ENTRAINING THE NATURAL FREQUENCIES OF RUNNING AND BREATHING IN GUINEA FOWL (*NUMIDA MELEAGRIS*)

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Summary

Lung ventilation of tetrapods that synchronize their locomotory and ventilatory cycles during exercise could be economized if the resonant frequency of the respiratory system matched the animal's preferred step frequency. To test whether animals utilize this strategy, the input impedance of the respiratory system of five anesthetized, supine guinea fowl (*Numida meleagris*) was measured using a forced oscillation technique. The resonant frequency of the respiratory system was 7.12±0.27 Hz (*N*=5, mean ± S.E.M.). No statistically significant difference was found between the resonant frequency of the respiratory system and the panting frequency used by guinea fowl at rest

(6.67±0.16 Hz, *N*=11) or during treadmill locomotion (6.71±0.12 Hz, *N*=8) or to their preferred step frequency (6.73±0.09 Hz, *N*=7) (means ± S.E.M.). These observations suggest (i) that, at rest and during exercise, panting guinea fowl maximize flow while expending minimal mechanical effort, and (ii) that natural selection has tuned the natural frequencies of the respiratory and locomotor systems to similar frequencies.

Key words: guinea fowl, *Numida meleagris*, locomotion, ventilation, resonant frequency, respiratory impedance, high-frequency oscillation.

Introduction

Recent studies in animal exercise have shown a coordination between the timing of ventilation and locomotion. A synchronization between these two systems, also known as locomotor/respiratory entrainment, has been demonstrated in trotting and galloping mammals (e.g. hares *Lepus californicus*, domestic dogs *Canis familiaris*, horses *Equus caballus*, humans *Homo sapiens*), marsupials (tammar wallabies *Macropus eugenii*), flying birds (e.g. European starlings *Sturnus vulgaris*, barnacle geese *Branta leucopsis*, pigeons *Columbia livia*) and, most recently, running birds (Canada geese *Branta canadensis*, guinea fowl *Numida meleagris*) (Bramble and Carrier, 1983; Baudinette et al., 1987; Berger et al., 1970; Butler and Woakes, 1980; Banzett et al., 1992b; Funk et al., 1993; Nassar, 1994). Subsequent work has focused on establishing a neural and/or biomechanical origin for this phenomenon (Young et al., 1992b; Banzett et al., 1992a), while the appearance of this coordination in such a phylogenetically wide array of cursorial tetrapods has led some researchers to suggest that locomotor/respiratory entrainment may be an important precursor for developing increased levels of aerobic stamina (Bramble and Jenkins, 1989; Carrier, 1991).

While much debate has ensued over the mechanism(s) causing locomotor/respiratory entrainment (biomechanical and/or neurological) (Ainsworth et al., 1996; Young et al., 1992b; Funk et al., 1989), only a handful of studies have

attempted to address the more significant question of whether entrainment actually improves either locomotor and/or respiratory performance (Funk et al., 1997; Bramble and Jenkins, 1993; Banzett et al., 1992a; Banzett et al., 1992b; Young et al., 1992b). It is known, for example, that in resting mammals and birds the metabolic cost of respiration is a modest fraction of the total metabolic rate, approximately 1–5 % of the total energetic resting budget (Lee and Banzett, 1997; Roussos and Zakynthinos, 1995; Lasiewski, 1972; Otis et al., 1950). Additional studies in humans, starlings and horses have suggested that locomotor forces and movements during exercise make only a minor contribution to lung ventilation, up to just 11 % (Banzett et al., 1992a; Banzett et al., 1992b; Young et al., 1992b). Thus, at the level of whole-body metabolism, synchronizing ventilation with locomotion may have no beneficial, not to mention measurable, effects.

However, respiratory performance in running animals might be enhanced if the ventilatory system had a natural frequency of oscillation matching that of the locomotor system (expressed as the preferred step frequency). Given that the rate of work of breathing during exercise in mammals can increase some 500-fold over resting levels (for maximal ventilation) (Otis et al., 1950), any savings that can be imparted to the respiratory muscles may be of some use in terms of improving aerobic performance. Since the axial muscles in tetrapods have been

shown to play a role in both locomotion and ventilation (Carrier, 1987; Carrier, 1996), entraining the locomotor and ventilatory cycles could also economize the work of breathing by reducing antagonistic motions between the two systems. Breathing at the natural, or resonant, frequency of the respiratory system would accomplish the dual aim of (i) maximizing the flow rate (minute volume) and (ii) minimizing the work required from the respiratory muscles, as has been shown in guinea pigs (*Cavia porcellus*), dogs and pigeons (Mead, 1960; Crawford, 1962; Crawford and Kampe, 1971). Running at the preferred step frequency would minimize the metabolic cost of transport ($ml O_2 g^{-1} km^{-1}$), as has been shown previously in horses (Hoyt and Taylor, 1981). Thus, entrainment at the natural frequencies of the two systems would both minimize any phase interference between the locomotor and ventilatory cycles and economize the work of the locomotor and ventilatory muscles.

Characterizing an 'optimal' breathing frequency

The idea of breathing at a frequency that maximizes flow rate but minimizes the work of the respiratory muscles (sometimes defined as an 'optimal' breathing frequency) was first proposed by F. Rohrer (Rohrer, 1925, cited in Mead, 1960), revived by Otis et al. (Otis et al., 1950) and further elaborated by Mead (Mead, 1960) and Hull and Long (Hull and Long, 1961). One of the many conclusions of Otis et al. (Otis et al., 1950) posited that 'the respiratory apparatus may be so designed that the conditions for minimal mechanical work are also those for maximal muscular efficiency' (Otis et al., 1950, p. 603), an idea inherent in the concept of breathing at resonance. Applying these predictions to (i) awake guinea pigs and (ii) resting and exercising humans, Mead (Mead, 1960) noted that the observed and predicted optimal breathing frequencies in these animals were similar, but also concluded that both breathed at frequencies requiring the least amount of force from the respiratory muscles themselves.

Working independently, Hull and Long (Hull and Long, 1961) used high-frequency oscillations (see also Long et al., 1962, and below) to formalize further the mechanical properties of the respiratory system. To model this mechanically, Hull and Long (Hull and Long, 1961) adapted the principles governing Ohm's law and applied them to ventilatory flow. Resistance in an electronic circuit is described by Ohm's law:

$$r = v/i, \quad (1)$$

where r is resistance, v is voltage and i is current, and the same concepts can be applied to model resistance in the respiratory system:

$$Z = P/\dot{V}, \quad (2)$$

where Z is respiratory system impedance, P is driving pressure and $\dot{V}$ is flow volume. From this, they defined Z as comprising three elements: resistance (R), inertance (I) and compliance (C) (also known as a three-element R-I-C model; the electrical analogues being, respectively, resistance, inductance and capacitance).

