Novel motor gestures for phonation during inspiration enhance the acoustic complexity of birdsong

Franz Goller[*] and Monica A. Daley[†]

Department of Biology, University of Utah, 257 South, 1400 East, Salt Lake City, UT 84112, USA

Sound generation based on a pulmonary mechanism typically occurs during the expiratory phase of respiration. Phonation during inspiration has been postulated for the calls of some amphibians and for exceptional sounds in some human languages. No direct evidence exists for phonation during inspiration in birds, but such a mechanism has been proposed to explain very long uninterrupted songs. Here, we report the first physiological evidence for inspiratory sound production in the song of the zebra finch (*Taeniopygia guttata*). Motor gestures of the vocal and respiratory muscles leading to the production of inspiratory phonation differ from those of silent inspirations during song as well as from those leading to phonation during expiration. Inspiratory syllables have a high fundamental frequency, which makes them acoustically distinct from all other zebra finch song syllables. Furthermore, young zebra finches copy these inspiratory syllables from their tutor song, producing them during inspiration. This suggests that physical limitations confine the production of these sounds to the inspiratory phase in zebra finches. These findings directly demonstrate how novel respiratory–vocal coordination can enhance the acoustic structure of birdsong, and thus provide insight into the evolution of song complexity.

Keywords: birdsong; phonation; inspiration; expiration; respiratory–vocal coordination

1. INTRODUCTION

In air-breathing vertebrates that use a pulmonic air stream to generate sounds phonation is typically restricted to the expiratory phase of the respiratory cycle. Sound production during inspiration is exceptional, but is known to occur in a few species of amphibian (*Bombina* and *Discoglossus*) (Schneider 1988). In human speech the use of a pulmonic ingressive air stream during phonation is very rare and may be restricted to a clicking sound in one African language (Fuller 1990; Traill 1991; Ladefoged & Zeitoun 1993; Crystal 1997).

Switching phonation from expiration to inspiration has been proposed as a possible mechanism to enable minute-long uninterrupted songs in the European nightjar (*Caprimulgus europaeus*) (Hunter 1980). However, no direct physiological evidence exists for phonation during inspiration in birds. In fact, in all birds for which respiratory patterns during song are known, phonation has been shown to occur only during the expiratory phase of the respiratory cycle (reviewed in Gaunt 1987; Brackenbury 1989; Suthers & Goller 1997; Suthers *et al*. 1999). This is also true of the majority of syllables in zebra finch (*Taeniopygia guttata*) song, which is typically produced during between three and seven expiratory pulses, separated by short inspirations (minibreaths) (Wild *et al*. 1998). Here, we show, however, that some male zebra finches also sing particular syllables of their motif during inspiration. We explore the neural control mechanisms responsible for their production by studying the peripheral motor correlates, and discuss potential evolutionary causes.

[*]Author for correspondence (goller@biology.utah.edu).
[†]Present address: Concord Field Station, Harvard University, Old Causeway Road, Bedford, MA 01730, USA.

2. METHODS

We recorded sub-syringeal air-sac pressures in 30 group-raised and nine tape-tutored male zebra finches. The methods for recording air-sac pressure and tracheal airflow have been previously described in detail (Hartley & Suthers 1989; Suthers *et al*. 1994). Briefly, for pressure measurements a flexible cannula was inserted under isoflurane anaesthesia into a thoracic air sac and connected to a miniature pressure transducer (Fujikura model FPM-02PG, Tokyo, Japan) on the back of the bird. The response of the pressure transducer is linear for the range of pressures measured here. Therefore, we report pressures as output voltages, which typically correspond to between $8 \, cm \, H_2O$ and $25 \, cm \, H_2O$ during song. In five male zebra finches, we recorded tracheal airflow simultaneously with air-sac pressure. A microbead thermistor (Thermometrics BB05JA202, Edison, NJ, USA) was implanted into the lumen of the base of the trachea. Current supplied by a feedback circuit (Hector Engineering Ellettsville, IN, USA) heated the thermistor bead to a constant temperature, such that the voltage needed to maintain temperature was proportional to the air flowing through the trachea. The output of the thermistor is nonlinear, so we calibrated the airflow data in three out of the five males. Within 15–20 min of the last physiological data during song being recorded, we anaesthetized the bird, injected known flow rates into the air-sac cannula and recorded the voltage output of the tracheal thermistor. Airflow through the cannula caused cessation of spontaneous respiratory movements, so the latter did not interfere with calibration. Calibration values were used to linearize the airflow data and to calculate inspiratory and expiratory volumes during song.

