

Mechanisms of frequency and amplitude modulation in ring dove song

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Summary

Birdsong assumes its complex and specific forms by the modulation of phonation in frequency and time domains. The organization of control mechanisms and intrinsic properties causing such modulation have been studied in songbirds but much less so in non-songbirds, the songs of which are often regarded as relatively simple. We examined mechanisms of frequency and amplitude modulation of phonation in ring doves *Streptopelia risoria*, which are non-songbirds. Spontaneous coo vocalizations were recorded together with concurrent pressure patterns in two different air sacs and air flow rate in the trachea. The results show that amplitude modulation is the result of the cyclic opening and closure of a valve instead of fluctuations in driving pressure, as is the current explanation. Frequency modulation is more complex than previously recognized and consists of gradual, continuous

time–frequency patterns, punctuated by instantaneous frequency jumps. Gradual frequency modulation patterns correspond to pressure variation in the interclavicular air sac but not to pressure variation in the cranial thoracic air sac or air flow rate variation in the trachea. The cause of abrupt jumps in frequency has not been identified but can be explained on the basis of intrinsic properties of the vocal organ. Air sac pressure variation as a mechanism for frequency modulation contrasts with the specialized syringeal musculature of songbirds and may explain why the fundamental frequency in non-songbird vocalizations is generally modulated within a limited frequency range.

Key words: birdsong, phonation, amplitude modulation, frequency, air sac pressure, non-songbird, *Streptopelia risoria*, ring dove.

Introduction

The avian vocal organ, the syrinx, produces sound through vibrating labia or membranes, driven by respiratory air flow (Goller and Larsen, 1997a,b; Larsen and Goller, 1999). Birdsong assumes its complex and specific forms by the gating (on–off switching) of this sound and by the modulation of its properties both in time and frequency domains. This is achieved by coordinated neuromuscular activity of respiratory, syringeal and craniomandibular systems (for a review, see Suthers et al., 1999), but part of the complexity may also result from intrinsic, nonlinear characteristics of the syrinx (Fee et al., 1998; Fletcher, 2000). Elucidating the organization of such control mechanisms and intrinsic properties is a major challenge in the understanding of birdsong complexity.

Songbirds as a group are widely considered to have a higher level of vocal virtuosity and complexity than non-songbirds. This difference is attributed to the more complex syringeal musculature of songbirds (Gaunt, 1983). The involvement of different peripheral systems in the modulation of phonation has been relatively well-studied experimentally in a number of different songbird species (e.g. Ruppell, 1933; Suthers et al., 1994; Goller and Suthers, 1996; Fee et al., 1998; Hoese et al., 2000; Larsen and Goller, 2002). Studies on modulation in non-

songbirds, however, are almost non-existent, although the gating of phonation and sound generation as such have received considerable attention (e.g. Youngren et al., 1974; Lockner and Murrish, 1975; Nottebohm, 1976; Gaunt et al., 1977; Brackenbury, 1980; Suthers and Hector, 1985; Goller and Larsen, 1997a; Larsen and Goller, 2002). A better understanding of the differences in constraints and possibilities in phonatory control mechanisms of songbirds and non-songbirds could provide an insight into the factors underlying the disparity in song complexity between these two groups. Mechanisms of sound modulation in non-songbirds are also interesting in their own right, since about half of all bird species are non-songbirds and vocal communication in these taxa seems to be no less important than in songbirds.

In the current study, we investigate the mechanism by which ring doves, *Streptopelia risoria*, produce frequency and amplitude modulation in their vocalizations. This non-songbird is particularly interesting because its vocal behaviour has been studied in a variety of contexts, including reproduction (e.g. Lehrman, 1965), development (Nottebohm and Nottebohm, 1971), genetics (Lade and Thorpe, 1964), neuroendocrinology (e.g. Cheng et al., 1998), behavioural ecology (e.g. de Kort and

ten Cate, 2001) and perception (Beckers et al., in press). Moreover, studies by Gaunt et al. (1982) on the mechanism of phonation and sound modulation in ring doves provide a basis for further study.

Ring doves are the domesticated form of the African collared-dove *Streptopelia roseogrisea*. The vocalizations of domestic and wild forms are not different (Goodwin, 1983; Slabbekoorn et al., 1999), and they are considered to be the same species (Baptista et al., 1997). Ring dove advertisement vocalizations ('perch coos') have been described as relatively simple and stereotypic coos (Nottebohm and Nottebohm, 1971; Slabbekoorn et al., 1999) that do not vary appreciably between birds (Goodwin, 1983). The development of normal coo vocalizations does not depend on learning (Nottebohm and Nottebohm, 1971), and their acoustic structure has been studied in detail by Gaunt et al. (1982) and Slabbekoorn et al. (1999). Ring dove coos consist of two sound elements (hereafter referred to as $e1$ and $e2$), separated by a silent pause (p), composed of a fundamental frequency (f_0) without overtones (Fig. 1A). The first part of $e2$ is amplitude modulated [and is therefore considered a separate note by Miller and Miller (1958)], which gives rise to a trill-like, rolling quality. Gaunt et al. (1982) and Miller and Miller (1958) report that there is little frequency modulation, although frequency varies slightly in the last part of $e2$. Slabbekoorn et al. (1999) did not give any specifics on frequency-modulation patterns but report that the frequency varies, on average, from 388 Hz to 822 Hz within coos.

To identify physiological correlates of phonation and modulation, Gaunt et al. (1982) recorded coo vocalizations, together with concurrent air pressure variation in the trachea and posterior thoracic air sac (PTAS; = caudal thoracic air sac), and electromyograms (EMGs) of syringeal and abdominal muscles. From this, they concluded the following: (1) syringeal muscles act to set the syrinx in vocalizing position but they are probably not important for modulation; (2) the overall two-element coo pattern is generated by airflow from two large peaks of air sac pressure, caused by activity of abdominal muscles, and (3) the trill-type amplitude modulation is due to pulsatile activity of the abdominal muscles, which cause an oscillation in driving air sac pressure. In addition, Gaunt et al. (1982) recognized a second, subtle type of amplitude modulation, which they explained by a muffler action of lateral tympaniform membranes (LTMs) and small differences in the vibration frequencies of median tympaniform membranes (MTMs). However, this explanation is probably incorrect, since Goller and Larsen (1997a) showed that the LTMs rather than the MTMs are the sound source in domesticated rock pigeons (*Columba livia*), a species that is closely related to ring doves (Johnson et al., 2001) and has a very similar syringeal anatomy.