Resistance results in the loss of energy through frictional heat and, as such, is considered dissipative opposition. Compliance and inertance are elastic and inertial elements that store potential and kinetic energy, respectively, and are considered non-dissipative oppositions (Hull and Long, 1961). Compliance is defined as the change in volume in response to a unit change in pressure. Inertance is a function of mass and volume acceleration and represents the pressure necessary to accelerate those elements having mass. The impedances of inertance and compliance elements (Z_i and Z_c, respectively) are frequency-dependent and are given by:

$$Z_i = i(2\pi f I) \quad (3)$$

and

$$Z_c = -i(2\pi f C)^{-1}, \quad (4)$$

where f is frequency and i is $(-1)^{1/2}$. Thus, impedance (Z) consists of both resistive (often referred to as real) and reactive (referred to as imaginary) parts, which can also be defined according to their polar notation, i.e. magnitude ($|Z|$) and phase (Θ) (Michaelson et al., 1975), respectively. The magnitude of an R-I-C system is given by:

$$|Z| = \{R^2 + [2\pi f I - (2\pi f C)^{-1}]^2\}^{1/2}. \quad (5)$$

The resonant frequency, f_o, is the frequency at which the impedances of the compliance and inertance elements are equal and opposite (thus canceling each other out), pressure and flow are in phase, and the total impedance is minimal. The resonant frequency is found by setting the reactance terms in equation 5 to zero and solving for f. Or:

$$2\pi f I - (2\pi f C)^{-1} = 0 \quad (6)$$

and thus:

$$f_o = 2\pi(IC)^{-1/2}. \quad (7)$$

Previous studies

The use of a forced ventilation technique (Hull and Long, 1961) to determine the resonant frequency of the respiratory system in resting dogs is based on the seminal work of DuBois et al. (DuBois et al., 1956), who developed the method of delivering pressurized oscillations to the airways at a range of frequencies to determine the impedance of the respiratory system in humans (for a similar study in cats, see also Brody and DuBois, 1956). Numerous variations on this technique have been developed, all dependent on where the initial pressure is applied to the body (through the mouth *via* a mask/tube or around the thorax *via* a whole-body plethysmograph) and where flow is measured (the mouth *via* a pneumotachometer or the thorax) (Hahn, 1990). Until the early 1970s, studies measuring the resonant frequency of the respiratory system applied pressures at each frequency of interest, with an upper limit usually under 20 Hz. Michaelson et al. (Michaelson et al., 1975) described a method of applying pressure signals made up of all frequencies simultaneously and using Fourier transforms to compute impedance. Methods of measuring pressure and flow have also improved, making it possible to measure impedance to much higher frequencies

(Habib et al., 1994). Because of the ease and rapidity with which impedance can be measured, such studies have become increasingly popular among physiologists, in particular for testing multi-element models of the mammalian respiratory system (DuBois et al., 1956) (e.g. Sobh et al., 1997). High-frequency impedance studies have been used to examine the respiratory mechanics of animals including rats (*Rattus norvegicus*; Jackson and Watson, 1982), guinea pigs (Sobh et al., 1997), dogs (Jackson et al., 1984), horses (Young and Tesarowski, 1994), cynomolgus monkeys (*Macaca fascicularis*; Madwed and Jackson, 1997) and human neonates with respiratory distress syndrome (Kalenga et al., 1998).

Only two studies have applied this technique to examine the respiratory mechanics of birds. Crawford and Kampe (Crawford and Kampe, 1971) applied a variation of the sinusoidal forced ventilation method to nine domestic pigeons and concluded that pigeons pant at the resonant frequency of their respiratory system (10.4 Hz). In an earlier study, Crawford (Crawford, 1962) applied a similar technique to conclude that dogs, too, pant at the resonant frequency of their respiratory system. Gillespie et al. (Gillespie et al., 1982) applied this same technique to 10 awake Pekin ducks (*Anas platyrhynchos*) and affirmed that, despite the morphological differences between birds and mammals, the respiratory system of birds could also be described by a multi-element (R-I-C) model similar to that described for mammals. The study of Gillespie et al. (Gillespie et al., 1982) determined that the natural frequency of the chest wall in ducks was 8.0 Hz, but hyperthermic behavior was not examined.

Young et al. (Young et al., 1992b) expanded on the study of Crawford (Crawford, 1962) by combining the visceral piston model of Bramble and Carrier (Bramble and Carrier, 1983) with the study of Heglund and Taylor (Heglund and Taylor, 1988), which demonstrated an allometric relationship between body mass and stride frequency at the trot–gallop transition. The primary focus of Young et al. (Young et al., 1992b) was to determine whether other ventilatory and physiological factors related to locomotor/respiratory entrainment in mammals could also be described by a similar relationship to body mass. They determined that a number of locomotor and respiratory variables scale similarly to body mass, including the preferred galloping speed and the natural frequency of the chest wall (see Discussion). While an important starting point, the study of Young et al. (Young et al., 1992b) was limited to mammals. With the exception of the study of Crawford and Kampe (Crawford and Kampe, 1971) on pigeons and the work of Gillespie et al. (Gillespie et al., 1982) on the respiratory mechanics of ducks, there have been few empirical studies on the mechanical properties of the avian respiratory system, particularly as they relate to exercise.

Aim of present study

Guinea fowl (*Numida meleagris*) typically synchronize their ventilation with their stride while running, particularly when undergoing thermoregulatory panting (Nassar, 1994). Ventilation and locomotion often remain phase-locked, with
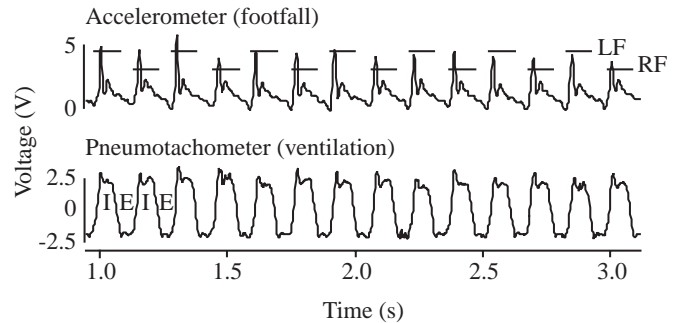

Fig. 1. Representative recordings showing the coordination between footfall and ventilation in running guinea fowl. Treadmill speed was $2.5\,\mathrm{m\,s^{-1}}$. The upper trace shows the signal from the accelerometer (footfall). The corresponding footfall pattern is shown superimposed over the accelerometer trace. RF, right foot; LF, left foot; the length of the horizontal line gives the time the foot is on the ground. The lower trace shows a recording of ventilation as measured by the pneumotachometer (I, inspiration; E, expiration). Note that the signals for footfall and ventilation are in phase at $2\,\mathrm{breaths\,stride^{-1}}$. All data channels were sampled at 200 Hz.

little or no drift occurring (Nassar, 1994). Guinea fowl are capable of utilizing several different patterns of entrainment. A typical pattern is shown in Fig. 1, in which two breathing cycles occur with every stride (giving an entrainment ratio of 2:1). Inspiration coincides with the initial support phase of the step cycle (deceleration of the body in both the horizontal and vertical planes) and expiration coincides with the second half of the support phase (acceleration of the body in both the horizontal and vertical planes). The panting frequency of guinea fowl also appears to be similar (approximately 6–8 Hz), regardless of whether hyperthermia occurred while the bird was resting (sitting in a cage illuminated by incandescent heat lamps) or exercising (running on a treadmill) (P. N. Nassar, personal observations). The consistency of this panting frequency during both rest and locomotion led to the hypothesis presented here: the resonant frequency of the respiratory system in running birds might be tuned to the natural frequency of the locomotor system. Because these systems entrain when the bird undergoes thermoregulatory panting, it is thought that guinea fowl may 'tune' both locomotion and ventilation at a natural frequency as a means of reducing the work of breathing on the respiratory muscles. We would argue that this tuning consists of both active (i.e. neurological) and passive (i.e. musculoskeletal) mechanisms. The aim of this study was to determine the natural frequency of the respiratory system and to investigate whether this is equivalent (or related) to the step frequency during rest and exercise. This study does not directly demonstrate, however, whether any advantage exists to tuning the respiratory and locomotor systems. This hypothesis is similar to that proposed by Young et al. (Young et al., 1992b), but in addition to studying this on an entirely different class and size of animal, the present study utilizes a different technique, high-frequency oscillations, to examine the natural frequency of the respiratory

system on living birds. This study also heralds the first application of a high-frequency oscillation protocol in characterizing the mechanical properties of the avian respiratory system.