In the tape-tutoring experiments, juvenile zebra finches were acoustically and visually isolated from adult males between the ages of 35 days and 110 days, and tape-tutored with edited song between the ages of 35 days and 55 days. Tutor songs were edited using Signal software (Engineering Design, Belmont,
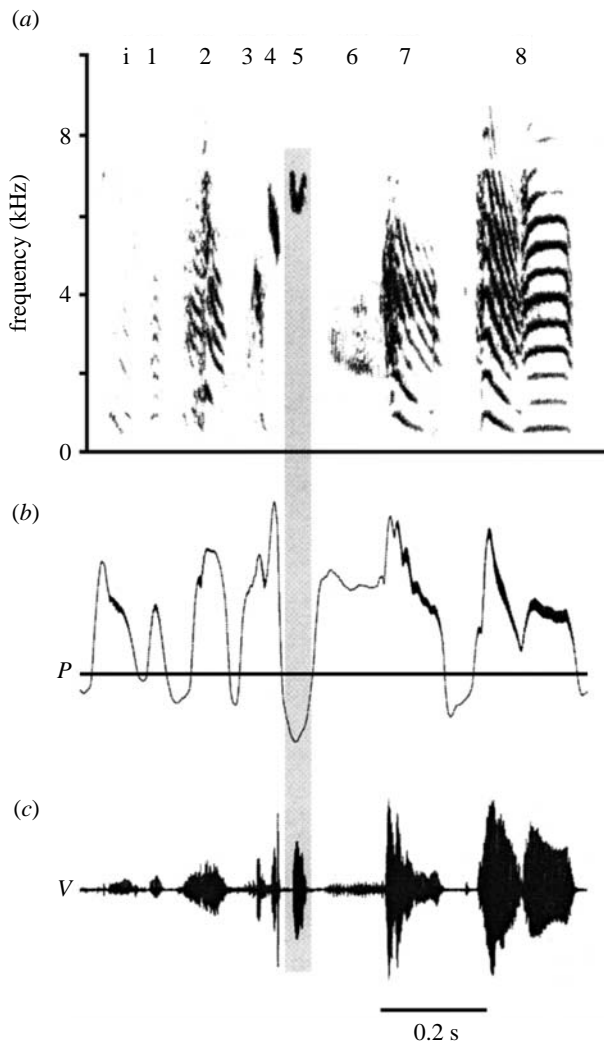
Figure 1. Zebra-finch song (shown as (*c*) an oscillogram and (*a*) spectrographically) illustrating various syllable categories including one inspiratory syllable (5, grey bar). The syllables of the motif are numbered consecutively and are preceded by an introductory note (i). (*b*) The air-sac pressure recording (the horizontal line indicates ambient pressure) allows determination of the phase of the respiratory cycle during which sounds are generated.

Figure 2. (*b*) Airflow (*F*) and (*c*) air-sac pressure (*P*) during inspiratory syllables (grey bars) differ from those during silent minibreaths (sub-atmospheric pressure; the horizontal line indicates ambient pressure) and those during expiratory sounds of the song (shown spectrographically in (*a*)). Transitions from inspiration to expiration are so rapid that airflow data do not reach zero during flow reversals. The marked peaks in airflow during the inspiratory syllables (grey bars) correspond to silent portions at the beginning and end of the minibreath, indicating that the syrinx is abducted prior to and after sound generation.

MA, USA). Two tutor songs were generated in which the inspiratory syllable was moved to an expiratory position, with a silent period in the space previously occupied by the syllable. Two and three juvenile males, respectively, were tutored with one of these two songs. We also created one song where the inspiratory syllable occurred during three out of the four minibreaths. This song was used to tutor four juvenile males. Songs and air-sac pressures from males tutored with one of these tapes were recorded after song crystallization (between the ages of 120 days and 180 days). Song syllables were spectrographed (Signal 3.1) and scored visually as being similar or different. Statistical analyses were performed using either SigmaPlot 5.0 (*t*-test; SPSS, Inc. Chicago, IL, USA) or Signal (linear correlation) software.

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah.

