximal airway, which could be related to the need to increase the branches to fill a space and with the higher flow velocities in the proximal zone associated with high breathing rate. These imply high Reynold's numbers and increased energetic costs by increased viscous work and turbulence flow (Massaro and Massaro, 2002). If that is the case, a lower physical optimum angle is expected (Kamiya et al., 1974) because it reduces the probability of turbulent flow. This is possible even in fully symmetric trees with more than three bifurcation generations (Andrade et al., 1998). The energetic saving for the bat may be relevant because better optimization values occur in the proximal zone of the airway where the main proportion of the resistance to air flow is generated. Furthermore, bats can reach very high breathing frequencies and tidal volumes during flight (Thomas, 1987). These increase both the turbulent and viscous respiratory work and making energetic saving important.

In the studied species, the average diameter scaling ratio in the distal zones ranged from 0.716 to 0.752. This feature may be critical because in symmetrical bronchial trees the flow resistance is highly sensitive to γ_d (Mauroy et al., 2004): low γ_d implies high resistance. One explanation for this feature is that the exponent 'n=3' in Murray's law used in the derivation of airway models that predict an optimal γ_d=0.7937, may be lower than that for transitional or turbulent air flow, conditioning both

a lower bifurcation angle and a lower γ_d. For example n-exponents have been reported from 2.4 to 2.9 for the airways of four mammals (Horsfield and Thurlbeck, 1981), and 2.8 for the human airway (Kitaota and Suki, 1997). Furthermore, the airways of most mammals are asymmetrical (Canals et al., 2002b). On average, the symmetry ratios of the studied species varied from 0.688 in the proximal zone to 0.798 in the distal zone. This feature could contribute towards decreasing the expected γ_d optimum, and consequently to reducing the dead space, thus requiring a lower tidal volume (Mauroy et al., 2004).

Tadarida brasiliensis showed evidence of the importance of the interaction between the diameter asymmetry of the bronchial tree, the diameter scaling ratio (γ_d) and the distance to the physical optimum in the proximal airway zone. This species showed the lowest distance, which was sustained by a better symmetry (larger D_r) and was accompanied by a scaling ratio closer to the optimum. These findings agree with the predictions of Mauroy et al. (2004), who estimated that a 4% of decrease in γ_d would nearly double the bronchial tree resistance, decreasing the air flow by 50%.

In brief, *T. brasiliensis* showed a high aerobic capacity that was sustained by modifications of both lungs and airways. The bats show reductions in the thickness of the blood–gas barrier, and modifications in lung volume and the geometry of the bronchial tree, but no modifications in the general design of the mammalian respiratory system. It showed the best physical optimization values in the proximal zone. This may be an important feature that saves energy and minimizes energy loss during breathing. Those features, together with previously reported optimization of cardiovascular parameters, such as the haematocrit, red blood cell number, heart mass and heart rate (Maina, 1998), probably facilitate satisfying the high energetic demands of flight in bats.

List of symbols and abbreviations

d_G	geometrical distance to the optimum value
$\mathbf{D}=(d_g,d_p)$	vector of distances
D_{LO_2}	mass-specific diffusion capacity (total, including plasma and red cells)
d_p	physical distance to the optimum value
D_r	diameter symmetry ratio (daughter:parent)
D_{sa}	alveolar surface density
D_{tO_2}	morphometric oxygen diffusion capacity (tissue)
L_r	length symmetry ratio (daughters)
Lv^*	lung volume estimator
Lv_p	parenchymal lung volume
M_b	body mass
MS	metabolic scope
RMR	resting metabolic rate
R	ratio of alveolar perimeter to field area
$\dot{V}_{O_2}$	oxygen consumption
$\dot{V}_{O_2max}$	maximum oxygen consumption
V_{RX}	volume of a box containing the lungs
γ	length scaling ratio
γ^*	optimum scaling ratio

γ_d diameter scaling ratio
κ Krogh's diffusion factor
θ bronchial bifurcation angle
τ_h harmonic mean thickness of the blood gas barrier

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