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Difference between the vocalizations of two sister species of pigeons explained in dynamical terms

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Abstract

Vocal communication is a unique example where the nonlinear nature of the periphery can give rise to complex sounds even when driven by simple neural instructions. In this work we studied the case of two close-related bird species, *Patagioenas maculosa* and *Patagioenas picazuro*, whose vocalizations differ only in the timbre. The temporal modulation of the fundamental frequency is similar in both cases, differing only in the existence of sidebands around the fundamental frequency in the *Patagioenas maculosa*.

We tested the hypothesis that the qualitative difference between these vocalizations lies in the nonlinear nature of the syrinx. In particular, we propose that the roughness of *maculosa*'s vocalizations is due to an asymmetry between the right and left vibratory membranes, whose nonlinear dynamics generate the sound.

To test the hypothesis, we generated a biomechanical model for vocal production with an asymmetric parameter Q with which we can control the level of asymmetry between these membranes. Using this model we generated synthetic vocalizations with the principal acoustic features of both species. In addition, we confirmed the anatomical predictions by making post-mortem inspection of the syrinxes, showing that the species with tonal song (*picazuro*) has a more symmetrical pair of membranes compared to *maculosa*.

Keywords

biomechanics; birdsong; asymmetry; vocal control; nonlinear dynamics

Introduction

Complex behavior emerges from the interaction of a nervous system and a peripheral device (Chiel and Beer 1997). In that framework it is interesting to unveil how much of that complexity is originated at the level of the nervous system, which is indeed capable of

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generating complex instructions, and how much is due to the nature of the peripheral device (Suthers et al. 2006, Amador et al. 2008). A well-studied example is birdsong, where a highly nonlinear phonating device is controlled by the song system in order to generate highly sophisticated sounds. In particular, it has been instructive to study the case of the zebra finch (*Taeniopygia guttata*) where the song presents a wide range of syllables and notes with different spectral features, many of those determined by the nonlinear nature of oscillations displayed by the syringeal labia (Sitt et al. 2008).

The non-linear nature of the vocal organ has been also proposed to explain part of the complexity of coo vocalizations of the turtle-doves (genus *Streptopelia*; Beckers et al. 2003, Beckers and ten Cate 2006). Perch coo vocalizations of pigeons and doves (hereafter referred to as songs) are innate, that is they do not need to go through a learning process to develop normally (Lade and Thorpe 1964; Nottebohm and Nottebohm 1971; Baptista 1996). They are also species-specific (Slabbekoorn et al. 1999; Mahler and Tubaro 2001) and field experiments have shown that individuals respond selectively to the songs of their own species and are sensitive to changes in their acoustic variables, suggesting that song is important for recognition of conspecifics and assessment of their quality (Slabbekoorn and ten Cate 1998; de Kort and ten Cate 2001).

In this work we compare the vocalizations of two sister species of New World pigeons: the Spot-winged pigeon *Patagioenas maculosa* and the picazuro pigeon *Patagioenas picazuro* (Johnson and Weckstein 2011), hereafter referred to as *maculosa* and *picazuro*, respectively) whose temporal pattern and modulatory control of the fundamental frequency is extremely similar and yet, the acoustic features of their vocalizations are very different. We will test the hypothesis that the qualitative difference between these vocalizations lies in the nonlinear nature of the avian vocal organ. In other words, our hypothesis states that in both species, the functioning of the avian vocal organ can be modeled by the same physical mechanisms, differing only in some configuration parameters. In this way, the two types of vocalizations correspond to the qualitatively different solutions that a dynamical system can present for different value of its parameters.

Following the temporal modulation of the fundamental frequency, the motor instructions used by the two species are presumably similar. In both cases, the song starts with an introductory note followed by three different cooing notes (this sequence of cooing notes is repeated a variable number of times, usually from 2 to 5 times), whose fundamental frequencies follow similar patterns (Fig. 1a,b left panels). The difference between the vocalizations of the two species is in timbric features (Fig. 1a,b right panels). In particular, an obvious difference is the existence of sidebands around the fundamental frequency in the case of the *maculosa* vocalizations. In terms of timbre, this feature consists of songs with a distinctive roughness (Goodwin 1964; Mahler and Tubaro 2001).

The avian vocal organ in pigeons is relatively simple: a pair of opposed vibratory masses at the trachea, identified as the lateral tympaniform membranes (LTM) by endoscopic observation during sound production (Goller and Larsen 1997; Larsen and Goller 1999) (see Fig. 2). Complexity of the sound implies a relatively high dimensionality of the equations

ruling the behavior of the masses modulating the airflow. Most of the studies in birdsong assume a low dimensional model for the dynamics of each vibratory membrane, and a complete synchrony between them. Therefore, an increase in the dimensionality of the problem is achieved if the description of each vibratory membrane is performed with the same level of simplicity, but the *ad hoc* requirement of the vibratory membranes synchrony is abandoned. In this work, we will explore the hypothesis of the left and right LTM presenting a somewhat independent dynamics (as it could happen if the vibratory masses were different) and that their asymmetry is what determines the existence of side bands around the fundamental frequencies in the song of *maculosa*. For doing this, we first analyze this hypothesis theoretically, showing that the existence of an asymmetry can lead to sound with the recorded acoustic features. Then, we perform post mortem inspections to test whether the species with atonal song has in fact more asymmetrical LTMs.

Materials and Methods

In order to test the hypothesis that the degree of left-right asymmetry is at the core of the complex sounds in *maculosa*, we have 1. Studied the solutions of an asymmetric phonating system and compare the synthetic sounds predicted by the asymmetric model of vocal production with the song recordings of *picauro* and *maculosa*, and 2. Performed a direct inspection of the syrinx of each of the two species under analysis in order to measure their degree of LTM asymmetry.

