RHYTHM DEVELOPMENT IN THE ZEBRA FINCH SONG

by

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Abstract

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When we listen to the zebra finch song, the song sounds very rhythmic and repetitive. Each bird has its own song rhythm. In order to study rhythm development in songbirds we introduced a new concept to bird song research, the quantification of rhythm and periodicity in song structure. By developing a technique that gives us insights into the song rhythm we have studied how song structure changes during different stages of the zebra finch life. We found that birds trained from an early age (day 43) exhibit two types of rhythm transformations: the first type is a smooth down-modulation of rhythm during several days of song development. The second type is an abrupt transition from one rhythm to a different rhythm. Smooth transitions might be caused by a slow shift in the timing of neuronal firing, while abrupt transitions might be caused by reorganization of the rhythm generator. We also examined why is it that zebra finches that are isolated until the end of their sensitive period and then trained do not imitate well. We discovered that their poor imitation correlates with inability to add new syllables to the songs. Zebra finches that sing a long motif before the onset of training were able to imitate better than finches with a short motif, perhaps because they have more raw materials to work with. Our findings suggest that at the end of the sensitive period vocal changes still occur, but imitation is constraint at the level of song rhythm and on the level of fine temporal structure.

Acknowledgements

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Chapter 1. General Introduction

Many organisms employ neural systems that produce cyclical sequenced behavior with temporal precision that generates rhythms. This is true for behaviors as basic and ancient as respiration (Feldman 1986; Feldman & Del Negro 2006; Suzue 1984) and locomotion (Arshavsky et al. 1986; Li et al. 2009; Zhang et al. 2008; Enzure & Tanaka 1997), it is also true for more complex behaviors that are acquired through experience such as egg laying (Kupfermann 1972; Pierce & Cheney 2004; Scheller & Axel 1984), dancing (Brown et al. 2005; Pierce & Cheney 2004; Brockmann & Robinson 2007; Alexander et al. 2007) and sports (Jonsson et al. 2006). The mechanism of temporally precise cyclical behaviors has been studied at the neural level (Suzue 1984; Li et al. 2009; Arshavsky et al. 1986; Enzure & Tanaka 1997). The aim of such research is to understand how temporally precise cyclical behavior is learned (Marler 1981; Marler & Peters 1982; Tchernichovski 2001; Marler 1970; Nottebohm 1972), what factors influence its production (Konishi 1965; Immelmann 1969; Nelson & Marler 1994) and the specific neural circuitry (Fiete et al. 2004; Bottjer et al. 1984; (Nottebohm et al. 1976; Nottebohm et al. 1982; Bottjer et al. 1989), and genomes (Kubikova et al. 2009; Clayton 2005; Mello & Jarvis 2009, Clayton 2005; Blanchi & Sieweke 2005; Blanchi et al. 2003; Feldman & Del Negro 2006; Wolfgang et al. 2009**;** Pierce & Cheney 2004) that underlie their accuracy. One neural system that has received particular interest is the network of neural circuit that controls singing behavior in songbirds.

The songbird's song is a complex learned behavior that is produced with varying degrees of stereotypy, depending on the species. Several features of birdsong cause it to be a preferred animal model for the study of motor development (Brainard & Doupe 2002):

i) Birdsong is a stereotyped and quantifiable behavior (Marler 1981; Marler & Peters 1982; Tchernichovski et al. 2001; Saar et al. 2009);

ii) There are similarities between the acquisition of birdsong and human speech (Brainard & Doupe 2002; Catchpole & Slater 2008; Doupe & Kuhl 1999).;

iii) Song production and song learning are controlled by a set of discrete brain nuclei (the song system) (Zaretsky & Konishi 1976; Fortune & Margoliash 1995; Vates et al. 1996; Janata & Margoliash 1999)

iv) Song development is short (e.g., 60 days in zebra finch as opposed to several years of human speech development) (Immelmann 1969);

In the frequently-studied zebra finch (Taeniopygia guttata), the adult's song consists of sequences of highly structured vocal units (Marler 1981; Tchernichovski et al. 2001; Deregnaucourt et al. 2004). Referred to as syllables, they are produced in a stereotyped order, or motif (Thorpe 1958). A typical song is usually preceded by one or more introductory notes, vocal units with stereotyped acoustic structure, which are then followed by multiple repetitions of the bird's motif (Figure 1.1). The adult zebra finch song consists of highly stereotyped sounds (syllables) delivered in a fixed order and rhythm (Marler 1981; Tchernichovski et al. 2001; Deregnaucourt et al. 2004). Let us define the song part:

Introductory note is a note in a series of identical notes, which precede the first motif of a bout.

Syllable is an acoustical distinct sound in a song motif that is generally separated from other sounds by a silent interval.

Motif is a regularly repeated and individually stereotyped sequence of distinct syllables and intervals.

Bout is an event of singing which typically starts with a few introductory notes followed by several more complex song syllables.