Materials and methods

Respiratory impedance

The input impedance (Z) of the respiratory system in anesthetized adult guinea fowl *Numida meleagris* L. (1.42±0.2 kg, N=5, four males, one female; mean ± S.D.) was measured at frequencies between 2 and 32 Hz using a forced oscillation technique. Guinea fowl were chosen for this study because of their demonstrated ability to exercise on a treadmill for extended periods and for their ability to entrain their ventilatory and locomotor systems at a variety of ratios.

Fig. 2 illustrates the experimental arrangement. Impedance measurements were made by digitally generating a pseudorandom noise signal containing frequencies from 2 to 32 Hz in increments of 1 Hz. This signal was passed through a digital-to-analog converter (DAS-16, Metrabyte), amplified and used to drive a 15.38 cm loudspeaker (Polydex, cn107pnpr) mounted centrally in a 19.72 cm high × 23.08 cm diameter cylindrical Plexiglas chamber. The ventilator apparatus was based on a design by Jackson and Vinegar (Jackson and Vinegar, 1979), later modified by Dorkin et al. (Dorkin et al., 1982). Details of the impedance measurements can be found in Chalker et al. (Chalker et al., 1992).

The birds were anesthetized (ketamine, 20 mg kg^{-1}; xylazine, 2 mg kg^{-1} by intramuscular injection), and a tracheostomy was performed. A short (5 cm long) brass cannula whose diameter matched that of the trachea (6 mm) was inserted into the trachea and connected in series to a pneumotachometer (to measure flow volume, $\dot{V}_{ao}$; Hans-Rudolph, model 8011D) and the ventilator. The cannula had an open dorsal port through which the pressure of the airway opening (P_{ao}) at the trachea could be measured with a pressure transducer (Microswitch, model 164). The distance from the tracheal incision to the entrance port of the ventilator was approximately 8 cm. The animal was lying supine because this was the most convenient way to connect the bird to the apparatus without obstructing breathing. Twenty impedance measurements (resistance and reactance) were performed and averaged for both the lower and upper airways of each bird. The lower airways consisted of the trachea, syrinx, air sacs and lungs. It was found that the upper airways, consisting of the trachea, larynx, pharyngeal regions and oral/nasal cavities, behaved as a closed tube when not actively involved in ventilation and were characterized by an infinite resistance. Thus, the trachea was transected as close to the mouth as possible, just caudoventral to the

mandible, and measurements of the upper airways were made only from the initial tracheal incision to the first or second tracheal ring. The added length/volume of the upper airways (larynx, mouth, etc.), if included, would tend to decrease the resonant frequency of the respiratory system. That is, the resonant frequency of the respiratory system in a non-tracheostomized bird would be less than that of a tracheostomized bird. All measurements were taken consistently during the end-expiratory pause of the breathing cycle, when the respiratory muscles and the chest wall of the animal were considered to be at their most relaxed.

Thermoregulatory panting

Panting is a thermoregulatory function defined by high-frequency, low-tidal-volume ventilation. Thermoregulatory panting in guinea fowl is easily observed by (i) an opening of the mouth and (ii) an accompanying movement of the gular in the lower jaw. Similar to other species of birds, movements of the 'gular flutter' in guinea fowl are synchronous with ventilatory movements of the body cavity (Calder and Schmidt-Nielsen, 1968; and P. N. Nassar, unpublished data). Visual comparison of pneumotachometer recordings showed that guinea fowl are capable of thermoregulatory panting during both rest and exercise.

The thermoregulatory panting frequencies of guinea fowl (1.45±0.39 kg, N=11, mean ± S.D.) were measured as they rested in cages warmed by heat lamps. The panting frequency of the 11 resting birds was determined using one of two methods. (i) Six birds were placed in a cage, and a dual thermistor bidirectional flow probe (Dwight Hector Products, Inc.) was secured over one nostril to measure flow direction (inspiration *versus* expiration). The cage was then heated with incandescent heat lamps and kept at a temperature of approximately 35–40 °C until the birds began panting. (ii) Five different birds were placed in a similar apparatus, but in this
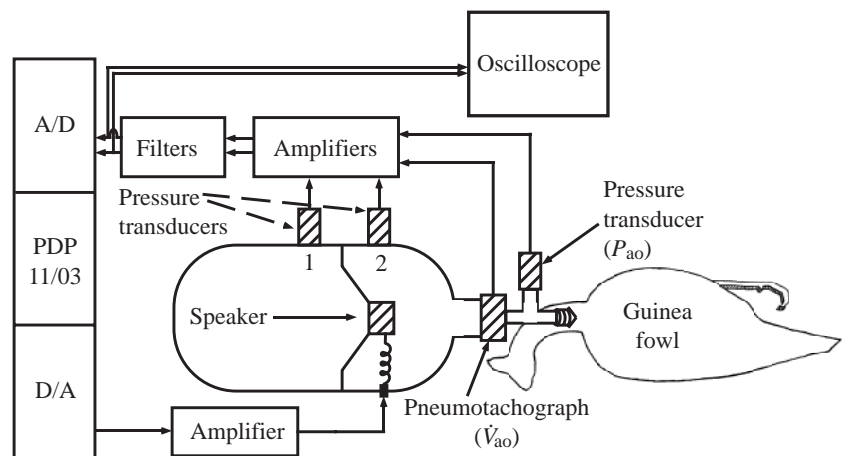

Fig. 2. Schematic diagram of the experimental apparatus used to measure respiratory system impedance. PDP 11/03, computer; D/A, digital-to-analog converter; A/D, analog-to-digital converter; P_{ao}, airway opening pressure; $\dot{V}_{ao}$, flow at the airway opening. The guinea fowl is anesthetized and lying supine (modified from Dorkin et al., 1988).

case panting frequency was measured by having the birds wear a tight-fitting acetate mask (see details below) constructed to be large enough to enable the bird to open its mouth while panting. A screen-mesh pneumotachometer was incorporated into the rostral end of the mask to measure breathing frequency and tidal volume. Experiments were halted after approximately 10–15 min of panting data had been collected. Rectal temperatures were not measured.

The thermistor method was used initially on six birds, and it was not until the following summer that the acetate mask was found to be more useful for exercise studies. From then on, all ventilatory measurements, whether rest or exercise, were acquired with a mask. Since we saw no conflict in results from either protocol, however, both datasets were used to determine resting panting frequency.

The panting frequencies of running guinea fowl (1.18 ± 0.12 kg, $N=8$, mean $\pm$ S.D.) were measured as they exercised on a motorized, variable-speed treadmill (2.60 m long $\times$ 0.56 m wide) at high speeds (2.78–3.33 m s^{-1}). The breathing frequency was determined for birds wearing an acetate mask (see below) and running at a pace that was fast but allowed each bird to maintain a constant speed for at least 20 min. Thermoregulatory panting usually began 5–10 min into the running trial period. Ventilatory rates (breaths s^{-1}) for resting and running were determined by calculating the time it took the bird to complete at least 50 breaths (after Perry et al., 1988).

The closed-mask design for measuring airflow was inspired by Silke Birlenbach. The cylindrical mask was constructed of Polyvinyl plastic (0.51 mm thick), measured 8.3 cm long $\times$ 6 cm diameter, and weighed 23 g. A stainless-steel wire cloth screen (38 µm mesh width; Small Parts, Inc., CX-400) 2.5 cm in diameter was incorporated into the front end of the mask to serve as a pneumotachometer. Two Tygon tubes (outer diameter 3.2 mm, inner diameter 1.6 mm, wall thickness 0.8 mm), one to measure ambient pressure and the other to measure pressure inside the mask, connected the mask to an external pressure transducer (Omega Inc., PX163-005D 5 V, $\pm$12.7 cmH$_2$O; 1 cmH$_2$O=98.1 Pa). The resulting air pressures were amplified on a direct current amplifier (constructed by D. R. Carrier), and the resulting signals were recorded on a multi-channel computer data-acquisition program (AcqKnowledge, v. 3.1.2, Biopac Systems).