The experiments reported here were designed to examine the physiological events responsible for the trill-type amplitude modulation, a common phenomenon in dove vocalizations, and to examine the mechanism of frequency modulation. Frequency modulation, although limited (Miller and Miller,

1958; Gaunt et al., 1982), is also present in many other dove species. Even small differences in frequency have been shown to have communicative meaning in Eurasian collared-doves (*Streptopelia decaocto*; Slabbekoorn and ten Cate, 1998; ten Cate et al., 2002). Insight into its mechanistic basis might also provide a better understanding of why frequency modulation is limited in the first place. To achieve this, we set out to supplement the measurements of Gaunt et al. (1982) by recording spontaneous coo vocalizations, together with concurrent air pressures in the interclavicular air sac (ICAS) and cranial thoracic air sac (CTAS) and air flow rate in the trachea. The results lead us to conclude that the mechanism for amplitude modulation is different from the current model. We also propose a novel mechanism for frequency modulation.

Materials and methods

Subjects

We used six adult male ring doves (*Streptopelia risoria* L.) as subjects, one of which was used for a control experiment. Four doves were obtained commercially in the USA, and two in the Netherlands. Data recorded from four of the birds have also been used for a study on pure-tone sound production. Results from this study are reported elsewhere.

Surgical procedures and recording of data

The procedure to record air sac pressure and tracheal flow rate is described in detail in Suthers et al. (1994). Therefore, we will only give a summary here.

After birds were anaesthetized with isoflurane (Abbott Laboratories, North Chicago, IL, USA), a midline incision was made in the skin between the clavicles to expose the trachea as it entered the ICAS membrane. Tracheal airflow was measured with a microbead thermistor probe (BB05JA202N; Thermometrics, Edison, NJ, USA) inserted into the tracheal lumen, just rostrally to the interclavicular membrane. Thermistor wires were routed subcutaneously to connectors on a backpack that the birds wore. The flow rate in the trachea was measured by a feedback circuit in which the current needed to maintain the heated thermistor at a constant temperature was non-linearly proportional to the rate of air flow (Hector Engineering, Ellettsville, IN, USA). The response of a clean thermistor to a step function in air flow is approximately 90% full scale in 6 ms. During *in vivo* measurements, however, its time constant may increase gradually over time due to mucus deposition on the thermistor tip. Both ICAS and CTAS pressures were measured by the same type of piezoresistive silicone diaphragm pressure transducer (FPM-02PG; Fujikura, Marietta, GA, USA), attached to an air sac cannula consisting of a flexible silastic tube (i.d.=1.02 mm; wall thickness=0.57 mm; Dow Corning, Midland, MI, USA). A cannula 18.5 cm long was inserted into the ICAS through a small hole in the interclavicular membrane. From there, it was routed subcutaneously to the backpack carrying the pressure transducer. The CTAS was cannulated by a similar tube 14 cm long inserted into the air sac through the

abdominal wall just posterior to the last rib and a few mm lateral to the ventral midline. The cannulae extended 13 mm into the air sacs, and tissue adhesive was used to ensure an airtight seal.

Recording of data started as soon as the animal started to coo, 1–4 days after surgery, and continued for 1–3 weeks. Vocalizations were recorded on a condenser microphone (Audio Technica AT835b or Sennheiser MKH 40) placed 0.5–1 m in front of the cage. All signals (emitted vocalization on microphone, ICAS pressure or tracheal flow rate, and CTAS pressure) were recorded digitally (20 kilosamples s^{-1}) on a rotary storage recorder (model RSR 512; Metrum Information Storage, Littleton, CO, USA) or on a DAT data recorder (model RD135T; TEAC). Coo vocalizations were recorded with either concurrent tracheal flow rate and CTAS pressure signals or with ICAS and CTAS pressure signals. We transferred the recorded signals from tape to a microcomputer by resampling (20 kilosamples s^{-1}) using a Data Translation DT-2821G board and a TTE J87 anti-aliasing filter (high cut-off at 8 kHz; stopband attenuation 60 dB per one-third octave).

Because ICAS cannulae were routed subcutaneously, movement of body parts during cooing (e.g. inflating crop) could have applied some external, time-varying pressure to the cannula wall, thereby adding artifactual pressure components to our ICAS recordings. To determine if forces on the cannula wall make a significant contribution to the recorded pressure signal, we recorded vocalization pressure patterns in an additional bird as described for ICAS recordings above, except that the air sac end of the ICAS cannula was sealed by a 3-mm plug of silicon-based dental impression medium (President, Coltène Inc., nr. 4667). Any pressure fluctuations recorded under this condition must be caused by tissue pressure on the wall of the cannula. The results show that such artifactual pressure components are negligible: peak-to-peak pressure amplitudes in recordings with the ICAS cannula plugged are only 0.4% of those in our normal ICAS recordings during vocalization.

The pressure transducers were calibrated and showed a linear response for the ranges that we encountered in our recordings. Ring doves keep their beak and nares tightly closed during cooing (Gaunt et al., 1982), and flow reversal in the trachea before and after phonation often does not coincide with an opened beak (G. J. L. Beckers, personal observation). This complicates the identification of atmospheric pressure levels in our recordings. Since we were mainly interested in how phonatory characteristics are associated with changes in air sac pressure, we therefore made no further attempts to determine absolute pressure levels but used relative pressures instead. For the analyses of tracheal flow rate, we were also restricted to the use of relative values, since the relationship between flow rate and thermistor output is nonlinear and changes over time due to gradual mucus deposition on the thermistor tip.

Data analysis

Recordings were analyzed with the software program Praat (available from Paul Boersma and David Weenink,

<http://www.praat.org>) version 4.0.5 for Linux. Air sac pressure and tracheal flow rate signals were low-pass filtered digitally at 100 Hz, using a built-in function of the Praat program (frequency domain filter, Hann-like shaped band, 100 Hz smoothing). Although all vocalizations were recorded with a microphone, we used oscillations associated with near field sound in the tracheal flow and oscillations in air sac pressure for analyses. These oscillations were retrieved by band-pass filtering the raw flow and pressure signals at 350–800 Hz (50 Hz smoothing). The reason for preferring such signals is that they do not suffer from the sometimes severe acoustic artifacts that are introduced in microphone signals if the recording room is not specifically designed for low-frequency sounds like dove coos (G. J. L. Beckers, personal observation; see Discussion). We looked for correlates of gating and frequency modulation in tracheal flow rate and ICAS and CTAS pressure patterns by printing and visually comparing them together with spectrograms of concurrent coo vocalizations. This was done for all recordings. We did not differentiate between nest-, bow- and perch coos since in ring doves there are no apparent differences in overall acoustic structure. For quantification of relationships between flow and pressure patterns and the modulation of coo frequency, we focused on a selection of 10 coos for each combination of recorded variables. If possible, we selected vocalizations from different recording sessions, and within sessions we selected recordings with a relatively high signal-to-noise ratio. For one recording combination (tracheal flow with CTAS pressure in RD2), however, we only obtained eight coos recorded in a single session. For each coo, we determined the fundamental frequency (f_0) in consecutive 3-ms time frames, using Praat's autocorrelation function (Boersma, 1993) and the mean values of available tracheal flow rate, and ICAS and CTAS signals in these frames. On average, this resulted in a series of 366 measurement sets per coo. All data were read into a matrix file, which was imported into SPSS for Windows, version 10.1, for statistical analyses. Associations between the modulation of coo frequency and the three recorded physiological variables were examined quantitatively by computing product-moment correlation coefficients between concurrent 60-ms time segments (which thus consisted of 20 consecutive 3-ms frames). This was only done for time segments in which the f_0 time–frequency contour was continuous, so we excluded segments with silent intervals and segments that contained frequency jumps (see Results). Very rarely, f_0 was almost constant within a time segment. Since correlation coefficients are not informative in such cases, we excluded segments in which the S.D. of frequency was less than 1 Hz. Significance tests of the obtained correlation coefficients are not appropriate because samples are not statistically independent in time series. Also the strength of correlation should not be given much explanatory power, because there is no reason to assume that associations, if any, would be linear. In the case of flow rate, the measurements are known to be nonlinear in themselves, so a comparison of flow–frequency coefficient magnitudes with those of pressure variables makes *a priori* no sense. Instead,