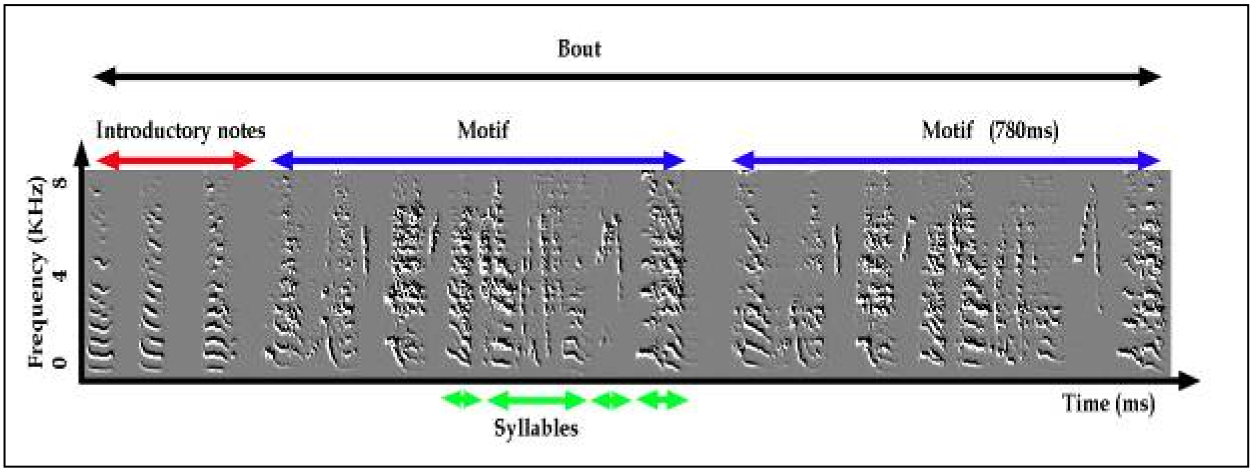


Figure 1.1 Examples of a song from an adult male zebra finches. Songs are shown as sonograms with acoustic frequency on the y-axis and time on the x-axis. Individual syllables are usually identified by their individual temporal acoustic structure. In a typical song, syllables are produced in the same sequential order multiple times. Each repetition of the sequence is referred to as a motif, and a song bout consists of one or more motifs. Song bouts are usually preceded by a variable number of introductory notes.

Like many other song birds, juvenile male finches acquire the temporal and acoustic structure of their song during two phases of development (Marler 1981) - a largely passive phase of listening to the songs of other adult males called the sensory period, and a phase of practice and self-monitoring referred to as the sensorimotor period, Figure 1.2. A juvenile male zebra finch exposed to a tutor's song transforms soft, squeaky sounds (known as subsong) (Brainard & Doupe 2002; Catchpole & Slater 2008; Doupe & Kuhl 1999) into structured syllables of distinct categories that are assembled to produce an imitation (Marler 1981, Marler & Peters 1982). The juvenile male (females do not sing) acquires his song during a sensitive period for learning that is divided into two overlapping parts called the sensory and sensorimotor phases. During the sensory phase, the male listens to and memorizes a tutor song. This memory is called the template. In many songbirds the sensory phase is followed by a sensorimotor phase, but in the zebra finch those stages are almost entirely overlapping. During the sensory-motor phase, the bird begins to sing, since he presumably "practices" the memorized song and modulates his vocal output to eventually match the template (Immelmann 1969; Eales 1985; Catchpole & Slater 1995). The acoustic structure stabilizes into the stereotyped sequence of elements (syllables).

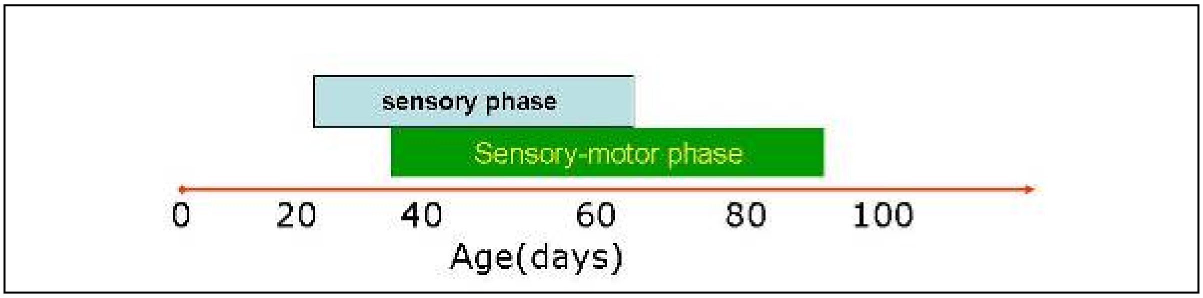


Figure 1.2 A Timeline illustrating the sensitive period timeline of a young male zebra. A largely passive phase of listening to the songs of other adult males called the sensory period, and a phase of practice and self-monitoring referred to as the sensorimotor period. (Marler 1981)

During both of these periods, auditory feedback is critical, as evidenced by the fact that song develops poorly in its absence (Konishi 1965). At about 90 days of age the Zebra Finch song reaches its final form, which the bird then produces on a daily basis for the rest of his life (Immelmann 1969). The closure of the sensitive period could be delayed by withholding exposure to the model (Eales, 1985; Morrison and Nottebohm, 1993; Jones et al., 1996). A similar pattern is followed in other species (Dietrich 1980, Clayton, 1987, 1988, 1989), although for some, such as canaries or sparrows, new songs are learned every breeding season and multiple songs types are produced (Kroodsma 1977). There is evidence that with proper incentives the adult Bengalese Finch can still change some of its features (Tumer & Brainard 2007), while inter-syllable gap and song features change naturally at a very late age (Chapter 5).