To minimize the effects of stress, all the birds were initially trained to run on a treadmill for 2 weeks without any instrumentation. They were then trained to wear a mask while running on a treadmill for an additional 2–4 weeks prior to gathering data. All connecting cables (two tubes to measure pressure, a third tube for bias flow, and the accelerometer cable) were suspended from above so as not to affect the bird's movements and could move freely with the bird fore and aft on the treadmill.

Step frequency and preferred running speed

Step frequencies and running speeds were measured for birds running on a treadmill as well as on a trackway. Step frequencies in both protocols were observed by securing a non-calibrated vertical accelerometer (Omega, Inc., ACC 103, mass 15 g) on the dorsal midline above the pelvis of the bird. In this manner, every step made by the bird (i.e. downward vertical acceleration) corresponded to a sharp 'spike' as shown on the accelerometer data-acquisition recording. The accelerometer was connected to the data-acquisition equipment *via* a lightweight cable that held enough slack to span twice the length of the trackway so as not to hinder the movement of the bird. Control studies to determine the speeds of the birds with and without the accelerometer–cable attachment were not performed, but we saw no adverse effect from these devices on the basis of the speeds the birds were able to attain (see Results). The timing of footfall was determined by correlating accelerometer measurements with high-speed video recordings (Peak Systems, Inc.). Step frequencies on the treadmill were measured in the same manner as described above for breathing frequencies (the rate at which the bird took 50 steps, measured in steps s^{-1}), and using the same eight birds. Running speed was determined by calibrating the treadmill belt.

Step frequencies on the trackway were determined by training guinea fowl (1.44 ± 0.1 kg, $N=7$, mean $\pm$ S.D.) to run in a straight line down an enclosed carpeted trackway (20 m long $\times$ 1 m wide). The initial training regimen was for one investigator (P. N. Nassar) to stand outside the track and pursue each bird to induce running and also to ensure that the bird maintained a relatively constant speed along the entire length of the trackway. A bird already trained to run on the trackway was also introduced to encourage the bird-in-training to follow. By the end of the training day, each guinea fowl was capable of running the length of the trackway multiple times, independently, without the need for the investigator to pursue actively. The final running speed was chosen by the bird. At least 17 (mean 26) runs were recorded for each bird over a 2 day trial period. Between 10 and 17 steps could be acquired per trial (limited by the length of the trackway), and accelerometer measurements were used only when the bird was running at a constant speed. Step frequencies on the trackway were measured by the rate at which the bird took *n* steps, measured in steps s^{-1}, based on the accelerometer recordings.

Running speed on the trackway was determined using 4–5 photocells spaced 1 m apart along the wall at the level of the bird's thigh. When the bird triggered the photocell as it passed by, a corresponding signal appeared on the computer data-acquisition system. Running speed (m s^{-1}) could then be determined by noting the rate at which the bird triggered successive photocells. Eight birds were used to measure running speed (the seven birds used in the trackway study and one additional bird). Trials in which the bird speeded up or slowed down were discarded. A mean value was taken from the range of running speeds each bird exhibited, and this mean was interpreted as the preferred running speed for that particular animal (after Perry et al., 1988).

All experiments were performed at Brown University, Providence, RI, USA, and the University of Utah, Salt Lake City, UT, USA, and animal handling and care were in accordance with institutional guidelines.

Results

Respiratory impedance

The impedance data from the upper and lower airways were each optimally fitted to the impedances of a three-element R-I-C model (for details, see Jackson and Watson, 1982). The resulting impedance values from the upper (u) and lower (l) airways were then combined, where:

$$R_c = R_u + R_l, \tag{8}$$

$$I_c = I_u + I_l \tag{9}$$

and

$$C_c = C_l, \tag{10}$$

where c is the combined resistance (R), inertance (I) or compliance (C) of the upper and lower airways (the upper airways, as we have measured them, do not have a compliant element). By using the values of these estimates for I and C in equation 7, we were able to determine the resonant frequency (f_o) for each bird.

Fig. 3 shows the mean values ± S.D. of the input impedance measurements of the combined upper and lower airways from

all five guinea fowl. The impedance data are shown as resistance (Fig. 3A) and reactance (Fig. 3B).

Resistance (Fig. 3A) remains unaffected by, and is independent of, respiratory frequency. When breathing normally, lying supine and anesthetized, the resonant frequency of the guinea fowl respiratory system (including both the upper and lower airways) was 7.12±0.27 Hz (N=5, mean ± S.E.M., Fig. 3B). From 0 to 7.12 Hz, the system is characterized by compliant elements, whereas from 7.12 to 32 Hz the system is characterized by inertial elements. The system is in resonance when the reactance is zero, or 7.12 Hz. This general pattern is similar to the results found in other species (e.g. Jackson and Watson, 1982). These results also correspond to the predictions made originally by Otis et al. (Otis et al., 1950), who calculated the work of breathing for a known alveolar tidal volume and constant dead space, and concluded that 'for a constant alveolar ventilation there is a (breathing) frequency which is optimal (in the sense of minimal work)' (Otis et al., 1950, p. 600). Hence, they predicted that, at low ventilatory frequencies, a large degree of elastic work would be performed to produce the necessary tidal volume (i.e. compliance elements would prevail); at high frequencies, the system would overcompensate to ventilate the dead space (i.e. inertial elements would dominate). This same pattern is shown in Fig. 3B.

Panting frequency: resting and running birds

The panting frequency of resting guinea fowl was 6.67±0.16 Hz (N=11, mean ± S.E.M.), while the panting frequency of running guinea fowl was 6.71±0.12 Hz (N=8, mean ± S.E.M.). Treadmill speeds ranged from 2.78 to 3.33 m s^{-1}. With the onset of thermoregulatory panting, ventilation was always closely coordinated to locomotion, at 2:1 (breaths:stride), even when the birds ran at close to their maximal sustainable speeds. Because of this tight coupling, step frequencies at these speeds were similar to breathing frequencies.