we just used the sign of correlation coefficients (positive or negative) as an indication of whether or not frequency and a particular physiological variable varied in the same direction within a 60-ms time segment. For each individual, we categorized the correlation coefficients of all time segments into two categories: positive and negative coefficients. If continuous frequency modulation is consistently associated with one or more of the recorded physiological variables, then we would expect almost all of their coefficients to fall into only one of these two categories.

Results

Table 1 gives an overview of which signal combinations were obtained for each bird, as well as the number of recorded coos. Figs 1 and 2 give representative examples of the two types of signal combinations that we recorded concurrently during vocalization. Visual comparison of the printed recordings shows that there is little variation in the overall patterns of flow and pressure within and between individuals. We therefore address amplitude and frequency modulation using these figures. Qualitative descriptions are valid for all recorded signals, unless stated otherwise. Overall pressure patterns before, during and after coo bouts look very similar to those reported by Gaunt et al. (1982). In the present study, we are interested in the mechanisms of sound modulation and will therefore focus on patterns during coo vocalizations.

Amplitude modulation and gating

Phonation is always accompanied by air flow through the trachea, while silent intervals within coos are always accompanied with a stop, or at least strong reduction, of air flow (Fig. 1A,B). This is also true for the amplitude-modulated part of *e2*. The ‘amplitude modulation’ is a series of short sound elements separated by silent intervals (Fig. 3) and could therefore be considered as a ‘trill’ consisting of separate ‘notes’. The heated thermistors record air flow rate but not the direction of air flow. Gaunt et al. (1982) have shown, however, that tracheal pressures are always much lower than air sac pressures during ring dove vocalizations, so all air flow during

phonation (Fig. 1B) must be in an expiratory direction through the syrinx.

Pressure patterns in the CTAS (Figs 1C, 2C) and ICAS (Fig. 2B) are high during phonation (*e1* and *e2*) and reduced during the silent interval *p*. A similar pressure pattern was reported for the PTAS by Gaunt et al. (1982). This overall pattern of high pressure during phonation and low pressure during silent intervals, however, does not hold for the amplitude-modulated part of *e2*. During the first part of *e2*, there is a continuous, gradual rise in both ICAS and CTAS pressure, with no reduction during the silent intervals between sound pulses (Fig. 3B). In the last part of the trill, pressures start to oscillate with increasing amplitude. Sometimes, a slight

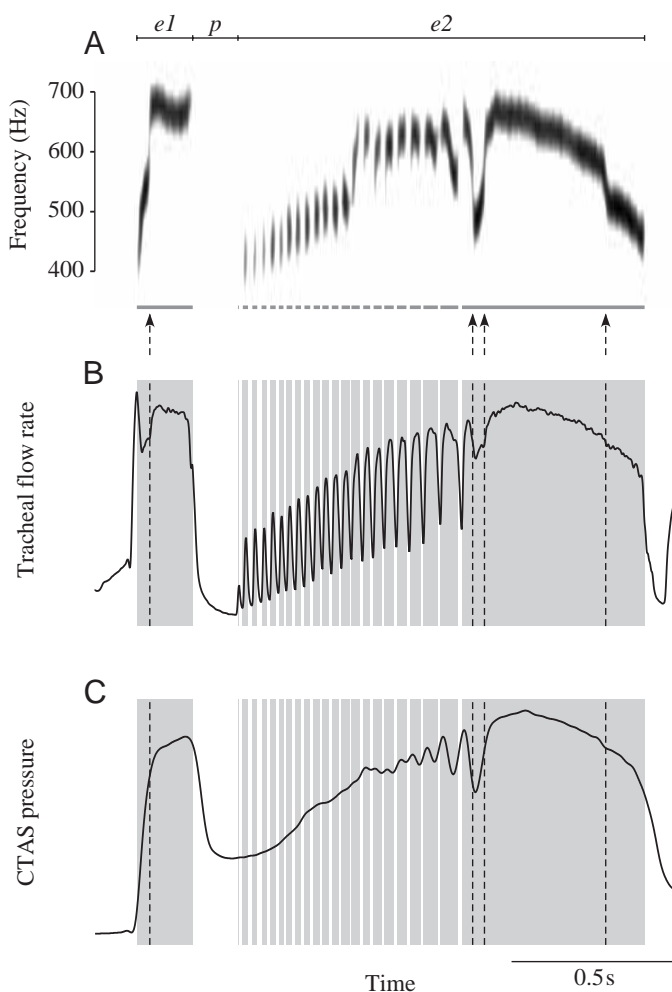


Fig. 1. Concurrent vocalization (A), tracheal flow rate (B) and cranial thoracic air sac (CTAS) pressure (C) signals for one coo. Grey bars beneath the spectrogram and grey areas in the flow and pressure plots indicate where sound is produced, as measured from the sound oscillogram (not shown). Arrows and broken lines indicate the location of frequency jumps. The flow rate recorded during the silent intervals of the amplitude-modulated part of *e2* may not reach zero because the microbead thermistor fails to track the very fast and large changes in flow rate. Note that flow rate and pressure signals have been low-pass filtered at 100 Hz to remove acoustic components and higher frequency noise.