Training young zebra finches with song playback and tracing moment-to-moment vocal changes suggests that some vocal changes are not simple (Figure 1.3), (Tchernichovski et al. 2001, Tchernichovski & Mitra 2002). For example, exposure of a young zebra finch to a song model will induce the prompt generation of repeated and structured sounds, which by the second day of training already lead to rapid changes in the distribution of syllable features. These changes are then followed within a few days by the establishment of distinct acoustic categories (Deregnaucourt et al. 2005; Saar et al. 2005; Tchernichovski et al. 2004; Deregnaucourt et al. 2005; Saar et al. 2009). The relatively short time (days and even hours) over which changes and transition in song learning occur make this system useful for measuring physiological variables associated with sensory-motor learning.

We know, in a very general way, how this sensory-motor conversion occurs. At the sensory level, the zebra finch can perceive and memorize many songs, but only one (or a few) of those are selected to become the *song template* for imitation. The bird uses auditory feedback to guide vocal changes. This is thought to be achieved by comparing feedback from the bird's own song (BOS) to the song template (Brainard & Doupe 2000), i.e., evaluating the acoustic error. We use the term "error", even though we do not know how, and based on what features, the brain evaluates the difference between the memory template and auditory input from the BOS. It is assumed that the error gives rise to an "instructive" signal: an action resulting from detection of the difference (Doupe et al. 2004). Examination of the details of song development gives us an opportunity to assess the structure of this instructive signal: When we observe vocal changes during development, we might assume that some of the changes we observe are driven by the postulated instructive signal (Doupe et al. 2004).

Not all vocal changes during development are driven by the acoustic error from the acquired template. Some features of song development evolve regardless of sensory experience in socially isolated, and even in deaf birds (Price 1979). In other words, song learning is the modulation of a more primitive (and perhaps a more fundamental) ongoing developmental process. Still, it is sometimes possible to identify vocal changes that are driven by specific features of the song template (Tchernichovski 2001).

We would like to distinguish between two possible different types of error signals, a simple and a complex error signal because they would be generated by different neuronal mechanisms. A simple binary error signal (e.g., classifying vocal performance as similar or dissimilar to the template) cannot drive any particular type of change, but it can guide "learning by experimentation" (Fiete et al. 2004). Its role would be primarily selective, resulting in the retention of one rather than another syllable or subsyllabic vocal gesture. In contrast, a complex error signal could reflect certain deviations from the template and then attempt to correct them. It could generate a very specific instructive signal to attempt specific corrections (e.g., elevate the pitch of a particular sound). As a result, vocal changes induced by a complex error signal should appear more structured, or even "planned", compared to vocal changes driven by a simpler error signal.