3. RESULTS

As has been reported for zebra finches (Wild *et al.* 1998), our males sang most song syllables during expir-
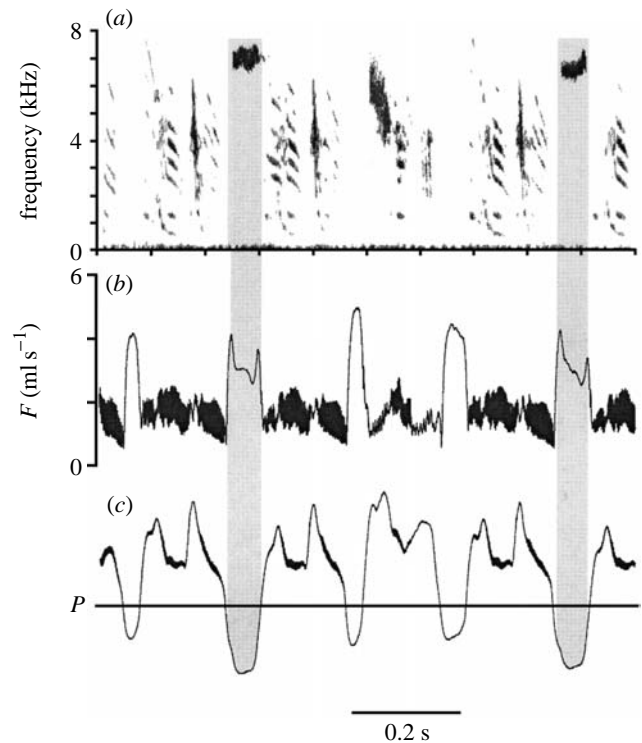
ation. In addition, however, we found that many males generate one or two distinct syllables of their song motif during one or more of the inter-syllable inspirations (inspiratory syllables) (figures 1 and 2). We recorded sub-syringeal air-sac pressure during song in 30 zebra finches, 16 (53%) of which had at least one inspiratory syllable in their song motif, five (16.7%) of which had two inspiratory syllables and one (3.3%) of which had three inspiratory syllables.

The motor gestures for sound production during the inspiratory phase differ from those leading to silent minibreaths and from those during expiratory sound generation. The air-sac pressure during the inspiratory syllables is twice that of silent minibreaths. The mean $\pm$ s.e.m. normalized voltage values were 0.47 ± 0.03 V for minibreaths and 0.96 ± 0.01 V for inspiratory syllables (means are significantly different: *t*-test, $p < 0.0001$, $n = 37$ and $n = 20$, respectively; figure 3*a*). Despite the increased inspiratory pressure, the rate of airflow during inspiratory syllables was lower than that during silent minibreaths, indicating that resistance to flow through the vocal organ, the syrinx, is greater during phonation. The rate of airflow during inspiratory syllables exceeds that observed during expiratory phonation at similar absolute pressure values (figure 3*a*).

As has been shown in canaries (*Serinus canaria*) (Hartley & Suthers 1989), inspiration during silent

minibreaths in zebra finches typically replenishes the volume of air expelled during phonation (figure 3*b*). Inspiratory syllables last longer than silent minibreaths (figure 3*c*), allowing the expired volume to be replenished despite the increased syringeal resistance. Therefore, the inspiratory notes, while using a different motor pattern, accomplish the same task of maintaining air supply as do silent minibreaths.

The inspiratory syllables are acoustically distinct from any syllables produced during expiration (figure 4). Expiratory phonation is dominated by syllables of a low fundamental frequency with a dense harmonic structure (e.g. syllables 1, 2, 7 and 8 in figure 1). Songs typically also include rapidly amplitude-modulated syllables (e.g. syllable 6 in figure 1), and sometimes very short down-modulated frequency sweeps with a high fundamental frequency (e.g. syllables 3 and 4 in figure 1). In contrast, all inspiratory syllables have an exceptionally high fundamental frequency (between 5 kHz and 7 kHz; syllable 5 in figure 1; figure 4) and often include frequency-modulated components. The amplitude of inspiratory syllables is similar to that of song syllables produced during expiration (figure 1).