1. Theoretical Methods

1.1 Biomechanical model of membranes—In the last years, there have been a growing number of studies dedicated to building mathematical implementations of computational models of birdsong production (Mindlin and Laje 2005). Those models allow integrating a number of different experiments dedicated to unveiling the relative role of the different anatomical structures involved. Beyond this integration, these models allow to formulate specific predictions, since they are operational models aimed at describing the dynamics of the parts of the avian vocal organ for certain values of the parameters. Some of these parameters are time dependent, and under the active control of the nervous system: this is the case of the activities of the muscles involved in the configuration of the syrinx. Others are anatomical, time independent parameters, and are characteristics of the species: the degree of symmetry of the avian vocal organ, for example.

There are many possible strategies to address the modeling of the phonating vocal organ. One can be interested in closely relating the elements in the model with the actual anatomical and physiological data. In that case, the physical model will include a variety of phenomenological terms accounting for the detailed functional and anatomical structure of the syrinx (Elemans et al. 2003). A different strategy consists on the use of low dimensional dynamical models. In that case one is interested in deliberately simplify to a bare minimum the number of elements in the description, in order to identify the dynamical processes behind a given phenomenon (Elemans et al. 2008). In our case, we will follow this latter approach.

Direct observations have shown that vocal folds in humans, or vibratory membranes in birds, do not behave as rigid masses. They are made of tissue capable of sustaining both lateral displacements and wavelike motions (flapping) of the cover layers. Ishizaka and Flanagan (1972) were the first to model this internal structure of the oscillating element in terms of two coupled masses. Many versions of this conceptual model have been proposed in the literature, but they all share a search of equilibrium between mathematical simplicity and a sensible description of the diversity of physical phenomena shaping the force on the oscillating tissue (Lucero and Koenig 2005; Lucero et al. 2015). The phase difference of the distances between LTMs at the top and at the bottom is ultimately responsible for the existence of a high pressure when the LTMs display a convergent profile, and a low pressure when the profile is divergent. This allows transferring energy from the airflow to the vibratory membranes. The main achievement of the two mass model is to capture the simultaneous existence of two modes: a lateral displacement and the flapping. Titze managed to design a low dimensional model whose only dynamical variable was the vibratory membrane's midpoint position, and yet it could capture the necessary interplay between the two modes (Titze 1988). He proposed a kinematic restriction on the LTMs, such that the area between the LTMs along the vertical direction would be compatible with the existence of a flapping. In his simplified model, the two LTMs were identical and symmetric copies.

A model accounting for differences between (flapping) left and right LTM allow us to progressively add complexity to the problem. In other words, we will test the hypothesis that it is possible to generate sounds with the acoustic features that we need by allowing flapping of the LTMs, but introducing a small difference between them. In this way, the dynamics of the midpoint positions of the LTM $\xi_{(l,r)}$ (left and right, respectively) would be ruled by the following dynamical system:

$$M \frac{d^2 \xi_{(l,r)}}{dt^2} + B_{(l,r)} \left(1 + \eta_{(l,r)} \xi_{(l,r)}^2 \right) \frac{d \xi_{(l,r)}}{dt} + K_{(l,r)} \xi_{(l,r)} = P_g \quad (1)$$

where M corresponds to the mass of the oscillating tissue, B is a dissipative constant, K accounts for restitution function and η is a nonlinear coefficient that accounts for energy dissipation at large amplitudes (Mindlin and Laje 2005). The mean pressure at the lumen between the LTMs (P_g), is given by

$$P_g = P_s \left(\frac{a_1 - a_2}{a_1} \right) \quad (2)$$

where P_s is the pressure at the trachea close to the LTMs, a_1 and a_2 are the lower and upper cross-sectional areas respectively. Then, the dynamics of the areas can be expressed by

$$a_{(1,2)}(t) = L[\xi_0 + \xi_r(t \pm \tau_r)] + L[\xi_0 + \xi_l(t \pm \tau_l)] \quad (3)$$

where L is the LTM's length, ξ_0 is the half lumen's width at rest position, τ_l and τ_r are the times that take either wave-like motion of the LTM to travel half the vertical size of the respective membrane (Titze 1988).

The time delays in these equations are due to the flapping nature of the LTM oscillations. At the bottom of the membranes, the relevant displacement variables from equilibrium can be related to the departures at the midpoints, at times shifted by $\tau_{(l,r)}$. Similarly, at the top the displacements can be estimated from the displacements at the midpoints of membranes at previous times $\tau_{(l,r)}$.

This allows us to re write the pressure at the lumen between the LTMs (P_g) in terms of the velocities of the LTM's midpoints ($\frac{d\xi_{(l,r)}}{dt}$). In the case of small displacements and small delays (i.e. expanding for small values of $\tau_{(l,r)}$), we get:

$$P_g \approx \frac{P_s}{\xi_0} \left(\tau_r \frac{d\xi_r}{dt} + \tau_l \frac{d\xi_l}{dt} \right) \equiv \beta \left(\frac{d\xi_r}{dt} + \frac{d\xi_l}{dt} \right) \quad (4)$$

Where we assumed that $\tau_l = \tau_r$. In this way, the final equations (after re scaling time with a factor γ , and assuming $\eta_r = \eta_l = \eta$, $M_l = M_r$ that) read as

$$\frac{d\nu_{(l,r)}}{dt} = \gamma \frac{(2\beta - B)}{M} \nu_{(l,r)} - \gamma \frac{\beta \eta}{M} \xi_{(l,r)}^2 \nu_{(l,r)} - \gamma^2 \frac{K_{(l,r)}}{M} \xi_{(l,r)} + \gamma \frac{\beta}{M} (\nu_{(r,l)} - \nu_{(l,r)}) \quad (5)$$

The first term $((2\beta - B)/M)$ accounts for the positive dissipation coefficient, which corresponds to the air-sac pressure (P). The non-linear restitution term $-K_{(l,r)} \xi_{(l,r)}$ should be an odd function, what means that $K_{(r,l)} = K_{0(r,l)} + K_{1(r,l)} \xi_{(r,l)}^2$ if we expand the function up to the first nonlinear term. The last term, corresponds to the coupling between the two membranes.