Table 1. Recorded combinations of sound signals

Dove	Vocalization	Recorded signal			<i>N</i>
		Tracheal flow rate	ICAS pressure	CTAS pressure	
RD1	+	+		+	86
RD2	+	+		+	8
RD2	+		+	+	208
RD3	+		+	+	88
RD4	+		+	+	39
RD5	+	+		+	27

Plus symbols in the same row indicate that signals have been recorded concurrently. *N* denotes the number of recorded coos. ICAS, interclavicular air sac; CTAS, cranial thoracic air sac.

oscillation of pressure can also be seen in the first part of the trill, but always in a gradually rising pressure pattern. Pressure oscillations continue for one cycle into the part of *e2* that is not trilled. The start and end of sound pulses always coincide with the start and end of airflow (Fig. 3A), but the relationship between sound pulses and pressure cycles is less simple. The pressure reduction phase of a cycle starts at or shortly after the start of a short sound element, but overall the correspondence between the cycle phases of phonation and pressure is variable (Fig. 3B). The lowest parts of the pressure cycles do not coincide with the silent intervals of the trill.

Frequency modulation

Frequency modulation patterns are considerably more complex than previously recognized. For the five doves tested, f_0 is modulated over a bandwidth averaging 354 Hz. The mean centre frequency of this band is at 563 Hz, so f_0 is modulated for about one octave in ring dove coos

All coos include two types of frequency modulation: continuous frequency modulation and abrupt frequency jumps. Continuous frequency modulation is characterized by a gradual

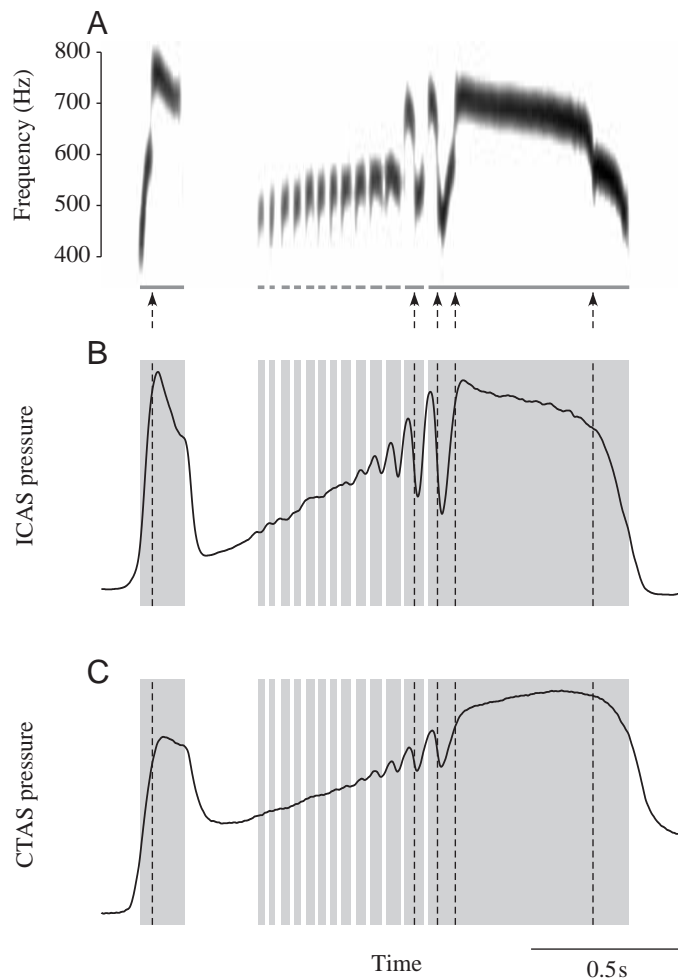


Fig. 2. Concurrent vocalization (A), interclavicular air sac pressure (ICAS) (B) and cranial thoracic air sac (CTAS) pressure (C) signals for one coo. For details, see Fig. 1.

change of f_0 over time. At frequency jumps, the gradual f_0 time–frequency contour is momentarily disrupted, as f_0 almost instantaneously (within 10 ms) jumps to a different frequency range. Frequency differences before and after jumps range from about 50 Hz to 150 Hz, either up or down (see example in Fig. 4). Phonation, however, is not interrupted at frequency jumps, although changes in amplitude can often be observed. The arrows and broken lines in Figs 1A, 2A indicate examples of frequency jumps in complete coo vocalizations. The occurrence and timing of jumps, however, can vary considerably between coos.

Frequency jumps are often accompanied by a sudden, small increase or decrease in tracheal flow rate, but the directions of change in frequency and rate of change in flow are not always the same. We did not observe any systematic changes in air sac pressures that can be linked to frequency jumps.

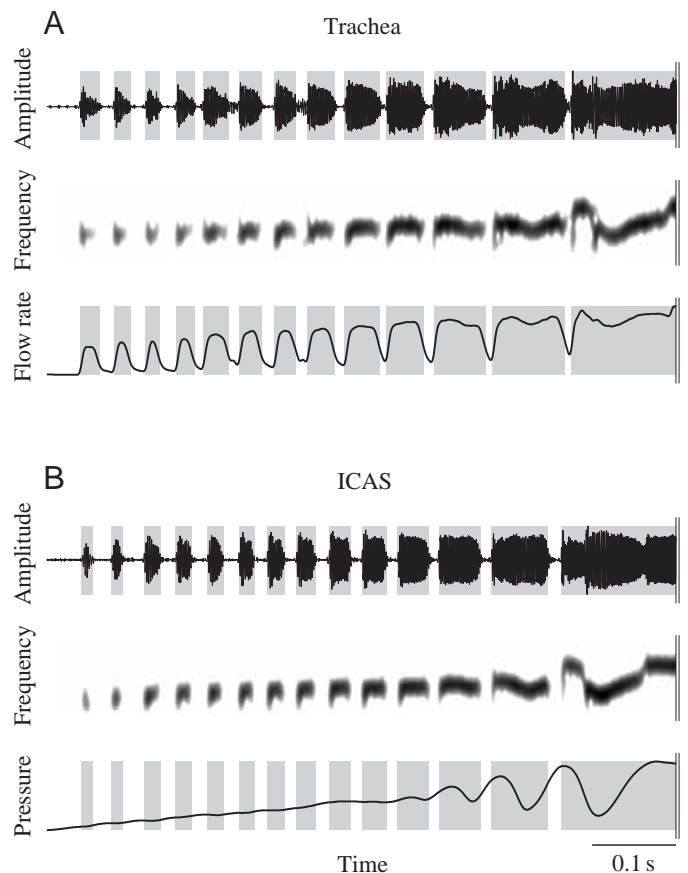


Fig. 3. Gating of sound in the amplitude-modulated part of *e2*, recorded in trachea (A) and interclavicular air sac pressure (ICAS) (B). Recordings A and B are from different coos but originate from the same individual. Both sound oscillograms in the trachea and ICAS show that phonation is completely interrupted by silent intervals of ≥ 10 ms. This modulation pattern corresponds to the pattern of flow rate in the trachea, which is reduced to zero or near-zero during silent intervals. ICAS pressure gradually increases in the first half of the amplitude-modulated part, although in some recordings, like in this example, the rate of increase is reduced during the sound pulses. Cranial thoracic air sac (CTAS) pressure patterns are similar to those in the ICAS in this part of the coo and are therefore not shown.