Learning plays an important role in the acquisition of inspiratory syllables during song development, as it does for expiratory syllables. Juvenile zebra finches copy inspiratory and expiratory syllables from tutor songs in a natural social learning environment, sometimes incorporating the entire song motif, and sometimes incorporating only the inspiratory syllable in an otherwise different song motif (figure 5). In order to test whether the inspiratory syllables could also be produced during expiration, we tutored nine juveniles using computer-edited song on tape. We artificially placed inspiratory syllables in various inspiratory and expiratory positions of the song motif. Out of the nine zebra finches that were tape-tutored, eight incorporated inspiratory syllables into their adult song motifs, while copying only between 9 and 44% of the entire tutor song motif. All the copied inspiratory syllables, however, were produced during inspiration, regardless of their respiratory position in the tutor song motif.

4. DISCUSSION

The physiological data strongly indicate that phonation during inspiration requires novel motor coordination of the muscle systems involved in sound generation. The muscles of the vocal organ control airflow and the acoustic parameters of sound. To maximize airflow during silent minibreaths, the valves of the vocal organ are normally abducted (Vicario 1991; Goller & Suthers 1996*a*). During inspiratory phonation, however, the increased syringeal resistance suggests that the syrinx is adducted. Additionally, high-frequency sounds are associated with very high electromyographic activity of the ventral syringeal muscles in all species that have been investigated (Goller & Suthers 1996*b*; Suthers & Goller 1997; Suthers *et al.* 1999). Typically, these muscles show only weak or no activity during inspiration. The motor patterns of the syringeal muscles during inspiratory phonation must, therefore, be different from those during quiet respiration. Furthermore, the two-fold
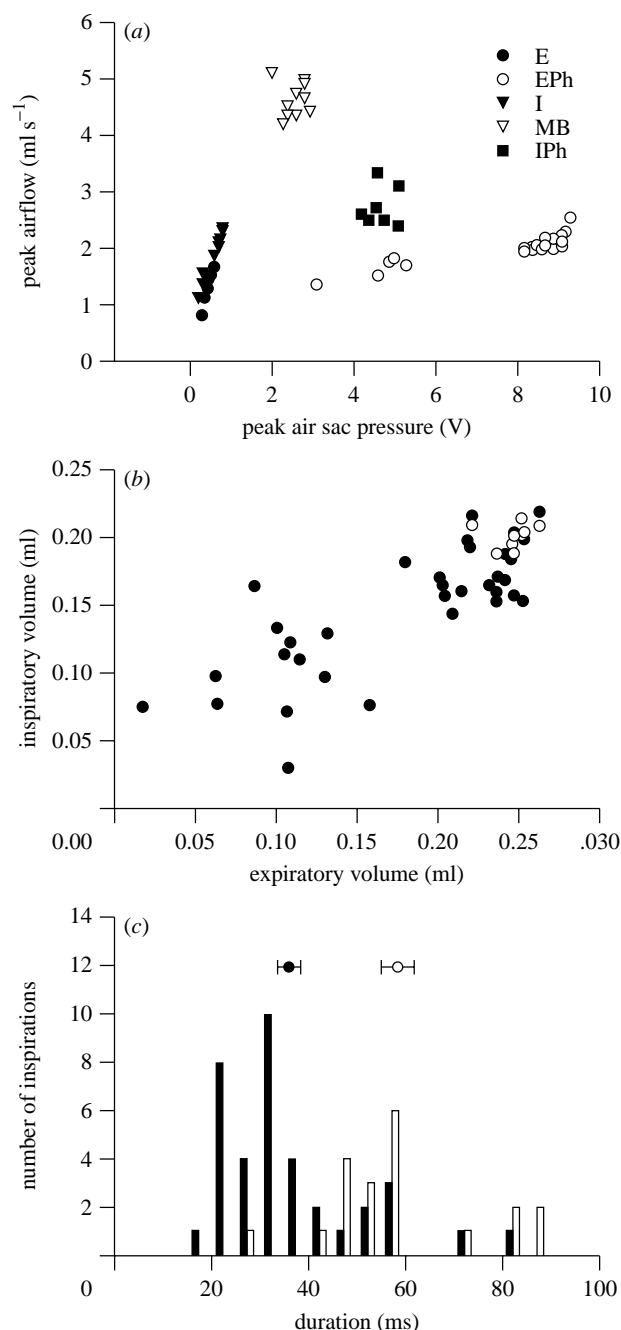