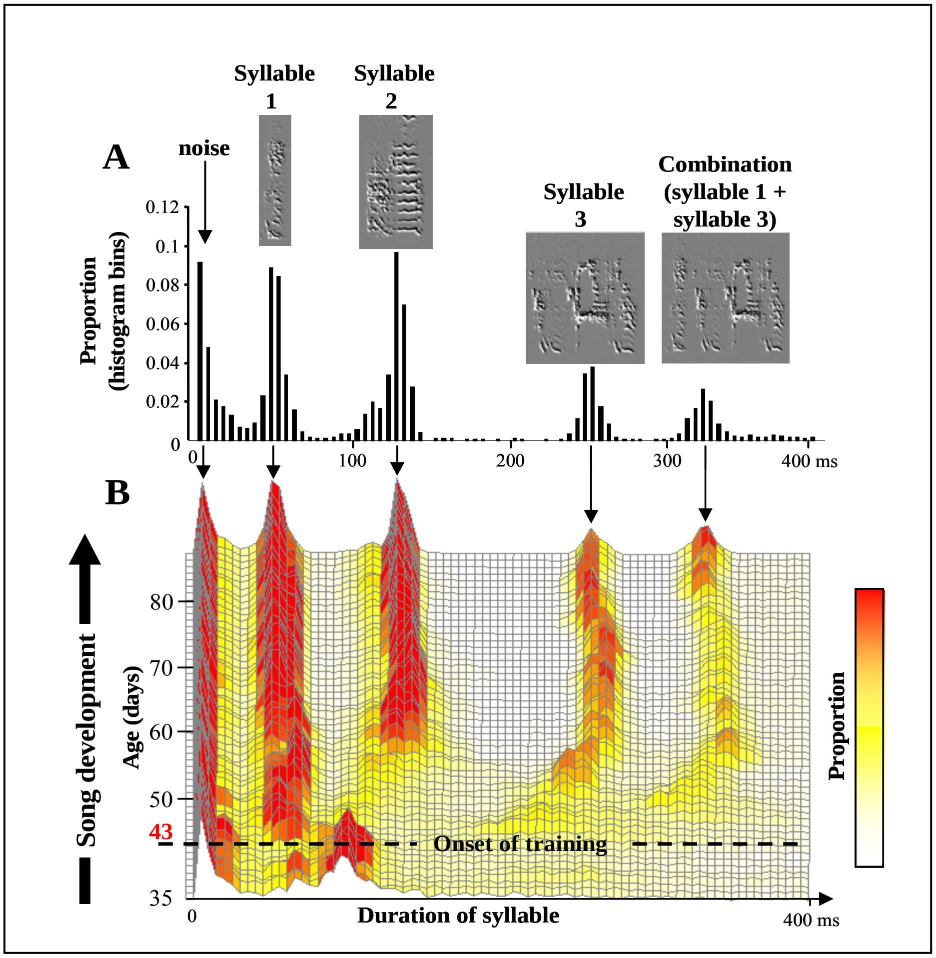


Figure 1.3 Dynamic Vocal Development (DVD) Map of syllable durations. A: a histogram of syllable durations in an adult bird. Each peak can be associated with a syllable type of unique duration. B: a trace of the evolution of syllables during song development. The histogram of the entire vocal ontogeny is plotted. Each row represents the histogram of syllable durations during one developmental day. Each syllable type can be traced to an early stage of song development even when duration changes, (Figure copied with permission from Tchernichovski). For a more detailed discussion of the construction of DVD maps see Chapter 2.

Recent studies (Olveczky et al. 2005; Scharff & Nottebohm 1991, Andalman & Fee 2009) have shown that inactivating a basal-ganglia-related circuit via its output, the lateral magnocellular nucleus of the nidopallium (LMAN) promptly reduces the degree of variability in song structure of a juvenile bird, making it as stereotyped as the song of an adult bird. This finding suggests that the variability of the juvenile song is actively “injected” into the premotor song nuclei via the forebrain song pathway. Injecting variability (Doupe 2004; Deregnaucourt 2005; Andalman & Fee 2009**)** into the song could allow vocal learning by experimentation, namely, by exploring different versions of song syllables while attending to the changes in the vocal output, and then selecting the most appropriate path (in the "production space") towards the model (Tchernichovski et al. 2001).

How song rhythm is generated?

In order to discuss the generation of song rhythm, we would like first to describe the neural system responsible for song learning and the production of that rhythm.

The avian song system

Anatomically, the neural system responsible for song learning and production can be divided intro three pathways – an auditory pathway, the anterior forebrain pathway and the motor pathway (Figure 1.4, 1.5). The first is the ascending auditory pathway, which transmits auditory input into the song system. In this pathway the thalamic nucleus Ovoidalis receives input from more peripheral structures, such as the cochlea and the auditory nerve, and projects to a region of auditory forebrain known as Field L, an area that is thought to be functionally analogous to mammalian auditory cortex (Zaretsky & Konishi 1976). Field L sends out a diverging array of projections, but one of its targets is a pathway through the caudal lateral mesopallium (CLM), the nucleus interfacialis of the nidopallium (Nif), and finally to the forebrain nucleus HVC (used as a proper name)

(Fortune & Margoliash 1995; Vates et al. 1996; Janata & Margoliash 1999), which is part of the motor pathway and the anterior forebrain pathway (AFP)

The AFP is a chain of forebrain and thalamic nuclei that are critical to song learning, but not to later song production after crystallization (Bottjer et al, 1984), Figure 1.4. In this pathway, HVC projects to Area X, a region within the avian basal ganglia (Farries 2001). As with the mammalian basal ganglia, the output of Area X projects to the thalamus, in this case specifically to the medial portion of the dorsal lateral nucleus of the anterior thalamus (DLM). DLM projects to the lateral portion of the magnocellular nucleus of the anterior nidopallium (LMAN), which then projects to another forebrain nucleus located at the telencephalic, the robust nucleus of the arcopallium (RA), which is also a critical component of the motor pathway (without RA zebra finches can not sing) (Nottebohm et al. 1976; Nottebohm et al. 1982; Bottjer et al. 1989). Lesions along this anterior pathway (Area X, DLM, or LMAN) during development prevent the acquisition of normal song, but lesions in singing adult males have little effect (Bottjer et al. 1984).

The third pathway is the motor pathway in the telencephalon (Figure 1.4). This neural circuit also includes HVC, which in addition to its AFP connections, also projects directly to RA. RA's projections are widely divergent, but most of its targets are brainstem nuclei that are critical for controlling the peripheral musculature during song production (Figure 1.4). The ventral region of RA projects to the tracheosyringeal portion of the hypoglossal nucleus (nXIIts), a region containing motor neurons innervating the syrinx, the avian vocal organ (Nottebohm et al. 1976; Vicario 1991). The dorsal region of RA sends projections to the dorsomedial nucleus of the intercollicular complex (DM), which has been implicated in the production of non-learned vocalizations, i.e. non learned calls (Vates et al. 1997; Striedter & Vu 1998). The dorsal RA and DM both send projections to respiratory nuclei in the brainstem, particularly nucleus retroambigualus (RAm) and nucleus paraambigualus (PAm) (Wild et al. 1997; Reinke & Wild 1998). These nuclei send spinal projections to neurons innervating the musculature of the air sacs, allowing them to control normal respiration, and presumably, the rapid respiratory cycles that occur during song production.