Continuous frequency modulation correlates to patterns of ICAS pressure change (for example compare Fig. 2A and Fig. 2B). Visual examination of all the recordings revealed that sound segments not interrupted by silent intervals or frequency jumps can always be scaled to match concurrent ICAS patterns. In low-noise recordings, even the fine structure of ICAS pressure and f_0 modulation is correlated (Fig. 5).

To verify whether or not there is a consistent pressure

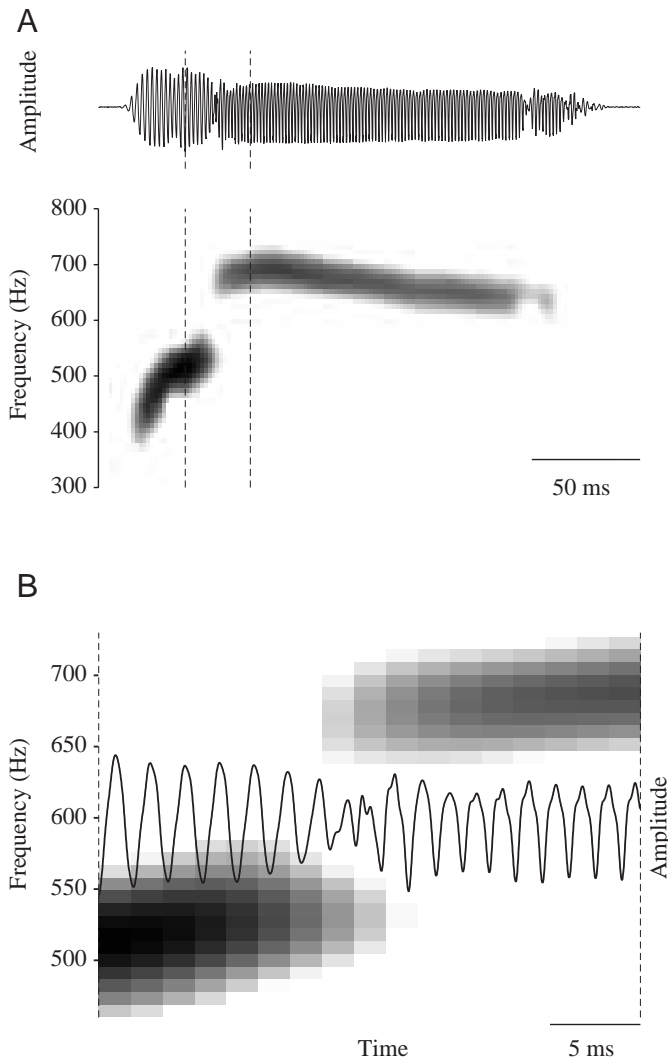


Fig. 4. An example of a frequency jump in *e1* [recorded in interclavicular air sac pressure (ICAS)], in which fundamental frequency (f_0) jumps from approximately 525 Hz to 675 Hz within 5 ms. (A) Oscillogram (top) and spectrogram (bottom) of complete *e1*. (B) Detail of jump (time frame is indicated by broken lines in A) in which the oscillogram is superimposed on the spectrogram. The oscillogram shows that sound is not interrupted during frequency jumps. Although harmonic overtones are present in ICAS-recorded coo vocalizations, spectrograms here show only f_0 , which is the sound component that radiates from the animal. Note that the shape of the sound waveform in the ICAS is different before and after the frequency jump. Spectrogram settings: time step, 1.8 ms; frame length, 25 ms; dynamic range, 10 dB. The signal has been pass-band filtered from 300 Hz to 2500 Hz.

association between ICAS pressure and f_0 modulation, we created scatterplots of ICAS pressure and coo frequency for each individual coo (three doves, 30 coos; e.g. Fig. 6A–C). All these scatterplots showed a positive, predominantly linear relationship between ICAS pressure and coo frequency within segments between frequency jumps. At frequency jumps, the slope of this relationship usually changes somewhat. The close correspondence between ICAS pressure and frequency modulation only breaks at the onset and offset of phonation in the short sound pulses of the amplitude modulation. However, these transients are maximally 10 ms in duration and constitute less than 5% of the coo sound. Moreover, scatterplots show that when this occurs, the overall relationship in amplitude-modulated parts remains linear and positive (see, for example, Fig. 6A, in which the yellow and light-green parts are from the amplitude modulation).

Tracheal flow rate and CTAS pressure are also often, but not always, positively correlated with continuous frequency-modulation patterns. Sometimes these parameters change in a direction opposite to that of frequency over major portions of *e1* or *e2* (compare, for example, the frequency pattern with CTAS signals in Figs 1 and 2). Scatterplots of the focal coo selections show segments where tracheal flow rate or CTAS pressure correlate negatively with frequency modulation (three doves, 28 coos, and five doves, 58 coos, respectively; e.g. Fig. 6D–F and Fig. 6G–I). Frequently, a switch from positive to negative relationships, or *vice versa*, occurs even within continuous segments of phonation.

A quantitative analysis of the coo selections reinforces these qualitative observations (Fig. 7). In 99.8% of all 60-ms segments of continuous phonation, correlation coefficients of ICAS pressures with f_0 are positive ($N=332$, three doves). For tracheal air flow and CTAS pressure, this is only 70.1% and

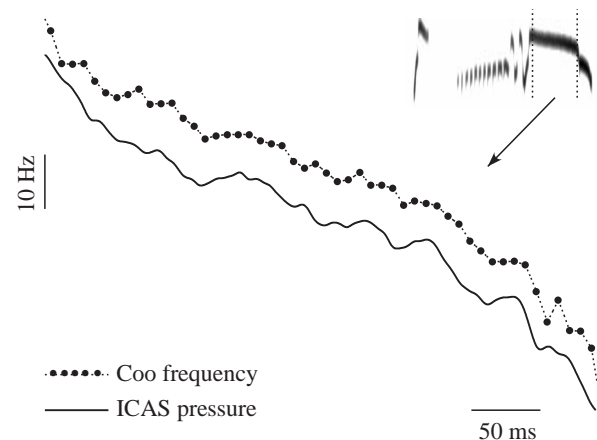


Fig. 5. Detail of concurrent modulation of frequency and interclavicular air sac pressure (ICAS) pressure in a segment of *e2*. The position of the segment within the coo is indicated in the spectrogram (top right-hand corner). Frequency of phonation was determined at 8-ms intervals with an autocorrelation algorithm (Boersma, 1993) using the microphone-recorded vocalization. Signals have not been adjusted horizontally for the delay of a few ms for the sound to reach the microphone.