Figure 3. (*a*) Representative example of the relationship between peak air-sac pressure and peak airflow, during quiet respiration and song, in one zebra finch (data for six motifs). E, quiet expiration; EPh, expiratory phonation, including introductory notes and syllables of the motif; I, quiet inspiration; MB, silent minibreaths, i.e. inspirations during the song motif; IPh, inspiratory syllables. (*b*) The expired and inspired volumes during the song motif are positively correlated ($r = 0.82$, $p < 0.001$). Closed circles: silent minibreaths, the inspiratory volume was calculated as the average of the two minibreaths on either side of one expiratory pulse; open circles: inspiratory syllables, flow rates during inspiration were calculated using calibration values for expiratory flow, which probably explains why the slope of this relationship deviates slightly from 1. (*c*) Inspiratory syllables (open bars) are significantly longer than silent minibreaths (filled bars). Frequency distributions of durations include inspirations during one song motif from 16 males with inspiratory syllables. Circles indicate the means $\pm$ s.e.m. for the two datasets.
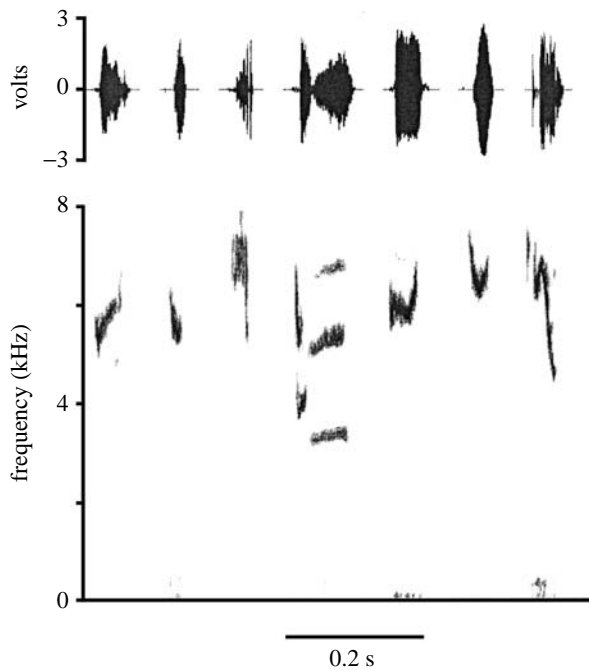
Figure 4. Inspiratory syllables have a high fundamental frequency and are distinct from sounds produced during expiration. These syllables represent the various types of inspiratory syllable found in our colony. The fourth syllable is the most complex inspiratory syllable; it is composed of two notes, both of which show independent contributions of the two sound sources (two-voice phenomenon).

increase in inspiratory pressure during phonation and the greater duration of the phonatory minibreaths suggest modification of the motor gestures of the inspiratory muscles as well.

The sounds generated by these unique motor gestures are acoustically distinct from the sounds produced by zebra finches during expiration. These syllables increase the acoustic complexity of zebra-finch song by extending the frequency range. Furthermore, by replacing silent periods of the song, inspiratory syllables increase the duration over which sound is present in the song motif. Acoustic density is increased and the temporal patterning is maintained by switching between acoustically distinct syllables. Thus, the inspiratory syllables contribute in a unique way to the acoustic and temporal complexity of the song. The fact that inspiratory syllables, when shared by many males, are produced during inspiration by all individuals suggests that an ingressive air stream is required to generate these acoustic features. This suggests, therefore, that physical constraints limit acoustic versatility, but that they can be circumvented by additionally phonating during the inspiratory phase. This indicates that novel motor gestures provide a mechanism for the evolution of song complexity. There is growing evidence that songbird females exhibit a preference for complex songs (Searcy & Andersson 1986; Searcy & Yasukawa 1996; Suthers & Goller 1997). In this context, it would be interesting to know whether female zebra finches prefer songs enhanced by inspiratory syllables.