Equation (5) accounts for the dynamics of a pair of symmetric membranes, then, in order to analyze the asymmetric case (where the two LTMs are not identical) we added a detuning parameter Q in the linear part of the restitution, i.e. $K_{0r} = QK_{0l}$. Notice that, although the detuning parameter affects the restitution coefficients ($K_{(l,r)}$), it does not imply that the difference between the LTMs would be in the tension generated by the muscles innervating the membranes, but in the resulting tension of all the forces acting on them.

The equations written above allow us to describe the motion of the LTMs, and therefore synthesize sound for a given set of parameters (see details in Results). Qualitatively different solutions can be obtained, depending on the parameters in the equations.

Numerical simulations for synthetic songs—In order to generate the synthetic songs we selected representative songs, one per species. Their sonograms are displayed in Fig. 1a for the *picazuro* and Fig. 1b for the *maculosa*.

Using Praat software (Broesma, 2002) we computed the fundamental frequency as a function of time, and the starting and ending times of each note in the song vocalized by *picazuro*. Then, a set of simple functions was used to generate synthetic time dependent parameters both for the pressure and the tension (P and K). For both species we used the same time dependent parameters (see Table 1).

Once we had the parameters we performed the numerical simulations with a fourth order Runge Kutta algorithm (Press et al. 1996).

2. Experimental methods

2.1 Subjects and songs

We used five adult males of *picazuro* and six adult individuals of *maculosa* (three males and three females) captured in Argentina, at Buenos Aires city, and at Buenos Aires, Entre Ríos and Cordoba Provinces. We also used recordings extracted from Straneck (1990a,b) and López-Lanús (2009) to analyze the acoustic characteristics of the song of these species.

2.2 Surgical procedures and anatomical measurements

In order to test the hypothesis that the rough sound uttered by *maculosa* compared to *picazuro* is due to the irregular modulation of the sound caused by the asymmetry between the left and right LTM, we excised the syrinx of the experimental subjects after sacrificing them with ketamine (60 mg/kg). The syringes were dissected and kept in 10% formaldehyde. Pictures against a mm page grid were taken with a HD Sony AVCHD camera. The images were analyzed using ImageJ software, (Schneider et al. 2012). Each picture of a lateral view was processed by 1. Setting a scale against the grid, 2. Identifying a membrane as the surface between the tracheal rings T1 and T2 and 3. Drawing a contour around each membrane (Fig. 2b). The software computed the area enclosed by the contour.

2.3 Statistical analysis

For each species we compared right and left LTM of each individual using a Wilcoxon matched-paired test. Interspecific comparisons were done using Mann.-Whitney U-test. All statistical analyses were performed with Statistica v7 software (Data Analysis Software System, www.statsoft.com).

Results

The origin of complexity in the spectrum

In order to understand the dynamics that can emerge from Equation(5), one can explore the regions of the parameter space where qualitatively different dynamics occur. A systematic analysis of the solutions of our dynamics system indicates that departing from $Q=1$, it is more difficult for the LTMs to stay locked. The smaller the value of Q , the larger β has to be for the LTMs to stay locked (Lucero et al. 2015). Fig. 3, displays a bifurcation diagram in terms of the air sac pressure (P) and the detuning parameter (Q) where different qualitative dynamics occurs.

The existence of a dynamics in the phase difference between the oscillations of the left and right LTM (φ) implies that the sound will present sidebands around the fundamental frequency. The reason is the following. First of all, since the flow will be the mean velocity of air through the lumen between the oscillating membranes times the area, and the area is proportional to the sum of the variables ($\xi_{(l,r)}$), the spectral content of the sound will be conditioned by the spectral content of these variables. That is particularly simple in the case of constant phase difference between the oscillations in each side (Fig. 3, red area).

Notice that each of the two variables will eventually converge to a dynamics consisting of a modulus R and a time evolving phase. In the locked case, the only difference between the dynamics of the left and right LTM will be a constant phase difference. This can be written as

$$\begin{aligned}\xi_l &= R_1 \cos(\varphi_1(t)) \\ \xi_r &= R_2 \cos(\varphi_2(t) + \Delta\varphi)\end{aligned}\quad (6)$$

If we increase the asymmetry, after a threshold value is crossed the phase difference will no longer converge to a stationary solution, and the radii display time periodic fluctuations (i.e., $R \rightarrow R_0 \cos(\omega_0 t)$). In this way, the pertinent variables will involve terms with the products of trigonometric functions of $\varphi(t)$, and $\varphi(t)$. It is from this product that the spectrum of the variables gets the sidebands (Fig. 3, grey area). For example, let us write the displacement of the right LTM as a time fluctuating radius times a trigonometric function:

$$\xi_r = \left(R_0 + \frac{A}{2} (e^{i\omega_0 t} + e^{-i\omega_0 t}) \right) \frac{1}{2} (e^{i\omega t} + e^{-i\omega t}) \quad (7)$$

$$= R_0 \cos \omega t + \frac{A}{2} (\cos((\omega_0 + \omega)t) + \cos((\omega_0 - \omega)t)) \quad (8)$$

In this way, the dynamics of the LTMs involves the frequencies $\omega_0 \pm \omega$, which appear as sidebands in the spectrum.