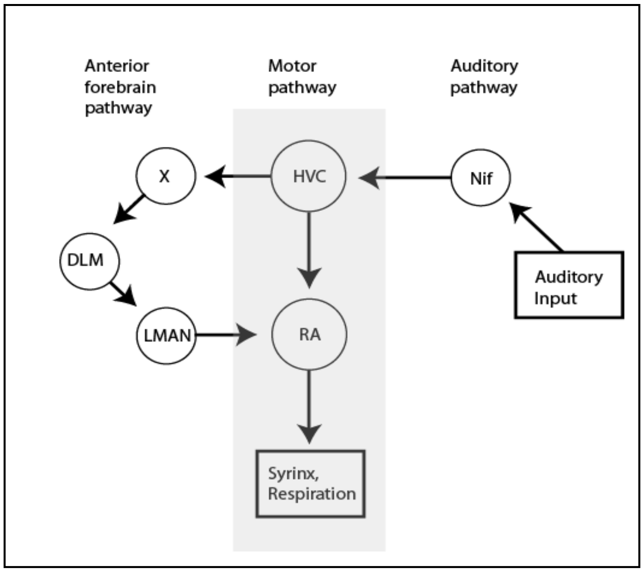


Figure 1.4 Schematic view of one hemisphere of the avian song system. The forebrain nucleus HVC (used as a proper name) sits at the intersection of three pathways: an auditory pathway bringing sensory information into the song system (right), the anterior forebrain pathway (AFP, left) which leaves HVC and projects ultimately to the robust nucleus of the arcopallim (RA) and is important for song learning, and the descending part of the forebrain motor pathway consisting of HVC, RA, and RA's projections to brainstem nuclei (center). For simplicity, numerous recurrent, convergent, and divergent pathways have been omitted (Figure copied with permission from Ashmore 2006) .

During the phase of song learning in zebra finches (age 25-90 PHD), the song control nuclei undergo massive growth (or, in the case of LMAN, shrinking) processes, which include the addition and turnover of neurons (Bottjer and Arnold 1997). Some neuronal populations then continue being replaced in the mature bird, albeit at slower rates. The projection neurons from HVC to RA, a population that exhibits sparse firing precisely time-locked to the song output (Yu and Margoliash 1996; Hahnloser et al. 2002), and a population of medium spiny neurons in Area X are two examples of continually replaced neuronal populations (Kirn and Nottebohm 1993; Scharff et al. 2000; Nottebohm 2004; Rochefort et al. 2007). Since recruitment rates of HVC→RA projection neurons are particularly high during vocal development, and increase again in seasonal songbird species as canaries during seasonal changes in the song repertoire, adult HVC→RA neurogenesis is thought to be linked to vocal plasticity and learning (Kirn et al., 1994). In juvenile birds, neurogenesis exists in HVC→X neurons. However, adult birds do not exhibit any neurogenesis in the HVC→X neurons.

How song rhythm is generated?

In young juveniles, AFP generates poorly structured subsong, whereas in adult birds, the HVC-motor pathway generates highly stereotyped motor sequences (Aronov et al. 2008). These pathways interact in the intermediate plastic-song stage (Ölveczky et al. 2005) to generate the partially structured but variable vocalizations upon which vocal learning operates. The transfer of functional dominance from AFP to the motor pathway during vocal learning might parallel their anatomical development. HVC does not reach its adult size until the late plastic-song stage (Alvarez-Buylla et al. 1992) and establishes functional synapses in RA later than LMAN does (Mooney 1992; Mooney & Rau 1994). Song maturation and the decrease in vocal variability have thus been attributed to the strengthening of inputs from HVC and the concurrent weakening of inputs from LMAN

(Herrmann & Arnold 1991; Akutagawa & Konishi 1994; Kittelberger & Mooney 1999; Stark & Perkel 1999).

Recent data indicate that bursts from individual RA-projecting HVC neurons (HVC-RA) are locked to specific times in the motif, both during syllables and the gaps of silence between syllables (Hahnloser et al. 2002). The activity in RA appears to be specific for sub-syllablic units (notes) regardless of the syllable in which they appear. Cooling the HVC was found to slow down the speed of the song across all timescales by up to 45 per cent while the acoustic structure remain similar, whereas cooling the downstream motor nucleus RA has no observable effect on song timing (Long & Fee 2008).

All the song control nuclei are bilateral – there is a song system in each hemispheres (Figure 1.5) and each song system projects ipsilaterally to one side of the syrinx. How are the two motor programs coordinated during singing? Since birds lack a corpus collosum, no connections exist across the midline to integrate activity at the forebrain level. This implies that song is actually under the simultaneous control of two separate descending motor pathways that only converge below the level of RA. This need for temporal precision with which song is produced (Cooper & Goller 2006; Glaze & Troyer 2006) arguably necessitates temporal precision at the neural level. In particular, given the presence of two descending pathways controlling song output, it suggests a need for precise coordination of activity across hemispheres.