Phonation during inspiration in zebra finches appears to be a natural phenomenon. Young zebra finches frequently copy inspiratory syllables from tutors in the
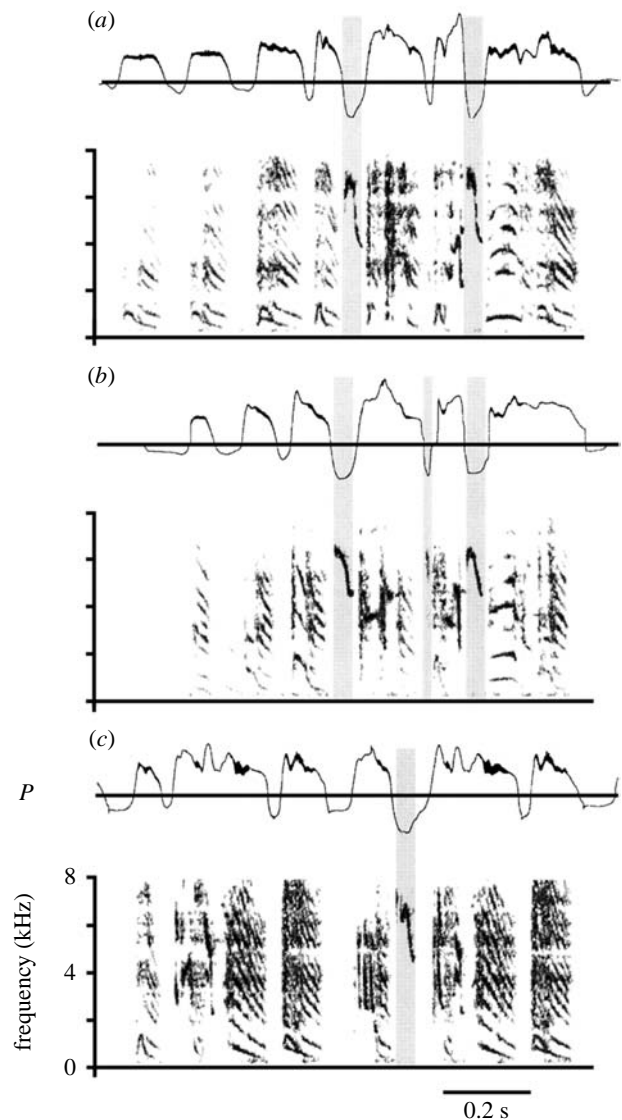

Figure 5. Examples of how inspiratory syllables were copied from the tutor song (*a*) in a breeding aviary. One male (*b*) produced a good copy of the entire motif of the tutor, but inserted a third short inspiratory syllable in place of a silent minibreath in the tutor song. Another male (*c*) copied only the inspiratory syllable, and incorporated it into a motif consisting of syllables copied from other males. Songs are represented spectrographically (bottom) and by the air-sac pressure pattern (*P*, top). The horizontal line on each pressure trace indicates ambient pressure. Note the overall similarity of pressure patterns in (*a*) and (*b*).

natural learning environment and in tape-tutoring experiments. Additionally, there is indirect evidence that inspiratory notes are also present in the songs of natural populations of zebra finches. The absence of a silent period in continuous song lasting more than 250 ms suggests that an inspiratory syllable takes the place of a silent minibreath. Based on this temporal characteristic and the unique acoustic structure of inspiratory phonation, we suggest that many of the syllables that have been identified as 'high' or 'tone' elements in the songs of wild zebra finches (occurring in 74% of song motifs) (Zann 1993) and in those of many captive colonies (Clayton 1988; Williams *et al.* 1989; Yu & Margoliash 1996; Solis & Doupe 2000) are inspiratory syllables.

It is interesting that this first physiological evidence for phonation during inspiration has been found in a species with a short song, and not in a bird with a very long uninterrupted song such as the nightjar (Hunter 1980). If the latter also employs inspiratory phonation, then different selective pressures may have led this species also to phonate during the inspiratory phase of the respiratory cycle. This pattern of using inspiratory airflow to generate specific sounds in zebra finches is different from the proposed phonatory pattern in some amphibians, where either calls are produced only during inspiration (Lörcher 1969) or production of the same call type during both expiration and inspiration is thought to generate an uninterrupted call series (Weber 1974). In humans it is not clear why the use of ingressive pulmonic production of rare speech sounds arose in very few language groups or how it might enhance speech. However, switching between the two respiratory phases takes considerably longer in humans than it does in small birds, creating long silent intervals, and possibly reducing the usefulness of alternation between expiratory and inspiratory phonation.

REFERENCES

Brackenbury, J. H. 1989 Functions of the syrinx and the control of sound production. In *Form and function in birds*, vol. 4 (ed. A. S. King & J. McClelland), pp. 193–220. New York: Academic Press.