67.1%, respectively ($N=221$, three doves, and $N=553$, five doves, respectively), and hence the association between f_0 and these variables is not consistent.

It is evident from our concurrent recordings of pressure in the CTAS and ICAS (Fig. 2) that, despite their obvious similarity, the pressures in these air sacs sometimes vary independently from each other in their direction of change during some portions of the coo. This unexpected finding has not been reported during vocalization in other birds. In this paper, we focus on the relationship between air sac pressure patterns and acoustic modulation. The possible significance of differences in air sac pressure patterns between air sacs will be addressed elsewhere.

Discussion

The main conclusions from our study are as follows: (1) the overall pattern of f_0 modulation in ring dove coos is strongly and positively associated with the change of air pressure in the ICAS; (2) the continuous and gradual f_0 contour is punctuated by instantaneous frequency jumps, not related to any observable systematic changes in pressure or flow patterns; and (3) the trill-like 'amplitude modulation' present in ring dove coos is not caused by an oscillation of subsyringeal pressure, as previously believed (Gaunt et al., 1982), but rather by the repeated opening and closure of a valve that is as yet unidentified but may consist of the lateral tympaniform membranes (LTMs). It is more appropriately described as a trilled sequence of notes.

The strong, positive association between f_0 modulation and ICAS pressure is likely to be a causal one. It has been shown in a related (Johnson et al., 2001) columbid species, *Columba livia*, that the source of coo vocalizations is the vibrating LTMs, located in the lateral walls of the tracheal part of the syrinx (Goller and Larsen, 1997b; Larsen and Goller, 1999). Phonation frequency is ultimately determined by the resonant frequency of these membranes, which depends on their geometry and density and the tension applied to them (Fletcher, 1992). The syrinx is located within the ICAS, and the external surface of the LTMs are in direct contact with ICAS space (King, 1989). It is therefore plausible that variation in ICAS pressure directly modulates LTM tension, and thus the frequency of phonation. Nevertheless, the possibility remains

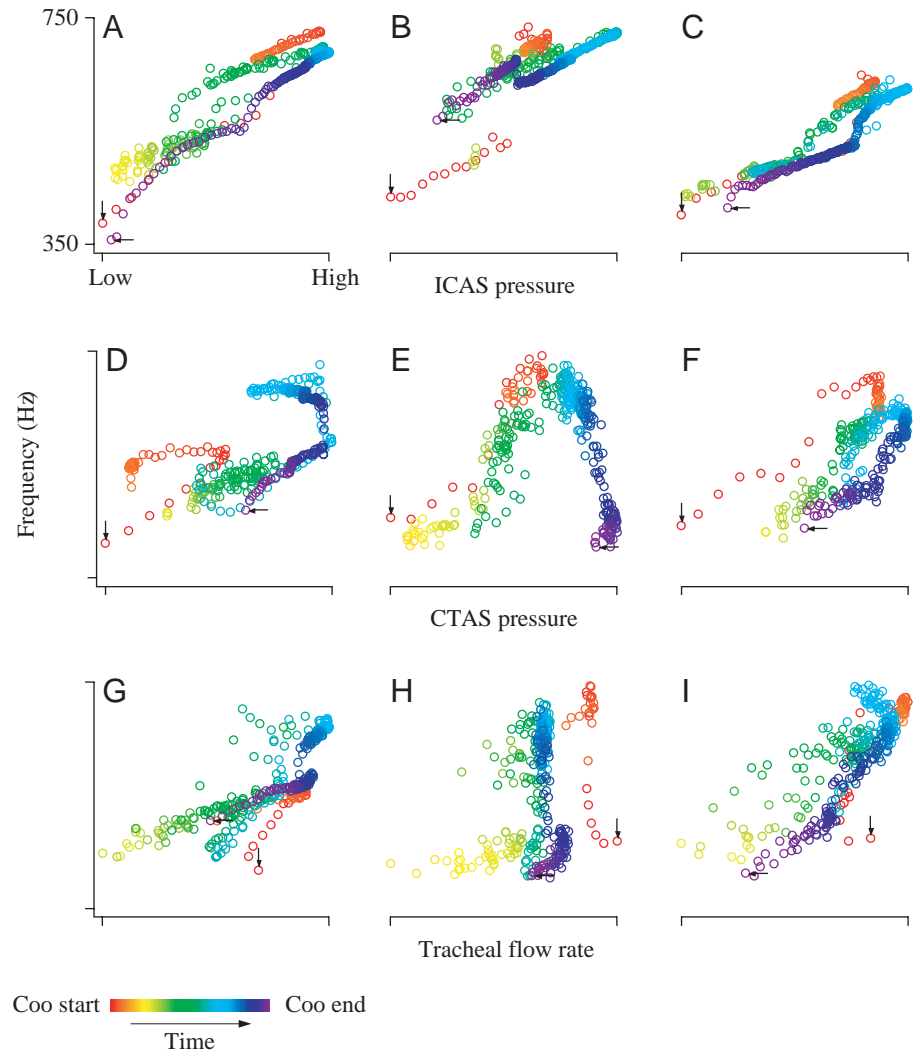


Fig. 6. Coo frequency plotted against interclavicular air sac pressure (ICAS) pressure, cranial thoracic air sac (CTAS) pressure and tracheal air flow. Each plot represents a single, complete coo, and circles indicate value pairs as measured in 3-ms time frames of that coo. Circles are coded on a colour scale so that the development over time within a coo can be traced. Vertical arrows and horizontal arrows indicate the beginning and end of a coo, respectively. Each row contains three representative coos, originating from different individuals.

that it is not ICAS pressure that modulates f_0 but a third factor that causes concurrent changes in both f_0 and ICAS pressure. However, in the light of our finding that tracheal flow rate is not consistently associated with f_0 modulation, it is difficult to envision a credible alternative explanation.

Several factors might play a role in regulating pressure in the ICAS. Perhaps the most obvious is the activity of various respiratory muscles responsible for expanding or compressing the air sacs. ICAS pressure might also be affected by changes in syringeal resistance, perhaps caused by an abductive action of the tracheolateralis muscles, the caudal ends of which insert directly on the external surface of the LTMs. There is a potential for complex interactions between control parameters that may help maintain a causal relationship between ICAS

pressure and f_0 over a wide range of physiological conditions. A full understanding of these mechanisms will require detailed knowledge of the roles of different muscle groups.