Beyond this qualitative analysis, we performed the numerical simulations with a fourth order Runge Kutta algorithm (Press et al. 1996), ran the codes in a Unix environment and analyzed spectrally the solutions using Praat Software (see Materials and Methods for a complete description) Using this procedure we were able to generate synthetic copies of the vocalizations for both *maculosa* and *picazuro*, which presents the same qualitative difference in the spectral properties (Fig. 1) (recorded and synthetic songs are available in the supplementary material). By introducing the asymmetry parameter $Q=0.45$, we generated the synthetic songs with the sidebands around the fundamental frequency, giving rise to sounds with the characteristic roughness of *maculosa's* vocalizations.

For each note, the parameters corresponding to the pressure and tension were obtained by integrating a first order linear differential equation driven by a set of functions listed in Table 1. All the silent times required pressure and tension obtained from linearly following $P(t)=0.001$ and $K(t)=0.01$ respectively. The other parameters used in the simulations are $\gamma=3500$, $B\eta/M=1$, $\beta/M=0.175$, $M=1/15$, $K_{1(r,l)}=0.9K_{0(r,l)}$. The only difference in the parameters used to synthesize the two songs displayed in the left and right bottom panels of Fig. 1 is the asymmetry parameter Q ($Q_{picazuro}=1$; $Q_{maculosa}=0.45$).

The parameter $Q=1$ allows synchronized solutions between the right and left labia, therefore generating an almost tonal sound, as can be seen in Fig. 1c. Fig. 1d shows a case in which the phase difference between the labia ($Q=0.45$) exhibits a nontrivial dynamics that is shown in the sound as sidebands around the fundamental frequency. The existence of sidebands in the *maculosa* vocalizations gives the sound its characteristic roughness.

The sounds obtained in this way are a simplification. Timbre emerges from the interaction between the source of the sound (syrinx in birds) and the upper track filter. The filter acts by enhancing (and diminishing) certain frequencies. The frequencies that are being enhanced are related to the size and shape of the different cavities. In the case of pigeons, they vocalize with a closed beak using inflation of the esophagus as a filter mechanism (like a Helmholtz resonator). Neck sizes are not identical in both species either, but in any case, all these features of the tracts involve linear filters. Therefore they do not explain the emergence of sidebands, and for that reason we do not describe their role in detail (see Beckers et al. 2003; Elemans et al. 2003; Beckers and Ten Cate 2006; Elemans et al. 2008 for a discussion).

Anatomical validation of the Asymmetry hypothesis

In *maculosa*, we found that all individuals have larger left LTM than right ones irrespective of their sex (see Table 2). In *picazuro*, about half of the males showed larger left LTM while the others possessed larger right LTM. In consequence, the syrinx of *maculosa* is significantly asymmetric (Wilcoxon matched pair test $Z=2.201$, $p=0.028$) while *picazuro's* syrinx is not (Wilcoxon matched pair test $Z=0.135$, $p=0.893$). Interspecific comparisons

showed that only right LTM differ, being smaller in *maculosa* compared with *picazuro* (Mann-Whitney test $Z=2.008$, $p=0.052$, two tailed), see Fig. 4.

Finally, we explored the asymmetry of the LTMs irrespective of which side is larger in each individual. In this case, we still found a larger absolute asymmetry (mean \pm standard deviation: $2.00 \text{ mm}^2 \pm 1.43 \text{ mm}^2$ vs. $1.33 \text{ mm}^2 \pm 0.83 \text{ mm}^2$) and relative asymmetry (15% vs. 10%) in *maculosa* than in *picazuro*. Moreover, males in *maculosa* seem to be more asymmetric than females ($3.27 \text{ mm}^2 \pm 0.30 \text{ mm}^2$ vs. $0.73 \text{ mm}^2 \pm 0.23 \text{ mm}^2$), and also more asymmetric than *picazuro* males (i.e. their 95% confidence intervals did not overlap).

Discussion

In this work, we generated a biomechanical model for vocal production that we applied to pigeon vocalizations but that can be used more generally for songbirds or humans. We worked with a flapping model accounting for possible differences between the right and left LTM. In particular, we introduced an asymmetry parameter Q with which we can control the level of asymmetry between the LTMs. Using this model, we were able to create a synthetic copy of the *picazuro* and *maculosa* vocalizations that share the same fundamental frequency but differ in their timbre, in close correspondence to the natural ones. In addition, we were able to confirm the anatomical prediction derived of this model, showing that the species with tonal song (*picazuro*) has more symmetrical LTM compared to *maculosa*. Hereafter, we will discuss these findings in relation to: a) the mechanism of song production, and b) the evolution of interspecific song differences.

Mechanism of song production in pigeon and doves

Behavior emerges from the interaction between a nervous system and a peripheral device. Due to its nonlinear nature, even when driven by simple instructions, a phonating vocal organ is capable of displaying complex behavior, and therefore, generating complex sounds. In this work we show a spectacular example: two non-vocal learner bird species, which modulate the fundamental frequencies in similar ways, generate different songs from the point of view of their timbres. Interesting enough, the songs are qualitatively different although the difference is not qualitative from the anatomical point of view. In fact, this is an example of what is an ubiquitous phenomenon in nonlinear systems: qualitative changes in the dynamics of a family of systems that undergo a smooth change in parameter space. In other words, this is an example of the nonlinear nature of the avian vocal organ being responsible for the acoustic features of the song.

In our case, the difference in timbres can be tracked down to the spectra very easily. The difference between the two sounds comes in side bands that can only be generated by nonlinear effects. Since the simplest models of sound production in birds are two dimensional, and it is not possible to embed solutions with side band spectra in two dimensions, higher dimensional dynamics had to be present in the problem (Guckenheimer and Holmes 1983). It is a common assumption in all the existing simple models of birdsong production that the two labia are synchronized, even if the natural anatomical configuration in all bird species has some degree of asymmetry. For example, tracheobronchial syrinxes

commonly found in oscine birds, possess two pairs of oscillating membranes, each pair consists of a lateral tympaniform membrane and a medial tympaniform membrane which are not identical, and therefore, essentially asymmetrical (Mindlin and Laje 2005).