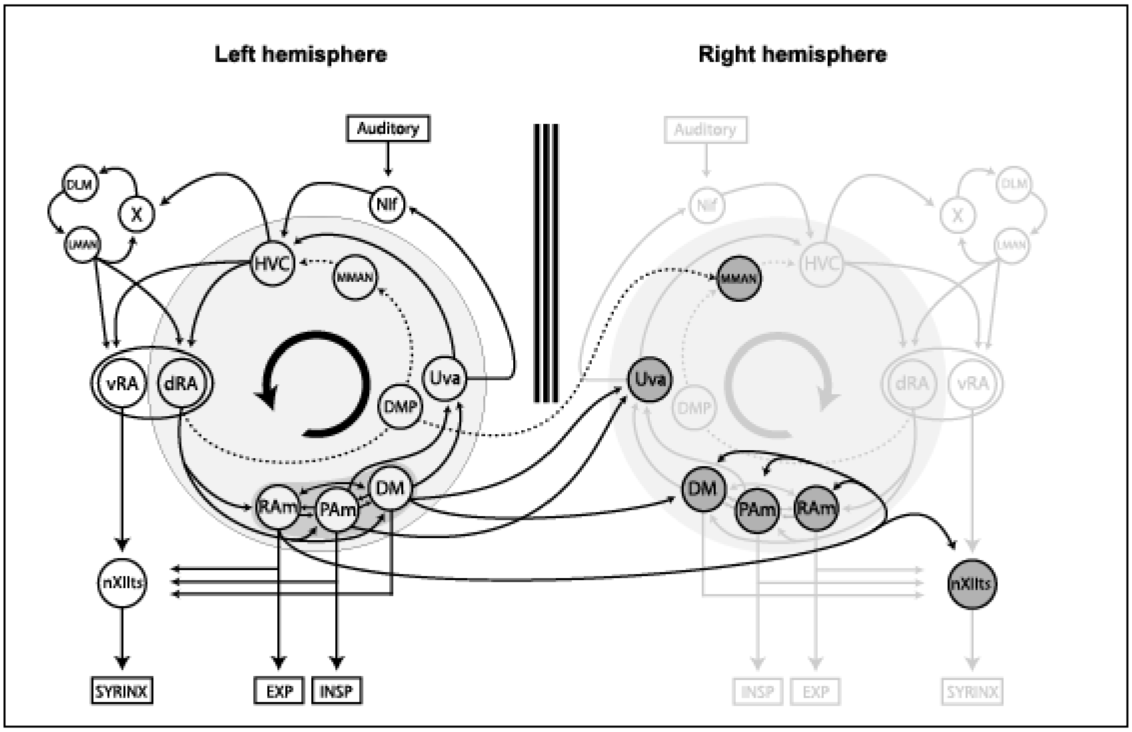


Figure 1.5 Diagram of the avian song system emphasizing its bilateral organization and the bilateral projections from the brainstem to the forebrain. Highlighted in gray is the portion of the song system that is thought to be involved in song pattern generation (the motor pathway). This loop consists of the forebrain nucleus HVC, the dorsal portion of RA (dRA), the vocal-respiratory network (highlighted in darker gray), and the thalamic nucleus uvaformis (Uva). The vocal-respiratory network is made up of nucleus retroambigualis (RAm), nucleus paraambigualus (PAm), and the dorsomedial nucleus of the intercollicular complex (DM). The circuit known as the anterior forebrain pathway (AFP), which is made up of Area X, DLM, and LMAN is not necessary for song production but plays an important role in song learning and maintenance. The three parallel lines between hemispheres illustrate the lack of commissural connections between forebrain song control nuclei. Many of the nuclei in the vocal motor system (DMP, RAm, PAm, and DM), however, project directly or indirectly to vocal motor nuclei in the contralateral hemisphere. Although these projections are bilateral, I only illustrate projections from the left to the right hemisphere for simplicity. Nuclei receiving contralateral inputs are highlighted in dark grey. The anatomical connections shown in this diagram represent the major projections in the song system. Weak projections have been left out. (Figure copied with permission from Schmidt et al. 2004)

The concept of rhythm

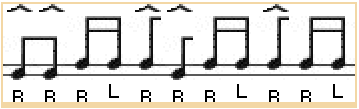
In the case of the song bird zebra finch, which sings again and again a single motif, we perceive as “main rhythm unit” the repeating acoustic alternations of the motif.

The notes reoccur every time the motif is sung. Hence the rhythm we perceive has the periodicity of the motif.

The popular concept of rhythm is not well defined. Olivier Messiaen once said "Most people believe that rhythm means the regular values of a military march. Whereas, in fact, rhythm is an unequal element, following fluctuations, like the waves of the sea, like the noise of the wind, like the shape of tree branches." In the Miriam-Webster dictionary “rhythm” has the following definitions: An ordered recurrent alternation of strong and weak elements in the flow of sound and silence in speech; The aspect of music comprising all the elements (as accent, meter, and tempo) that relate to forward movement; The movement, fluctuation, or variation marked by the regular recurrence or natural flow of related elements. And, the regularly recurrent quantitative change in a variable biological process (for example a circadian rhythm).

Rhythms in music

In music rhythm is the musical time. Musical rhythm is usually calculated by the relative duration of the sounds. To identify the rhythm, we first identify the "size" of each measure (bar). The measure is a segment of time defined as a given number of beats of a given duration. The regular pulsations of the music are called the beat. Measures of music divide a piece into time-counted segments. Strong beats (referred to as "accented" beats) occur in patterns. For instance, in 4/4 time, the conductor would beat a strong beat on the first beat of every measure and another accented beat - although not as strong - on the third count of the measure. Because the conductor's arms move downward on strong beats, especially those that begin a measure, accented beats are also referred to as "downbeats" (Kamien 1999, Backus 1977). Another example is the middle eastern rhythm “baladii” which has a time signature of 4/4 and goes like this:



This would be repeated (with variation) through a whole tune, or sometimes just played by itself as an accompaniment to dancing.