After prolonged running at sub-maximal treadmills speeds, guinea fowl stopped panting and switched their breathing pattern to a lower entrainment ratio of 1 breath stride^{-1}. In this mode of ventilation, tidal volume increased slightly while breathing frequency dropped, although both values remained well above resting and early warm-up levels. This ventilatory behavior was similar to a type exhibited by oxen (*Bos taurus*; Bianca, 1962), sheep (*Ovis aries*; Hales and Webster, 1967) and domestic fowl (*Gallus gallus*; Randall and Hiestand, 1939; Kassim and Sykes, 1982) known as second-phase breathing. Second-phase breathing, which is always preceded by high-frequency ventilation, is marked by (i) an increase in tidal volume, (ii) a decrease in respiratory rate, (iii) an increase in minute volume and (iv) an increase in arterial hypocapnia. When running at high speeds on the treadmill, guinea fowl showed the first three of the above behaviors. No blood-gas analyses were performed during these experiments, however, so this phenomenon cannot yet be fully confirmed in guinea fowl. It is also not understood why guinea fowl change their
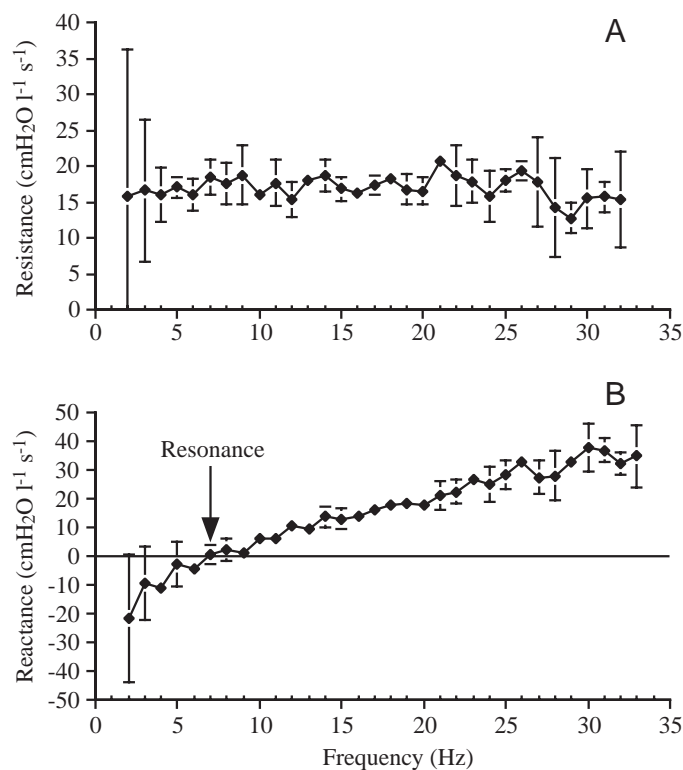

Fig. 3. Input impedance measurements for the combined upper and lower airways from five guinea fowl. Values are means ± S.D. (A) Resistance *versus* frequency. (B) Reactance (compliance + inertance) *versus* frequency. From 0 to 7.12 Hz, the system is characterized by compliant elements, whereas from 7.12 to 32 Hz, the system is characterized by inertial elements. The system is in resonance when the reactance is zero, i.e. at approximately 7.12 Hz. Impedance data were sampled at 128 Hz. Error bars not visible are contained within the symbol. 1 cmH$_2$O=98.1 Pa.

ventilatory behavior in this manner and slow down their breathing frequency. It appears that guinea fowl, like other animals, exhibit this ventilatory behavior as a prolonged thermoregulatory response. Observations of second-phase breathing in other species, however, have all been noted on resting animals, with the exception of the study of Entin et al. (Entin et al., 1999) on exercising sheep. Exercise-induced muscular fatigue may also be a key mechanism in promoting second-phase breathing, either from running for too long at one speed or from reaching a point at which the bird has attained its aerobic maximum to match the speed of the treadmill. Tests in resting cattle and sheep, however, have shown that these animals switch back to rapid, shallow panting when they are suddenly placed back in cooler ambient conditions (Hales and Webster, 1967); thus, it appears that the stimulus for second-phase breathing is primarily temperature-controlled.

Step frequency and preferred running speed

The mean step frequency of guinea fowl running down an enclosed trackway was 6.73 ± 0.09 steps s^{-1} (N=7, mean $\pm$ S.E.M.). The preferred running speed of these birds (measured separately from mean step frequency) was 4.21 ± 0.16 m s^{-1} (N=8, mean $\pm$ S.E.M.). It should be noted, that despite numerous trials in some subjects, the guinea fowl did not pant at high frequencies while performing these tasks (although some began to become hyperthermic in the resting periods in between). Later tests with a loose-fitting pneumotach/mask showed that mechanical coupling still persisted between ventilation and trackway locomotion, but at a much lower ratio of two breaths:three strides.

Statistical analyses

To determine whether the panting frequencies (both at rest and while running) and preferred step frequencies were similar to the resonant frequencies of the respiratory system, the data were subjected to both an unpaired *t*-test and a Mann–Whitney *U*-test (Sokal and Rohlf, 1995). For all tests, $P>0.1$, leading us to conclude that the differences between these measurements were not significant.

Discussion

These results suggest a tight coupling between locomotor and ventilatory function in guinea fowl on the basis of the close correlation between the resonant frequency of the chest wall and lungs (7.12 ± 0.27 Hz, N=5), the panting frequency at rest (6.67 ± 0.16 Hz, N=11), the panting frequency during exercise (6.71 ± 0.12 Hz, N=8) and the preferred step frequency (6.73 ± 0.09 Hz, N=7) (means $\pm$ S.E.M.).

First, these results demonstrate that guinea fowl pant at the resonant frequency of their respiratory system, during both rest and exercise. This suggests that guinea fowl maximize their ventilatory flow for thermoregulation while minimizing the work of the respiratory muscles and supports the conclusions reached by previous studies (Crawford, 1962, in panting dogs; Crawford and Kampe, 1971, in panting pigeons). That this

occurs during exercise as well as rest, however, implies that this strategy may impart some metabolic savings for the respiratory muscles, although how much of a savings remains to be quantified and is beyond the scope of the present study. Research into this critical question has shown that mechanically linking ventilation with locomotion imparts a savings of 1–26 % on the work of the respiratory muscles (Funk et al., 1997, in geese; Ainsworth et al., 1996, in dogs; Banzett et al., 1992b, in starlings) but, as mentioned above, with the exception of the study of Funk et al. (Funk et al., 1997), all investigations into this matter have been *via* indirect or theoretical means only (for a review, see Lee and Banzett, 1997). This metabolic savings could have implications not only for respiratory muscle work but, on a broader scale, for economizing aerobic performance. Breathing at resonance and having the respiratory system tuned to the same natural frequency as the locomotory system ensure not only that guinea fowl thermoregulate with a high degree of efficiency, but also that their respiratory system maximizes its rate of aerobic gas exchange by delivering oxygenated air to the lungs with minimal mechanical resistance. As Otis et al. (1950) noted, 'it is also recognized that for optimal conditions of gas exchange a certain relationship should be maintained between blood flow and ventilation. Since the breathing apparatus is a blood pump as well as an air pump, it may play an important part in the regulation of this ventilation/blood flow ratio' (Otis et al., 1950, p. 606).

Second, no statistically significant difference was found between the natural frequency of the respiratory system and the preferred step frequency at which the bird runs, i.e. both systems appear to be tuned so as to have the same natural frequency. It has been shown in decerebrate preparations in geese and ducks (Funk et al., 1989; Funk et al., 1992) that entrainment of ventilation with locomotion (in the above case with wingbeat frequency) in birds is possible at the level of the brainstem/spinal cord without the benefit of afferent (sensory) feedback. Interestingly, the introduction of afferent feedback in their experiments not only maintained the coordination between the two systems but allowed the animal to modulate further its breath:wingbeat ratio. In addition, entrainment of ventilation with exercise has been demonstrated in a variety of physical tasks in humans, ranging from running, through cycling to finger-tapping (Lee and Banzett, 1997). Thus, at one level, the appearance of such an entrainment in running birds may seem unremarkable because the appearance of coordination does not imply necessarily a mechanical linkage (Lee and Banzett, 1997). However, to find that both ventilation and locomotion are tuned to a similar frequency that may also be capable of economizing respiratory and aerobic performance argues for a more advanced neuromuscular integration in the evolution of these two systems than has previously been considered.

Discrepancy in preferred running speeds

It may be noted from these results that the preferred running speed as measured on the trackway (4.21 m s^{-1}) was not the

same as the preferred running speed measured for the treadmill-run birds (2.78–3.33 m s^{-1}). This is an artifact of treadmill *versus* trackway studies. Guinea fowl, like many other animals (e.g. dogs), do not appear to be comfortable exercising at high speeds on a treadmill. In this study, this may have been due to the dimensions of the treadmill, the steel tread of the belt and/or noise from the engine. The fastest speed at which the guinea fowl appeared to be comfortable running on the treadmill was approximately 3.33 m s^{-1}. Speeds faster than this simply caused most of the birds to stop running and slide off the back end of the treadmill into the waiting arms of the investigator.