Clayton, N. S. 1988 Song tutor choice in zebra finches and Bengalese finches: the relative importance of visual and vocal cues. *Behaviour* **102**, 281–299.

Crystal, D. 1997 *The Cambridge encyclopedia of language*, 2nd edn. Cambridge University Press.

Fuller, M. 1990 Pulmonic ingressive fricatives in Tsou. *J. Int. Phonation Assoc.* **20**, 9–14.

Gaunt, A. 1987 Phonation. In *Bird respiration*, vol. 1 (ed. T. J. Seller), pp. 71–94. Boca Raton, FL: CRC Press.

Goller, F. & Suthers, R. A. 1996a Role of syringeal muscles in gating airflow and sound production in singing brown thrashers. *J. Neurophysiol.* **75**, 867–876.

Goller, F. & Suthers, R. A. 1996b Role of syringeal muscles in controlling the phonology of bird song. *J. Neurophysiol.* **76**, 287–300.

Hartley, R. S. & Suthers, R. A. 1989 Airflow and pressure during canary song: direct evidence for minibreaths. *J. Comp. Physiol.* A **165**, 15–26.

Hunter Jr, M. L. 1980 Vocalization during inhalation in a nightjar. *Condor* **82**, 101–103.

Ladefoged, P. & Zeitoun, E. 1993 Pulmonic ingressive phones do not occur in Tsou. *J. Int. Phonation Assoc.* **23**, 13–15.

Lörcher, K. 1969 Vergleichende bio-akustische Untersuchungen an der Rot- und Gelbbauchunke, *Bombina bombina* (L.) und *Bombina v. variegata* (L.). *Oecologia* **3**, 84–124.

Schneider, H. 1988 Peripheral and central mechanisms of vocalization. In *The evolution of the amphibian auditory system* (ed. B. Frizsch, M. J. Ryan, W. Wilczynski, T. E. Hetherington & W. Walkowiak), pp. 537–558. New York: Wiley.

Searcy, W. A. & Andersson, M. 1986 Sexual selection and the evolution of song. *A. Rev. Ecol. Syst.* **17**, 507–533.

Searcy, W. A. & Yasukawa, K. 1996 Song and female choice. In *Ecology and evolution of acoustic communication in birds* (ed. D. E. Kroodsma & E. H. Miller), pp. 454–473. Ithaca, NY: Cornell University Press.

Solis, M. M. & Doupe, A. J. 2000 Compromised neural selectivity for song in birds with impaired sensorimotor learning. *Neuron* **25**, 109–121.

Suthers, R. A. & Goller, F. 1997 Motor correlates of vocal diversity in songbirds. In *Current ornithology*, vol. 14 (ed. V. Nolan Jr, E. D. Ketterson & C. F. Thompson), pp. 235–288. New York: Plenum Press.

Suthers, R. A., Goller, F. & Hartley, R. S. 1994 Motor dynamics of song production by mimic thrushes. *J. Neurobiol.* **25**, 917–936.

Suthers, R. A., Goller, F. & Pytte, C. 1999 The neuromuscular control of birdsong. *Phil. Trans. R. Soc. Lond.* B **354**, 927–939. (DOI 10.1098/rstb.1999.0444.)

Traill, A. 1991 Pulmonic control, nasal venting, and aspiration in Khosian languages. *J. Int. Phonation Assoc.* **21**, 13–18.

Vicario, D. 1991 Contributions of syringeal muscles to respiration and vocalization in the zebra finch. *J. Neurobiol.* **2**, 63–73.

Weber, E. 1974 Vergleichende Untersuchungen zur Bioakustik von *Discoglossus pictus*, Otth 1837 und *Discoglossus sardus*, Tschudi 1837 (Discoglossidae, Anura). *Zool. Jb. Physiol.* **78**, 40–84.

Wild, J. M., Goller, F. & Suthers, R. A. 1998 Inspiratory muscle activity during bird song. *J. Neurobiol.* **36**, 441–453.

Williams, H., Cynx, J. & Nottebohm, F. 1989 Timbre control in zebra finch (*Taeniopygia guttata*) song syllables. *J. Comp. Psychol.* **103**, 366–380.

Yu, A. C. & Margoliash, D. 1996 Temporal hierarchical control of singing in birds. *Science* **273**, 1871–1875.

Zann, R. 1993 Variation in song structure within and among populations of Australian zebra finches. *Auk* **110**, 716–726.