In this work we tested the hypothesis that the dynamical origin of spectral sidebands in pigeons is the existence of an asymmetry between the two oscillating membranes (in the case of pigeons and doves, their LTM). We showed with a simple model that for asymmetries above a certain threshold the solutions do not synchronize and give rise to vocalizations with spectral sidebands. This also leads naturally to the prediction that *maculosa* (the species with a rough song) presents a larger degree of asymmetry compared to *picazuro* (the one with a tonal song), and we tested this prediction by exploring comparatively the two species.

In fact, we found that the left LTM is larger than the right one in all *maculosa* individuals, in contrast with *picazuro* that only exhibit a kind of fluctuating asymmetry with different individuals showing alternatively a larger right or left LTM. In absolute and relative terms the asymmetry of LTMs is larger in *maculosa* than in *picazuro* (especially if we consider only males) and although these differences appear small they can be enough to produce qualitatively different vocalizations given the non-linear nature of the phonation mechanism proposed here.

It is interesting to note that the existence of closely related species differing in timbre aspects of their songs is rather common among other pigeons and doves. Beckers and ten Cate (2006), noted that fourteen species of *Streptopelia* doves have pure-tone perch-coos, but at least one species, *S. orientalis*, presents a “biphonation regime” with two harmonically unrelated frequencies. This species together with *S. tranquebarica* and *S. lugens* also vocalize notes with complex sounds. In addition, *Streptopelia*'s vocalizations also present sudden frequency shifts and transitions from harmonic to complex states, and between harmonic and subharmonic regimes. They interpret these observations as indicative of the non-linear phenomena involved in the mechanism of sound production.

Based on the phylogeny of mourning doves (genus *Zenaida*) published by Lapiedra et al. (2013) and recordings from different sources (Xenocanto, Macaulay Library of Natural Sounds) we found that *Z. graysoni* has a rough vocalization compared to its closely related (sister) species *Z. macroura*, and something similar happens in the species pairs *Z. asiatica*-*Z. meloda*, and *Z. auriculata*-*Z. aurita*, with the first species in each pair having the rough timbre. Moreover, in galliforms, another group of birds with relatively simple syringes, we found contrasting types of vocalizations including some with very harsh songs like the ones of both chachalacas (genus *Ortalis*) and junglefowl (genus *Gallus*) and others with much more tonal ones such as those of songwood-quails (genus *Odontophorus*) or peafowls (genus *Pavo*). Also in this order, Francolins (genus *Fracolinus*) are characterized by having tonal and whistling calls, in contrast to the atonal, raucous grating advertisement calls of the Spurfowls (genus *Galloperdix*, Milstein and Wolff 1987; Mandiwana-Neudani et al. 2011). We suspect that our asymmetric model of bird phonation could potentially explain much of this variation. Although we do not know much about the anatomy of the syrinx of these

species, our model predicts that those species with rough vocalizations should have more asymmetric oscillating membranes compared to those with tonal songs. Additionally, future studies on the syrinx anatomy of these species are clearly needed in order to test this prediction.

Interspecific song divergence and the nonlinear nature of phonation mechanism

Perch-coos are considered important in species recognition in pigeon and doves (Goodwin 1983; de Kort and Ten Cate 2004). In *Streptopelia*, Slabbekoorn et al. (1999) found that most species can be differentiated by song, mainly by temporal features rather than by frequency ones, and suggested that the poor performance of the latter is due to morphological constraints. In contrast, Mahler and Tubaro (2001) showed that both temporal and frequency variables were useful to differentiate species and groups of related species in the New World pigeons. In particular, they attributed the lower frequencies in the songs of heavier species to differences in the morphology of the vocal tract correlated with body size. Now we have a much more specific explanation for the vocal divergence between two sister and mostly sympatric species *Patagioenas maculosa* and *P. picazuro*. Both species have essentially the same temporal structure in the song, but are qualitatively distinct in frequency spectra. According to our model this is the result of a difference in the degree of asymmetry between left and right LTM area being larger in *maculosa* (the noisy species) than in *picazuro* (the tonal one), as could be confirmed by the anatomical study of their syrinxes. In other words, the interspecific differences are consequence of the nonlinear nature of the phonation mechanism that allows bifurcations: i.e. qualitative changes in a response as the result of a continuous and progressive subtle change in the parameters.

Beckers et al. (2003) and Beckers and ten Cate (2006) also explored the possibility that certain complex acoustic phenomena in vocalizations of *Streptopelia* doves were caused by the nonlinear dynamics of their vocal organ and concluded that strong interspecific vocal differences might have resulted from minor changes in control parameters without the need for large changes in syringeal structure or function. We believe that bifurcations in the dynamics of the phonation device of pigeons and doves could facilitate interspecific divergence and, under certain conditions, even contribute to the speciation process. A possibility is that isolated and diverging populations rapidly acquire qualitative vocal differences in allopatry through a small change in the asymmetry of their LTM areas. These differences could contribute to maintaining the species isolated if they become sympatric.

Most of the discussion about the possible role of song in the diversification process has been focused on learned vocalizations instead of the innate ones, because learning easily produces population differences in songs that could restrict gene flow among populations and ultimately result in speciation (Nottebohm 1970, but see Baptista and Trail 1992, Slabbekoorn and Smith 2002 for varied opinions in this regard). In contrast, it is assumed that innate songs are unable to change so easily, requiring modifications in the anatomy and physiology of the vocal organ and its neural control mechanisms (Edwards et al. 2005). However, we demonstrate that a small and quantitative modification in the degree of asymmetry in the area of the oscillating membranes can produce a large and qualitative change in the song output that can eventually act as a prezygotic isolation mechanism.