While the birds obviously ran faster on the trackway, measurements of footfall show that the step frequencies on the two surfaces were clearly similar (6.71 steps s^{-1} on the treadmill compared with 6.73 steps s^{-1} on the trackway). Thus, while running on the trackway, guinea fowl are not taking fewer strides while running at higher speeds, they are simply increasing their stride length. A longer, wider treadmill belt, a longer training period (more than 2 months) or different training regimens might enable these birds to attain higher treadmill speeds. The increased friction from the trackway may also have been a contributing factor in helping these birds achieve higher speeds. The lack of high-frequency panting in these animals while running on the trackway may be due to the short length of the trackway (i.e. it was not long enough to allow the bird to achieve a sustained speed for more than a few steps).

Do animals optimize ventilation during exercise?

The results of this study are consistent with the hypothesis that guinea fowl optimize ventilation during exercise, but do not directly test this in exercising birds. Direct tests of optimization of ventilation during exercise are rare (see below), probably because of the difficulty of measuring the work of breathing in an exercising animal. Indirect tests (including the present study) have made some headway into this issue, however. Young et al. (Young et al., 1992b) hypothesized that certain ventilatory and locomotor attributes in cursorial mammals should scale at a similar level relative to body mass. These attributes included breathing rate during galloping, stride frequency at the trot–gallop transition (chosen because both stride and ventilatory rates are close to maximal at this juncture), preferred galloping speed, the power output of the diaphragm and the natural frequency of the diaphragm/thorax/viscera. The first three variables were measured through observations of animals running in their natural habitat (e.g. greyhounds, a rhinoceros *Ceratotherium simun*) or on a treadmill (gerbils *Meriones unguiculartus*, horses). The maximal power output of the diaphragm was measured previously (Altringham and Young, 1991) in mice (*Mus musculus*), rats and rabbits (*Oryctolagus cuniculus*) *via* phasic stimulation of isolated diaphragmatic muscle fibers over a range of frequencies. The natural frequency of the diaphragm was determined (in mice, rats, rabbits and wallabies) by calculating the stiffness of the diaphragm and the thorax. This

was achieved by measuring the resisting force of the diaphragm when it was subject to pressures akin to that of the abdominal viscera. The resultant force *versus* displacement curve was then used as a measure of thoracic stiffness and also to compute the natural frequency of the system.

Young et al. (Young et al., 1992a) later demonstrated that the visceral piston model does not adequately drive ventilation during exercise in horses. This unfortunately counters their original hypothesis that entrainment is being driven by periodic oscillations of the viscera colliding with the diaphragm, which in turn would influence pressure changes in the thorax (Young et al., 1992b). In addition, one shortcoming with the chest wall measurements is that they only account for one dampening agent incurred by the thorax during exercise (other agents, for example, might include the compression of the ribcage when the forelimbs strike the ground). Nevertheless, Young et al. (Young et al., 1992b) found a close allometric relationship between all the above variables and body mass and, thus, proposed that locomotor/respiratory entrainment could indeed be seen as a means of optimizing ventilatory performance. Young et al. (Young et al., 1992b) demonstrated that the natural frequency of the thorax, diaphragm and viscera (nf_t) in mammals scaled to body mass (m) as:

$$nf_t = 5.02m^{-0.18}. \qquad (11)$$

There may be a host of additional physiological elements necessary to optimize ventilation (e.g. neurological synchrony between the two systems, coordination of gas exchange in the lung), not to mention any number of factors that might be prevailing to work against it. While Young et al. (Young et al., 1992b) made an encouraging start with their novel study, more research needs to be done in this field to sort out these issues.

In addition to the above studies looking at scaling effects in ventilation, Spells (Spells, 1969/70) and Hahn (Hahn, 1990) independently arrived at a similar relationship between the resonant frequency of the respiratory system in mammals and body mass after conducting extensive literature reviews of studies in which such tests had been performed on resting mammals. Hahn (Hahn, 1990) concluded that the resonant frequency of the respiratory system (f_o) scaled to body mass as:

$$f_o = 22m^{-0.28} \qquad (12)$$

(r=0.8). It should be noted, however, that Hahn's survey, which was the more comprehensive of the two, consisted of 24 studies representing only seven species, ranging from guinea pigs (0.31 kg) to calves (120 kg). Given the refinement of forced ventilation procedures over the past decade and its broadening clinical applicability, a larger and more varied sample size of subjects should become available for more accurate scaling analyses.

While many of the above studies examined how the respiratory system deals with artificially increased respiratory loads (e.g. increased atmospheric CO_2 levels), with the exception of the work of Young et al. (Young et al., 1992b), few of these studies correlate respiratory behavior directly with locomotion. This is surprising, especially considering the

implications of the hypothesis of Young et al. (Young et al., 1992b): that endotherms entrain their breathing with their locomotion as a means of optimizing exercise performance (or to a lesser degree as a means of economizing the work of the respiratory muscles during locomotion). If this is a condition found in mammals as well as birds, it suggests either the retention, from a common ancestor, of a series of primitive traits that still carry some selective advantage or that these characters evolved independently in two disparate lineages (much like endothermy). It is, however, difficult to demonstrate directly that locomotor/respiratory entrainment serves any significant beneficial role in terms of reducing ventilatory work, in part because it is difficult to unlink the two systems naturally and also because the devices needed to perform such measurements (e.g. esophageal balloons to measure thoracic pressures) may hamper the animal's ability to perform. In addition, 'significant' would have to be precisely defined and better quantified. Such a study is feasible, in theory, but would have to start by measuring the actual work of the respiratory muscles themselves during coupled and non-coupled exercise. Funk et al. (Funk et al., 1997) have made a striking first attempt, demonstrating that coordinating wingbeat and ventilation in geese can reduce the work of ventilation by at least 26 %. Until more studies like this appear, however, we are left, somewhat frustratingly, only with indirect methods, analogies and the use of negative evidence to otherwise support or dispute these claims.

Limitations of this study

The primary limitation to this study is that the high-frequency measurements of the resistance of the chest wall/lung were taken while the bird was anesthetized and resting in a supine position, which is clearly not a natural postural state for birds. Ideally, these measurements should be taken in an awake, standing animal wearing a form-fitting face mask with minimal dead space, and also while the animal is exercised at gradually increasing speeds on a treadmill. The primary drawback to this method, and why it was not used for this experiment, is that such a mask would need to be tightly bound to the face to minimize dead space. Because it was thought that this procedure would stress the birds unduly (thus influencing their ventilatory behavior), the anesthesia protocol was chosen instead. Keeping the bird in a supine position was also the clearest way for the bird to breathe without constricting the expansion of the thorax/abdomen. Yet studies in other animals have shown that respiratory impedance can vary depending on the position of the body and the tone of the hypaxial muscles (Gustin et al., 1988). The use of ketamine/xylazine as an anesthetic also depresses muscle tone and lowers respiratory rates in birds (Ludders and Matthews, 1996), thus increasing the compliance of the chest wall and, thereby, lowering the resonant frequency of the respiratory system. Clearly, the stiffness of the chest wall will change constantly throughout the ventilatory cycle and will change even more during the phasic exertions of running, so the natural frequency of the respiratory system will change in a cyclical pattern and may never be set at just 'one' point. That the natural frequencies presented in this study are not significantly different from those actually used by the running bird, however, is an encouraging sign that such a linkage does exist.