The occurrence of sudden frequency jumps in bird vocalizations has been reported for Eurasian collared doves, *Streptopelia decaocto* (Gürtler, 1973; ten Cate, 1992; Ballintijn and ten Cate, 1998), which are closely related to ring doves (Johnson et al., 2001). In this species, frequency jumps have been shown to have communicative meaning (Slabbekoorn and ten Cate, 1998; ten Cate et al., 2002). Frequency jumps have also been reported to occur in vocalizations of zebra finches, *Taeniopygia guttata*, where they are attributed to mode locking in the syringeal dynamics (Fee et al., 1998). Mode locking happens when a nonlinear interaction constrains two oscillating components of a system to maintain a small integer ratio of frequencies. Mode-locking transitions may occur because the characteristic frequency of one component is

changed relative to the other, and the oscillation frequency suddenly jumps to achieve a new stable integer ratio. We believe that mode-locking transitions are also a likely explanation for frequency jumps in ring doves, because other types of dynamical behaviour that the nonlinear interaction of two oscillatory components can lead to, i.e. sudden transitions to subharmonic and chaotic phonation (Wilden et al., 1998), are apparent in the normal vocalizations of related dove species (such as *Streptopelia tranquebarica* and *Streptopelia orientalis*) and, occasionally, in aberrant ring dove coos (G. J. L. Beckers, personal observations).

The existing model for the trill-type amplitude modulation in ring dove coos, namely an oscillatory driving air sac pressure caused by the pulsatile action of expiratory muscles (Gaunt et al., 1982), is not compatible with our results. We did find oscillations in air sac pressures but only during the last part of the trill. A close re-examination of the figures published by Gaunt et al. (1982) shows that this is also the case for PTAS pressure. Amplitude modulation is already present well before air sac pressures start to oscillate, when air sac pressures gradually increase, but tracheal air flow and phonation nevertheless stop in cyclic intervals (Fig. 3).

The absence of pressure oscillations in the first part of the trill is not due to a limit in the high-frequency response of the pressure transducer. The expected oscillation frequency here of about 25 Hz is well below the 100 Hz low-pass filter that we used to remove acoustic pressure oscillations of 350–800 Hz. In songbirds, this transducer system records sound pressure fluctuations up to at least several kHz. Besides the lack of strong air sac pressure oscillation in the first part of the trill, there is other evidence that oscillating air sac pressure is not the cause of the trill. If the cyclic reduction in air sac pressure were the cause of a reduction or cessation of phonation, then the lowest parts of the pressure cycle should coincide with the silent intervals between sound pulses. However, this is not the case (Fig. 3B). The lowest parts of the pressure cycles are located during the sound pulses, and silent intervals often occur when pressure is relatively high. Furthermore, if air sac pressure oscillation caused amplitude modulation, we would expect amplitude modulation to end during or shortly after the last pressure cycle. Yet our recordings show that the amplitude modulation always ends before the start of the last pressure cycle (Fig. 3B).

We can only explain the pressure and flow patterns during the trill by the repetitive opening and closing of some kind of valve regulating airflow. The rapid series of notes thus appears to be produced by a mechanism of pulsatile expiration in which the airway repetitively opens and closes while the continuous activity of expiratory muscles generates a gradually increasing air sac pressure. Each sound is produced by the opening of a pneumatic valve to release a puff of air through the syrinx. The timing of each sound pulse, then, is determined by the gating of airflow, not by modulation of expiratory muscle activity.

It is interesting that ring doves appear to generate rapid trills using the same mechanism of pulsatile expiration as do various songbirds such as canaries (*Serinus canaria*; Suthers, 1997)

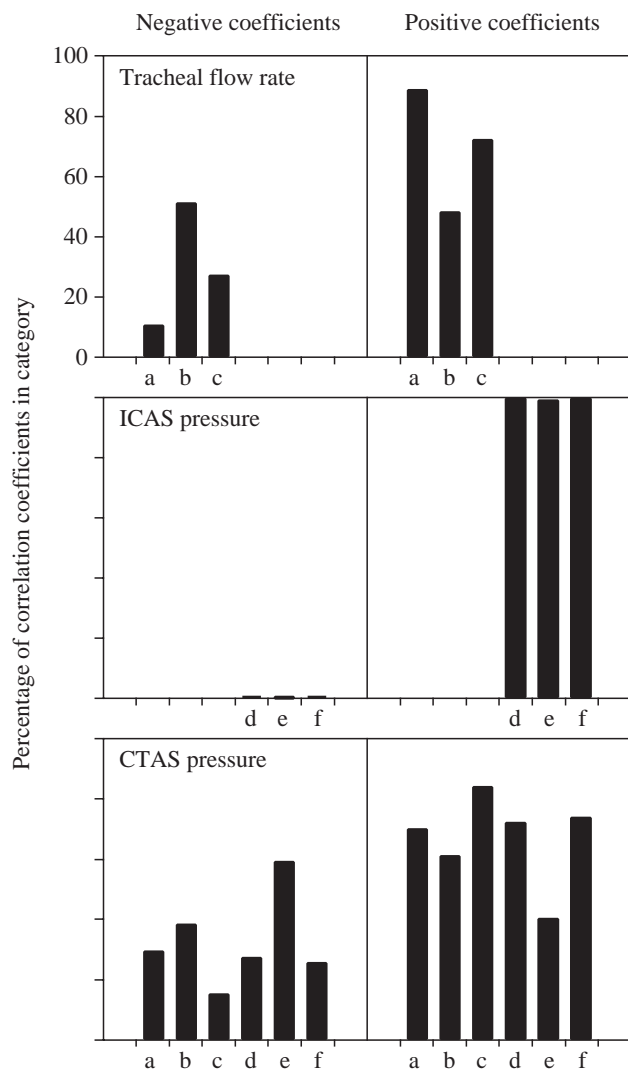


Fig. 7. Association of coo fundamental frequency with tracheal flow rate, and interclavicular air sac pressure (ICAS) and cranial thoracic air sac (CTAS) pressure. Lower-case letters correspond to the doves listed in Table 1: a, RD5; b, RD1; c, RD2; d, RD2; e, RD3; f, RD4.

and northern cardinals (*Cardinalis cardinalis*; Suthers and Goller, 1997). In songbirds, the medial and lateral labia appear to act as a pneumatic valve at the cranial end of each bronchus. In ring doves, such action might be performed by completely adducting the LTMs. The valve is apparently located in the syrinx instead of the glottis since tracheal pressure remains low during closure (Gaunt et al., 1982). Gaunt et al. (1982) have shown that the syringeal muscles (sternotrachealis and tracheolalealis) exhibit pulsatile EMG patterns during the amplitude-modulated part of ring dove coos. Since these muscles are likely to cause adduction and abduction of the LTM membranes, this suggests that the LTM membranes could indeed be involved in such gating action. Pulsatile expiration, presumably controlled by syringeal muscles, is also used by budgerigars (*Melopsittacus undulatus*) to produce a rapid sequence of notes (Suthers, 2001).