Notice that in this paradigm (see Fig. 3), a species with high asymmetry could still generate tonal sounds by vocalizing with high enough pressure.

Previous studies in another pair of sister species of doves, *Streptopelia capicola* and *S. vinacea*, showed that they can discriminate conspecific from hetero-specific songs in allopatry. However, in a relatively small and recent area of sympatry there are intermediate “hybrid” songs, and the response of individual hybrid males is not linked to the similarity between their own signal and the playback signal (de Kort et al. 2002; den Hartog et al. 2008). Thus, it seems that the song differences between *vinacea* and *capicola* originated in allopatry, and the lack of coupling between song and response within the sympatric hybrid zone casts doubts about the importance of song differences in maintaining their species identity.

The case of *maculosa* and *picazuro* is clearly different to the one involving the two *Streptopelia* species mentioned in the previous paragraph because *maculosa* and *picazuro* are largely sympatric but, in spite of this, hybrids between them have not been reported (Gray 1958; Baptista et al. 1997; Gibbs et al. 2001; McCarthy 2006). As a consequence of their sympatry, we do not know if their vocal differences arose during speciation or after the process was completed (i.e. whether this difference played a role in the speciation process or not). Irrespective of this, playback studies comparing male and female responses to homospecific vs. heterospecific songs in the areas of sympatry should inform about the importance of timbric differences in maintaining *maculosa* and *picazuro* isolated. In addition, there are areas of allopatry, in which only one of these species is present: *picazuro* is absent in a disjunct area of the distribution of *maculosa* in central Bolivia and Peru and *maculosa* is not present in a sector in the northeastern part of the distribution of *picazuro*. Comparing the structure of the songs of the two species in these areas to those in the areas of sympatry could shed some light on the influence of the interaction between these two species on their vocalizations. Analyzing responses to playbacks with homospecific and heterospecific songs in these areas and comparing them to those in the sympatric areas could also be informative about the role of song differences in interspecific interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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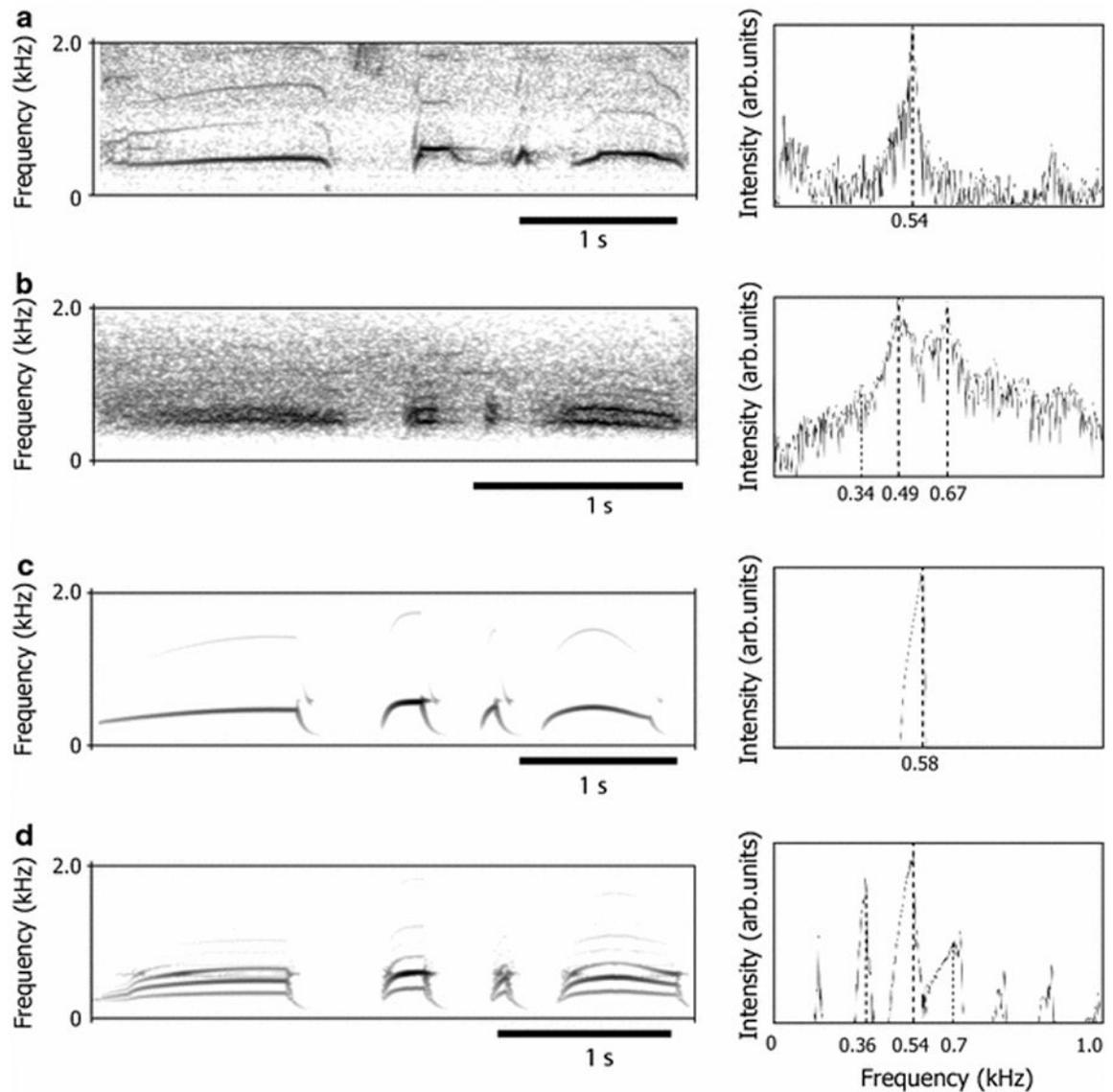