Note that the popular use of the word beat is different! In popular use, it often means the tempo of a piece or a particular sequence of individual beats, the meter, rhythm or groove (Kamien 1999, Backus 1977). Along with rhythm comes the idea of rate or pace. Tempo is the musical term that indicates the overall pace of an arrangement. Tempo markings include grave which means extremely slow or allegro which means fast.

An *ostinato* (derived from Italian: "stubborn") is a motif or a phrase that is persistently repeated in the same musical voice. The repeating idea may be a rhythmic pattern, part of a tune, or a complete melody. An *ostinato* happens in context. For example - in a piano piece, the right hand plays a melody, and the left hand plays the repeating element (the ostinato) (Kamien 1999, Backus 1977).

A famous short piece of *ostinato* can be found in the theme music to the movie *Jaws* composed by John Williams. It uses the two notes in the bass section of the scale, repeated in various tempos to express the different activities of the killer shark. Electronic music, especially that of the dance variety, relies on ostinato-like elements that repeat as the basis of a piece underneath variations. In classical music, examples of ostinatos can be found in the basso continuo part from Pachelbel's Canon in D, the military 5/4 rhythm in Gustav Holst's "Mars" from The Planets and the Confutatis movement of Mozart's Requiem. In Indian Classical Music, during Tabla or Pakhawaj solo performances, a conceptually similar melodic pattern known as the Lehara (sometimes spelt Lehra) or Nagma is played repeatedly throughout the performance. The basic idea of the lehara is to provide a steady melodious framework for rhythmic improvisations (Kamien 1999, Backus 1977).

A melody, also called tune, voice, or line is a succession of musical tones which is perceived as a single entity, i.e. a sequence of pitches and durations. Melodies often consist of one or more musical motifs, and are usually repeated throughout a song in various forms. Melodies may be described by their melodic motion, their pitches or the intervals between pitches (Kamien 1999, Backus 1977).

Different musical styles use melody in different ways. For example Jazz musicians use the melody line, called the "lead" or "head", as a starting point for improvisation. Rock music, melodic music, and other forms of popular music and folk music tend to pick one or two melodies (verse and chorus) and stick with them. The phrasing and lyric of the songs might change. Indian classical music relies heavily on melody. In western classical music, composers often introduce an initial melody, or theme, and then create variations. Often melodies are constructed from motifs or short melodic fragments, such as the opening of Beethoven's Fifth Symphony (Kamien 1999, Backus 1977).

A recent paper calculates rhythms by segmenting sonograms of human music into a binary signal (pulse train), where 1 means that the energy of the music is higher than a threshold, and then uses a wavelet transformation to study rhythms on different time scales (Cheng et al. 2009). The wavelet approach facilitates understanding of rhythms across multiple time scales- i.e, it's a multi dimension (time scale) approach. This multi dimension approach conveys a lot of information, but it is hard to interpret. When analyzing the zebra finch's song development, we wanted to reduce the complexity of the analysis and focus on one aspect in our rhythm analysis- the repeating unit of the Zebra Finch's song- the motif.

Rhythms in biology

Unlike the definition of rhythms in music, rhythms in biology span longer timescales. In biology, rhythm is defined as cyclic pattern of a biological process. Rhythms that vary according to the time of day are called circadian rhythms or diurnal rhythms. Examples of such rhythms are the opening and closing of flowers or, in humans, the changes in body temperature, blood pressure, and urine production during the day. Physiological rhythms are also present in the activity of individual organs, e.g., the beating of heart muscle and the activity of electrical waves of the brain. (Luce 1971; Brady 1982; Glass & Mackey 1988).

One can say that the duration of the zebra finch's motif is its biological rhythm since the relevant biological process is the neuronal firing pattern of the rhythm generator. In the next chapter we will present a method for calculating the zebra finch rhythms, but first, I would like to examine traditional ways of calculating biological rhythms.

Circadian activity rhythms can be measured by activity maps of individuals (Sarty 2006). Activity maps are raster plots of the individual's activity. In this dissertation we will utilize raster plots to explain song development. However, the disadvantage of raster plots is the need to align the data on a certain temporal marker. It is hard to identify a consistent temporal marker in the juvenile bird. During development, that temporal marker might change, and hence alignment will be compromised. Furthermore, if we have a periodic structure (syllable or note) that is "jittered" with respect to temporal alignment marker, raster plots will be affected. Such "onset-unlocked" periodic structure is likely to be present in the song during development while song rhythm has not crystallized. Aniruddh Patel who discovered spontaneous synchronization (entrainment) to music in the sulphur-crested cockatoo (Cacatua galerita eleanora) measured rhythms by comparing the timing of head bobs of sulphur-crested cockatoos to the timing of the musical beats (Patel 2008). We could apply this technique on the zebra finch song by identifying temporal markers or meaningful song segments. However, segmentation and syllable prototype are not robust in early song. One of the factors which makes segmentation at a young age difficult is that the juvenile bird's song is variable and silence gaps are not consistent. Therefore, we often see merging or truncation of syllables at those ages.