The oscillation of air sac pressures during the last part of the trill could be caused by rhythmic contractions of expiratory muscles, perhaps to achieve frequency modulation and add more complexity to the coo. However, an alternative explanation is that pressure oscillations are a consequence of the gating action of a pneumatic valve. The 'burst' of air flow at the onset of a sound pulse, immediately after a silent interval with reduced or zero flow, may momentarily cause a reduction in subsyringeal air pressure, which, after a time lag, is compensated for by the action of abdominal muscles. Three observations fit this idea: (1) pressure begins to drop at, or shortly after, the start of a sound pulse; (2) pressure oscillations become evident only at relatively high flow rates and pulse durations, and their amplitude increases as both the air flow rate and duration of a sound pulse increase (Fig. 1); and (3) Gaunt et al. (1982) report, with respect to the pressure oscillation, that "abdominal muscular activity is highest during dips in the pressure curve. Each EMG pulse begins shortly after pressure begins to drop from a maximum and continues until pressure again reaches the level at which activity began". Such an EMG pattern suggests a compensatory action of expiratory muscles against the sudden pressure drop.

Gaunt et al. (1982) recognized two types of amplitude modulation in ring doves: the trill-type amplitude modulation that we addressed above and a more subtle, beat-like amplitude modulation, which we do not address in this study. In microphone-recorded vocalizations, we too have observed a phenomenon of partial or complete beat cycles occurring, intermittently, in all parts of the coo. Coo sounds in tracheal flow or air sac pressure recordings, however, never show this phenomenon. Hypothetically, it is possible that such modulation originates from interaction of the sound source signal with a resonance filter elsewhere in the animal. Resonance filtering of source signals has recently been found in ring doves (G. J. L. Beckers, R. A. Suthers and C. ten Cate, unpublished data). However, interference through reflected sound waves and the formation of standing waves in enclosed rooms can also generate beat-like amplitude modulation (Kinsler et al., 2000). To test whether room acoustics could indeed introduce such strong beat modulations as observed by

us and reported by Gaunt et al. (1982), we created synthetic ring dove coos with normal frequency modulation but constant amplitude using the analytic signal technique as described by Mbu Nyamsi et al. (1994). Analysis of such signals generated by a speaker revealed that, indeed, strong beat-like amplitude modulations had been introduced, even when recorded in 'acoustic chambers' padded with acoustic foam. Recordings outside in the open field did not show such beat phenomena. Because we cannot distinguish between beat-like amplitude modulation produced by the dove, if any, and that caused by the acoustics of the room, we do not further investigate this phenomenon.

Our finding that pressure in the CTAS and ICAS can vary independently is new and, potentially, has implications for sound production. Brackenbury (1971) reported that during quiet respiration there is only a small gradient between the cranial and caudally located air sacs of geese (*Anser anser*). The direction of this gradient is reversed with the respiratory cycle. Gaunt et al. (1973) recorded pressure in the posterior thoracic, anterior thoracic and interclavicular air sacs of starlings (*Sturnus vulgaris*) and noted a similar pressure gradient only during distress calls, which they deemed to be of no significance to vocalization. The differences in pressure patterns that we report here in vocalizing doves, however, have not been described in other birds and suggest the possibility of independent control of pressure in different air sacs, not simply a caudocranial gradient between them. The means by which such pressure differences are produced is unknown. Possible mechanisms include changes in the rate of air flow into or out of individual air sacs or the possibility that certain respiratory muscles act selectively on different air sacs. If the pressure in the ICAS can be varied independently from the pressure inside the syrinx, it could alter the pressure gradient across the LTMs and affect sound production by changing their position or tension.

Our spectrograms of ring dove coos (Figs 1, 2) show that frequency modulation is much more complex than previously reported (Nottebohm and Nottebohm, 1971; Gaunt et al., 1982). Undoubtedly, a major reason for this difference in interpretation is the current availability of superior, digital techniques for spectrographic representation. An additional reason may be that frequency ranges in birdsong are often viewed spectrographically on an absolute and linear frequency scale, usually spanning a considerable part of our hearing range. Frequency modulation between 0.4 kHz and 0.8 kHz does not appear impressive on such a scale. Perceptually, however, both to humans (e.g. Moore, 1997) and birds (e.g. Dooling, 1982), it is more appropriate to consider f_0 variation in terms of proportions. Our measurements show that from such a point of view the difference between the minimum and maximum f_0 in individual ring doves is about a factor of two (one octave), which is not negligible. Acoustic analyses of turtle-dove perch-coos by Slabbekoorn et al. (1999) show that this is also typical for the other 15 species in the *Streptopelia* genus. Moreover, even limited frequency modulation has important communicative function in the Eurasian collared

dove, the sister species of the ring dove (Slabbekoorn and ten Cate, 1998; ten Cate et al., 2002).

Recently, it has been shown that much of the complexity of the song of canaries (Gardner et al., 2001) and chingolo sparrows (*Zonotrichia capensis*; Laje et al., 2002) can be modelled by smooth and simple variations of only a few parameters. Despite their complexity, frequency modulation patterns in ring dove coos may also arise from only two relatively simple, centrally coordinated motor variables. First, simple and smooth air sac pressure gestures determine the overall phonation and continuous frequency modulation patterns. Second, amplitude modulation is caused by the cyclic gating action of a valve. Much of the remaining complexity, such as oscillatory frequency modulation and frequency jumps, can be explained on the basis of feedback and intrinsic nonlinear properties of the syrinx.

What is the significance of our findings with respect to other bird species? We provide, to our knowledge for the first time, a mechanistic explanation for frequency modulation of phonation in a non-songbird. ICAS pressure may also modulate frequency in the vocalizations of other non-songbirds, given the fact that LTMs occur in many non-songbirds (King, 1989) and assuming that the finding of LTMs as the sound generator in a pigeon and a parrot species (Goller and Larsen, 1997b) can be extended to other non-songbirds. Frequency modulation in songbirds (Miskimen, 1951; Goller and Suthers, 1996; Larsen and Goller, 2002), and possibly parrots (Larsen and Goller, 2002), is achieved through the action of specialized syringeal musculature. A mechanism of frequency modulation by ICAS pressure variation could explain why many non-songbird species often exhibit rather limited frequency modulation, on an absolute scale, as compared with songbirds. Muscles that modulate ICAS pressure can only indirectly vary tension of the sound-generating structures and are not specialized in this task, as modulation of ICAS pressure is also essential for, and possibly constrained by, other physiological functions such as respiration. Moreover, the level of fluid power during bird vocalization depends on the level of air sac pressure (Brackenbury, 1977) and is positively correlated with sound intensity for the species investigated (Suthers and Goller, 1997; Gaunt et al., 1976). The overall modulation of frequency and sound intensity are thus likely to be coupled in ring doves, and possibly other non-songbirds. A future study should investigate the possibility that maximizing vocal intensity, an important property of long-distance signals, may constrain frequency modulation.

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