Fig. 1. Recorded and synthesized vocalizations of *Patagioenas picazuro* and *Patagioenas maculosa* Left panels: (a) Typical *picazuro* and (b) *maculosa* vocalizations, (c) synthetic songs of *picazuro* and (d) *maculosa* Right panels: Power spectrum from the third cooing note. Notice that in recorded songs high pass filters were used to attenuate background noise that, in the case of *maculosa* resulted in a considerable reduction of the first sideband. Recorded and synthesized songs are provided in the supplementary material.

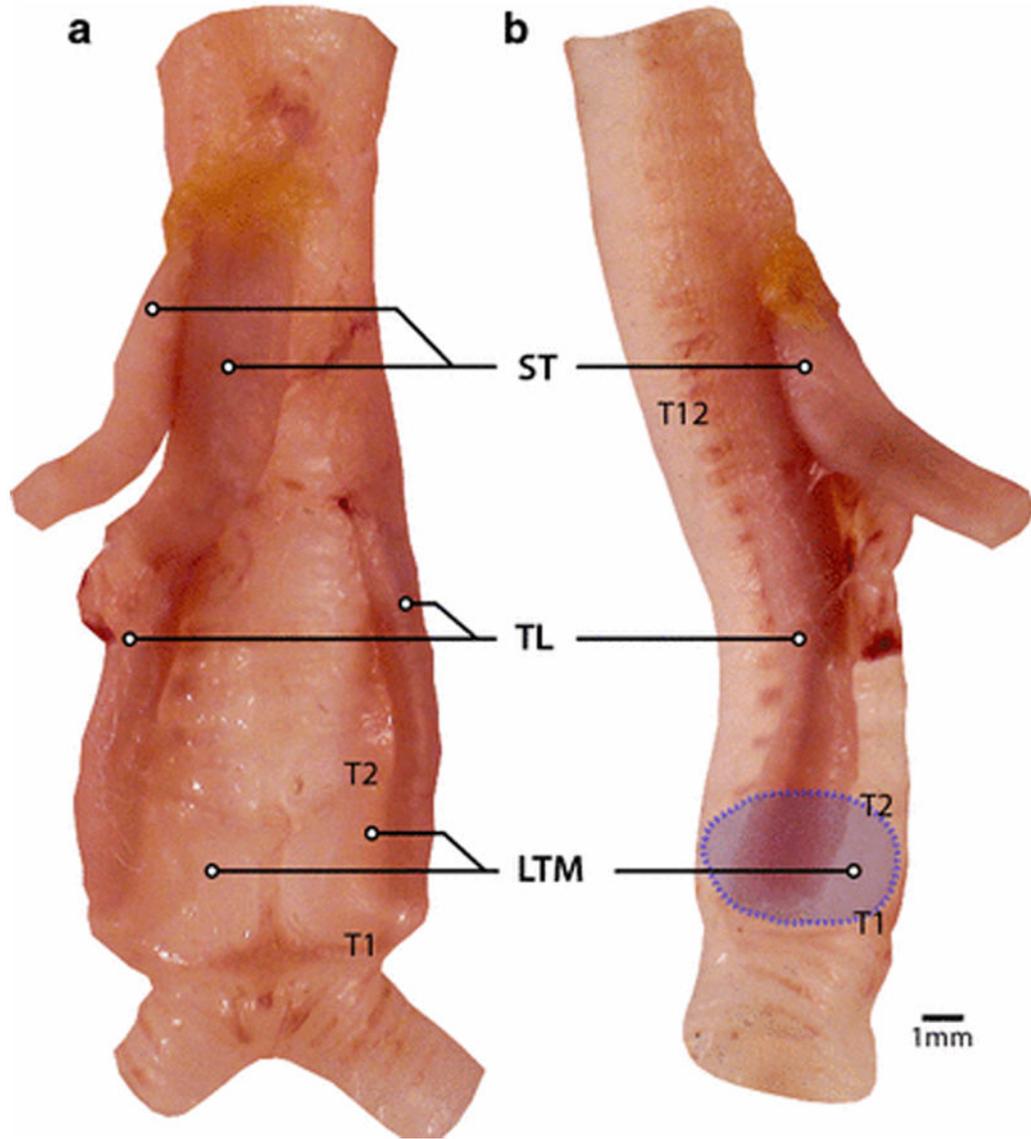


Fig. 2. Syrinx morphology

(a) Ventral view of *maculosa*'s syrinx. Tracheolateralis muscle (TL) inserts on the Lateral Tympaniform Membrane (LTM), between the tracheal rings T1 and T2. Left and right sternotrachealis (ST) muscle, run together and splits around the tracheal ring T12 into the interclavicular air sacs. (b) Lateral view. Area surrounded by blue dots corresponds to the computed surface of LTM membrane.