Drawbacks to linking ventilation with locomotion

There are some potential hazards to a tight mechanical linkage between ventilation and locomotion, and these have been discussed by Lee and Banzett (Lee and Banzett, 1997). One key disadvantage in keeping the two systems entrained is that ventilation might not be able to cope with the changing metabolic and thermal demands incurred during exercise. If ventilation is locked to locomotion and unable to keep pace with rising energetic costs (running up a steep incline, for example), then gas exchange, not to mention thermoregulation and other systems, would be compromised as a result. Guinea fowl, however, like most endothermic tetrapods, use different ventilatory strategies depending on their activity levels and, at least while running on level surfaces (inclines or other variants were not tested in the present study), most of these alternative ventilatory strategies appeared to be linked at some ratio to stride frequency, although these birds are capable of temporarily decoupling the two. It is conceivable that guinea fowl can change the resonance of their system simply by changing the system's 'stiffness' or, in this case, the compliance of the respiratory muscles during exercise. Thus, these birds may be capable of 'tuning' the two systems to take full advantage of the work being done by the respiratory muscles. Empirical evidence to support or refute this idea is, however, lacking.

Flying birds are known to coordinate their wingbeat cycle with their ventilation, although the ratio of the two frequencies is more variable than that found in most mammals, ranging from 1:1 in crows (*Corvus brachyrhynchos*), to 3:1 in Canada geese to 5:1 in quail (*Coturnix coturnix*) and pheasant (*Phasianus colchicus*; Berger et al., 1970; Funk et al., 1993). As mentioned above, pigeons pant at the resonant frequency of their respiratory system (between 9 and 11 Hz) (Crawford and Kampe, 1971) and, like crows, they coordinate their breathing with their wingbeat at 1:1 (breaths:wingbeat) (Hart and Roy, 1966). The wingbeat frequency of pigeons flying in a wind tunnel has been observed to reach 11.17 Hz (Butler et al., 1977). This similarity in wingbeat and ventilatory frequencies suggests that flying pigeons may also use the resonant qualities of their respiratory and flight systems to economize ventilatory flow. However, more accurate measures of respiratory variables, wingbeat frequencies and preferred flying speeds are needed from flying birds. Birds that use coupling ratios other than 1:1 (e.g. 1:2, 1:3, etc.) may possess locomotor systems with natural frequencies that run at the second (or higher) harmonic of the resonant frequency of their ventilatory system.

Despite the dramatic differences that exist in the locomotor and respiratory systems between birds and mammals (Gatesy and Biewener, 1991; Fedde, 1986; Duncker, 1971), the results

of the present study, in conjunction with previous work (Young et al., 1992b), suggest that birds and mammals have independently evolved the ability (i) to entrain their locomotor and respiratory systems and (ii) to tune these two systems to a similar natural frequency. That this convergence is found in the two lineages (birds and mammals) of modern tetrapods with increased aerobic capacity (Carrier, 1987; Bramble and Jenkins, 1989) suggests that resonant tuning may be important to the evolution of locomotor stamina in the terrestrial environment. In addition, the observation of resonant tuning indicates that the locomotor and ventilatory systems are integrated to a much greater extent than has previously been realized. Developmental, neurological, musculoskeletal and other evolutionary modifications that influence the stride frequency of running animals must be met with changes that alter the oscillatory frequency of the thoracic musculoskeletal system and *vice versa*.

References

Ainsworth, D. M., Smith, C. A., Henderson, K. S. and Dempsey, J. A. (1996). Breathing during exercise in dogs – passive or active? *J. Appl. Physiol.* **81**, 586–595.

Altringham, J. D. and Young, I. S. (1991). Power output and the frequency of oscillatory work in mammalian diaphragm muscle: the effects of animal size. *J. Exp. Biol.* **157**, 381–389.

Banzett, R. B., Mead, J., Reid, M. B. and Topulos, G. P. (1992a). Locomotion in men has no appreciable mechanical effect on breathing. *J. Appl. Physiol.* **72**, 1922–1926.

Banzett, R. B., Nations, C. S., Wang, N., Butler, J. P. and Lehr, J. (1992b). Mechanical independence of wingbeat and breathing in starlings. *Respir. Physiol.* **89**, 27–36.

Baudinette, R. V., Gannon, B. J., Runciman, W. B., Wells, S. and Love, J. B. (1987). Do cardiorespiratory frequencies show entrainment with hopping in the tammar wallaby? *J. Exp. Biol.* **129**, 251–263.

Berger, M., Roy, O. Z. and Hart, J. S. (1970). The co-ordination between respiration and wing beats in birds. *Z. Vergl. Physiol.* **66**, 190–200.

Bianca, W. (1962). Tolerance to severe heat and the behaviour of respiratory minute volume in cattle. *Nature* **195**, 1208–1209.

Bramble, D. M. and Carrier, D. R. (1983). Running and breathing in mammals. *Science* **219**, 251–256.

Bramble, D. M. and Jenkins, F. A., Jr (1989). Structural and functional integration across the reptile–mammal boundary: the locomotor system. In *Complex Organismal Functions: Integration and Evolution in Vertebrates* (ed. D. B. Wake and G. Roth), pp. 133–146. Chichester, New York: John Wiley & Sons.

Bramble, D. M. and Jenkins, F. A., Jr (1993). Mammalian locomotor–respiratory integration: implications for diaphragmatic and pulmonary design. *Science* **262**, 235–240.

Brody, A. W. and DuBois, A. B. (1956). Determination of tissue, airway and total resistance to respiration in cats. *J. Appl. Physiol.* **9**, 213–218.

Butler, P. J., West, N. H. and Jones, D. R. (1977). Respiratory and cardiovascular responses of the pigeon to sustained level flight in a wind tunnel. *J. Exp. Biol.* **71**, 7–26.

Butler, P. J. and Woakes, A. J. (1980). Heart rate, respiratory frequency and wing beat frequency of free flying barnacle geese *Branta leucopsis*. *J. Exp. Biol.* **85**, 213–226.

Calder, W. A. and Schmidt-Nielsen, K. (1968). Panting and carbon dioxide in birds. *Am. J. Physiol.* **215**, 477–482.

Carrier, D. R. (1987). The evolution of locomotor stamina in tetrapods: circumventing a mechanical constraint. *Paleobiol.* **13**, 326–341.

Carrier, D. R. (1991). Conflict in the hypaxial musculo-skeletal system: documenting an evolutionary constraint. *Am. Zool.* **31**, 644–654.

Carrier, D. R. (1996). Function of the intercostal muscles in trotting dogs: ventilation or locomotion? *J. Exp. Biol.* **199**, 1455–1465.

Chalker, R. B., Celli, B. R., Habib, R. H. and Jackson, A. C. (1992). Respiratory input impedance from 4 to 256 Hz in normals and chronic airflow obstruction: comparisons and correlations with spirometry. *Am. Rev. Resp. Dis.* **146**, 570–576.

Crawford, E. C., Jr (1962). Mechanical aspects of panting in dogs. *J. Appl. Physiol.* **17**, 249–251.

Crawford, E. C., Jr and Kampe, G. (1971). Resonant panting in pigeons. *Comp. Biochem. Physiol.* **40**A, 549–552.

Dorkin, H. L., Jackson, A. C., Strieder, D. J. and Dawson, S. V. (1982). Interaction of oscillatory and unidirectional flows in straight tubes and an airway cast. *J. Appl. Physiol.* **52**, 1097–1105.

Dorkin, H. L., Lutchen, K. R. and Jackson, A. C. (1988). Human respiratory input impedance from 4 to 200 Hz: physiological and modeling considerations. *J. Appl. Physiol.* **64**, 823–831.

DuBois, A. R., Brody, A. W., Lewis, D. H. and Burgess, B. F., Jr (1956). Oscillation mechanics of lungs and chest in man. *J. Appl. Physiol.* **8**, 587–594.