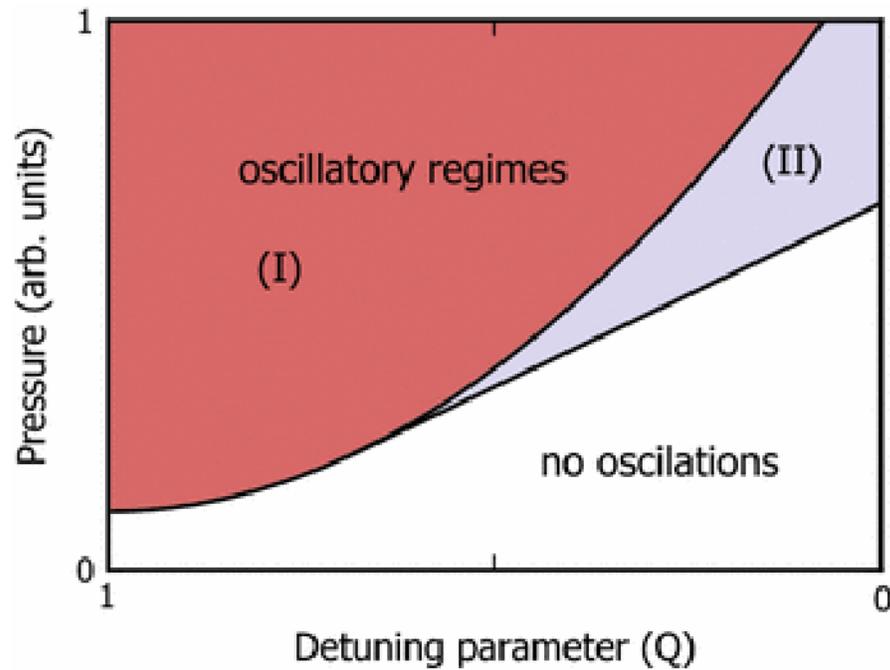


Fig. 3. Bifurcation Diagram

The figure displays a qualitative bifurcation diagram in terms of the air sac pressure (P) and the detuning parameter (Q), which accounts for the degree of symmetry between the LTM. White area corresponds to a non-phonatory region. In region I (red area) the membranes oscillates locked with 1:1 entrainment, which corresponds to tonal sounds. In region II (grey area), the membranes display nontrivial dynamics and sounds presents sidebands around the fundamental frequency. (Adapted from Lucero et al. 2015)

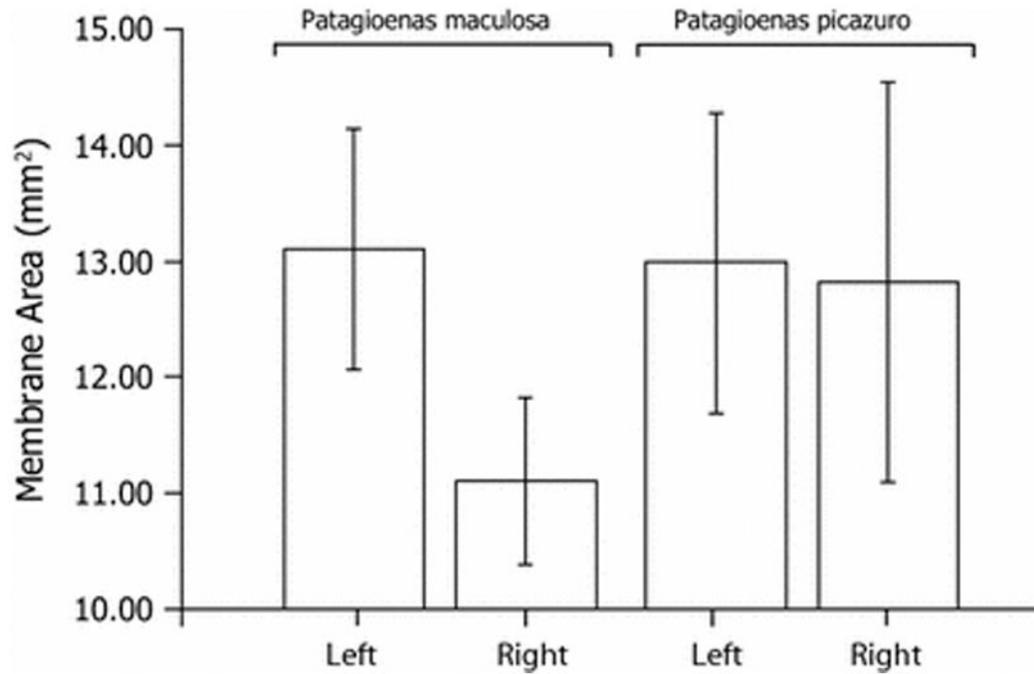


Fig. 4. Experimental evidence of asymmetrical syrinx

Column heights correspond to the average areas of left and right lateral tympaniform membranes (LTM) of *maculosa* (n=6) and *picazuro* (n=5). Error bars accounts for the standard deviation of the medias.

Table 1
Summary of the time-dependent parameters used for the simulations

	Introductory note		Note 2	Note 3	Note 4
Time interval (s)	[0.57,1.07]	[1.07,1.98]	[2.51,2.77]	[3.14,3.24]	[3.52,4.22]
Pressure (P)	$P(t)=(t-0.57)$	$P(t)=0.5\sin(2.198(t-1.07))+0.55$	$P(t)=1.35$	$P(t)=1.35$	$P(t)=-0.3\cos(7.85(t-4.22))+1.05$
Tension (K)	$K(t)=0.026$	$K(t)=0.033$	$K(t)=0.04$	$K(t)=0.036$	$K(t)=0.033$

Table 2

Anatomical measurements of left and right Lateral Tympaniform Membranes (LTM) areas.

Species	Subject	Left LTM area (mm ²)	Right LTM area (mm ²)	Sex
<i>Patagioenas maculosa</i>	1	14.70	11.27	M
	2	13.66	10.21	M
	3	12.39	11.27	F
	4	11.70	11.38	F
	5	13.27	10.35	M
	6	12.90	12.15	F
<i>Patagioenas picazuro</i>	7	13.84	15.21	M
	8	14.19	13.14	M
	9	10.87	12.45	M
	10	12.89	10.39	M
	11	13.13	12.91	M

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