Duncker, H.-R. (1971). The lung air sac system of birds. *Adv. Anat. Embryol. Cell Biol.* **45**, 1–171.

Entin, P. L., Robertshaw, D. and Rawson, R. E. (1999). Effect of locomotor respiratory coupling on respiratory evaporative heat loss in the sheep. *J. Appl. Physiol.* **87**, 1887–1893.

Fedde, M. R. (1986). Respiration. In *Avian Physiology*, fourth edition (ed. P. D. Sturkie), pp. 191–220. New York: Springer-Verlag.

Funk, G. D., Milsom, W. K., Sholomenko, G. N. and Steeves, J. D. (1989). Role of the telencephalon in the synchronization of locomotor and respiratory frequencies during walking in Canada geese. *J. Exp. Biol.* **145**, 283–301.

Funk, G. D., Sholomenko, G. N., Valenzuela, I. J., Steeves, J. D. and Milsom, W. K. (1993). Coordination of wingbeat frequency and respiration in Canada geese during free flight. *J. Exp. Biol.* **175**, 317–323.

Funk, G. D., Steeves, J. D. and Milsom, W. K. (1992). Coordination of wingbeat and respiration in birds. II. 'Fictive' flight. *J. Appl. Physiol.* **73**, 1025–1033.

Funk, G. D., Valenzuela, I. J. and Milsom, W. K. (1997). Energetic

consequences of coordination wingbeat and respiratory rhythms in birds. *J. Exp. Biol.* **200**, 915–920.

Gatesy, S. M. and Biewener, A. A. (1991). Bipedal locomotion: effects of speed, size and limb posture in birds and humans. *J. Zool., Lond.* **224**, 127–147.

Gillespie, J. R., Gendner, J. P., Sagot, J. C. and Bouverot, P. (1982). Impedance of the lower respiratory system in ducks measured by forced oscillations during normal breathing. *Respir. Physiol.* **47**, 51–68.

Gustin, P., Dhem, A. R., Lomba, F., Lekeux, P., Van de Woestijne, K. P. and Landser, F. J. (1988). Measurement of total respiratory impedance in calves by the forced oscillation technique. *J. Appl. Physiol.* **64**, 1786–1791.

Habib, R. H., Chalker, R., Suki, B. and Jackson, A. C. (1994). Airway geometry and wall mechanical properties estimated from subglottal input impedance in humans. *J. Appl. Physiol.* **77**, 441–451.

Hahn, G. (1990). Resonant frequency of the chest–lung system by analysis of the respiratory flow curve. *Comp. Biochem. Physiol.* **96**A, 499–502.

Hales, J. R. S. and Webster, M. E. D. (1967). Respiratory function during thermal tachypnoea in sheep. *J. Physiol., Lond.* **190**, 241–260.

Hart, J. S. and Roy, O. Z. (1966). Respiratory and cardiac responses to flight in pigeons. *Physiol. Zool.* **39**, 291–306.

Heglund, N. C. and Taylor, C. R. (1988). Speed, stride frequency and energy cost per stride: how do they change with body size and gait? *J. Exp. Biol.* **138**, 301–318.

Hoyt, D. F. and Taylor, C. R. (1981). Gait and the energetics of locomotion in horses. *Science* **292**, 239–240.

Hull, W. E. and Long, E. C. (1961). Respiratory impedance and volume flow at high frequency in dogs. *J. Appl. Physiol.* **16**, 439–443.

Jackson, A. C. and Vinegar, A. (1979). A technique for measuring frequency response of pressure, volume and flow transducers. *J. Appl. Physiol.* **47**, 462–467.

Jackson, A. C. and Watson, J. W. (1982). Oscillatory mechanics of the respiratory system in normal rats. *Respir. Physiol.* **48**, 309–322.

Jackson, A. C., Watson, J. W. and Kotlikoff, M. I. (1984). Respiratory system, lung and chest wall impedances in anesthetized dogs. *J. Appl. Physiol.* **57**, 34–39.

Kalenga, M., Battisti, O., Francois, A., Langhendries, J.-P., Gerstmann, D. R. and Bertrand, J.-M. (1998). High frequency oscillatory ventilation in neonatal RDS: initial volume optimization and respiratory mechanics. *J. Appl. Physiol.* **84**, 1174–1177.

Kassim, H. and Sykes, A. H. (1982). The respiratory responses of the fowl to hot climates. *J. Exp. Biol.* **97**, 301–309.

Lasiewski, R. C. (1972). Respiratory function in birds. In *Avian Biology*, vol. II (ed. D. S. Farmer and J. R. King), pp. 287–342. New York: Academic Press.

Lee, H.-t. and Banzett, R. B. (1997). Mechanical links between locomotion and breathing: can you breathe with your legs? *News Physiol. Sci.* **12**, 273–278.

Long, E. C., Hull, W. E. and Gebel, E. L. (1962). Respiratory dynamic resistance. *J. Appl. Physiol.* **17**, 609–612.

Ludders, J. W. and Matthews, N. (1996). Birds. In *Veterinary Anesthesia*, third edition (ed. J. C. Thurmon, W. J. Tranquilli and G. J. Benson), pp. 645–669. Baltimore: Williams & Wilkins.

Madwed, J. B. and Jackson, A. C. (1997). Determination of airway and tissue resistances following antigen and methacholine in non-human primates. *J. Appl. Physiol.* **83**, 1690–1696.

Mead, J. (1960). Control of respiratory frequency. *J. Appl. Physiol.* **15**, 325–336.

Michaelson, E. D., Grassman, E. D. and Peters, W. R. (1975). Pulmonary mechanics by spectral analysis of forced random noise. *J. Clin. Invest.* **56**, 1210–1230.

Nassar, P. N. (1994). A dual role for the abdominal muscles of running birds. *Am. Zool.* **34**, 15A.

Otis, A. B., Fenn, W. O. and Rahn, H. (1950). Mechanics of breathing in man. *J. Appl. Physiol.* **2**, 592–607.

Perry, A. K., Blickman, R., Biewener, A. A., Heglund, N. C. and Taylor, C. R. (1988). Preferred speeds in terrestrial vertebrates: are they equivalent? *J. Exp. Biol.* **137**, 207–219.

Randall, W. C. and Hiestand, W. A. (1939). Panting and temperature regulation in the chicken. *Am. J. Physiol.* **127**, 761–767.

Roussos, C. and Zakynthinos, S. (1995). Respiratory muscle energetics. In *The Thorax*, second edition, part A, *Physiology* (ed. C. Roussos), pp. 681–749. New York: Marcel-Dekker.

Sobh, J. F., Lilly, C. M., Drazen, J. M. and Jackson, A. C. (1997). Respiratory transfer impedance between 8 and 384 Hz in guinea pigs before and after bronchial challenge. *J. Appl. Physiol.* **82**, 172–181.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*, third edition, pp. 427–431. New York: W. H. Freeman & Company.

Spells, K. E. (1969/70). Comparative studies in lung mechanics based on a survey of literature data. *Respir. Physiol.* **8**, 37–57.

Young, I. S., Alexander, R. McN., Woakes, A. J., Butler, P. J. and Anderson, L. (1992a). The synchronization of ventilation and locomotion in horses (*Equus caballus*). *J. Exp. Biol.* **166**, 19–31.

Young, I. S., Warren, R. D. and Altringham, J. D. (1992b). Some properties of the mammalian locomotory and respiratory systems in relation to body mass. *J. Exp. Biol.* **164**, 283–294.

Young, S. S. and Tesarowski, D. (1994). Respiratory mechanics of horses measured by conventional and forced oscillation techniques. *J. Appl. Physiol.* **76**, 2467–2472.