In song birds, Franz Goller and Brent Cooper researched the social-context-dependent changes in the rhythm of the song motor program. They measured rhythms according to the duration of the expiratory and inspiratory pulses of the air sac pressure (Cooper & Goller 2006). However, this measurement of rhythm does not provide an insight into the periodicity of the rhythm, i.e. the stereotypy and repetitions of the motif.

Central pattern generators

In this dissertation we will try to improve our understanding of the rhythm generation in the song system. The simplest to understand rhythm generator is the Central pattern generator (CPG). Could a central pattern generator generate the Zebra Finch rhythm? Central pattern generators are neural networks that can endogenously (i.e. without rhythmic sensory or central input) produce rhythmic patterned outputs; these networks underlie the production of most rhythmic motor patterns (Graham-Brown 1911, Marder & Calabrese 1996, Stein et al. 1997). The first evidence that rhythmic motor patterns are centrally generated was the demonstration that the locust nervous system, when isolated from the animal, could produce rhythmic output resembling that observed during flight (Wilson 1961, Marder & Calabrese 1996). Subsequent work showed that, in a wide variety of animals, nervous systems isolated from sensory feedback could produce rhythmic outputs resembling those observed during rhythmic motor pattern production, e.g., leech heartbeat rhythm generator (Olson et al., 1995) or movements of the pylorus (most posterior region of the stomach) in decapod crustacea such as lobsters and crabs (Harris-Warrick et al., 1992).

As animals mature, there are changes in the rhythmic motor patterns they express. For instance, tadpoles swim, but frogs hop; chicks hatch, but then walk; humans crawl, then walk, then run. Evidence in vertebrates suggests that:

(1) CPG development does not require movement-induced sensory feedback, or even muscle innervations (Windhorst 2007);

(2) Later rhythmic motor patterns come about by modification of the CPGs that generated earlier patterns (Windhorst 2007) ;

(3) The ability to produce motor patterns that are expressed at only one developmental stage (e.g. hatching) is not lost as the animal matures, but can be re-induced by applying the proper sensory input at more mature stages (Sillar, 1996, Casasnovas and Meyrand 1995).

The zebra finch song has a clear rhythmic property because of the repeating nature of the song. It evolves and changes according to the environment and depends on sensory feedback (a zebra finch song accelerates when it sings directed song to a female) therefore it is not a CPG. Throughout the bird's development its rhythm changes; we do not know how the new rhythm is acquired, nor if the transition from one rhythm to another is random or constrained in some way.

Rhythm generators

Two forebrain nuclei in particular have been implicated in the control of the temporal structure of song: HVC and RA. The HVC cooling experiment (Long & Fee 2008) suggest that the dynamics underlying the sparse sequential activation of HVC neurons reside at least partly within HVC (Long & Fee 2008). Additionally, portions of the midbrain and respiratory areas project back to HVC through thalamic nucleus Uvaeformis (Uva) (Striedter & Vu 1998, Ashmore & Renk 2008), raising the further possibility that syllables, which are tightly linked to respiratory patterning (Goller &

Copper 2004), may be timed by respiratory oscillator circuits (Ashmore et al. 2005; Cooper & Goller 2006).

While the nature of song-related activity in the vocal-respiratory network is presently unknown, sensory information derived from both the lung-air sac system and the syrinx may form functionally important inputs to this network. Pulmonary and syringeal afferents terminate separately in adjacent regions of the parasolitary subnucleus of the nucleus of the solitary tract, which, in turn, projects upon PAm (Wild, 2004). Inputs from the vocal-respiratory network to the forebrain could serve the role of a clock (Williams, 1989; Williams and Vicario, 1993), driving or modulating HVC activity at syllable or sub-syllable intervals. This clock would serve to inform forebrain song control nuclei of the current respiratory state, to maintain a tempo that is consistent with the physical constraints of the respiratory system, and to synchronize the two hemispheres throughout song production (Ashmore et al. 2005).

Review of existing behavioral analysis techniques

This dissertation utilizes existing behavioral analysis techniques previously developed and optimized for song bird research of developmental learning. Developmental learning takes place early in life but its effects may last the entire lifetime of the individual. Developmental learning is difficult to study because the behavioral changes span many time scales: The challenge is to relate the developmental neuronal and peripheral changes to behavioral changes, which can occur within hours as well as across daily cycles of wakefulness and sleep and over developmental stages. The study of developmental song learning in birds provides a unique model system for examining this process in detail.

Vocal changes can be detected in song (or sub-song) even in very early stages of development. For example, the juvenile chaffinch gradually manipulates the acoustic structure of its innate begging calls (Nottebohm 1968) until they become similar to its tutor's syllables. Detailed analysis of vocal changes in the zebra finch uncovered that syllable types observed in the fully developed song can be traced back, often until early development (Liu et al. 2004; Tchernichovski 2004; Saar et al. 2009). For each syllable type, structured trajectories of vocal change can be observed. The analytical challenge is quantifying vocal trajectories.

One measure of our understanding of the song development process is our ability to survey the entire trajectory of development, formulate some hypotheses about the specific process in a given bird and after describing the "current state" of its song structure, attempt to make predictions e.g. what is the bird going to do next? For example, comparing different versions of a syllable in a current production, can we predict, perhaps based on similarity to the template, the direction in which the trajectory of vocal changes will proceed?

If we only look at the time-frequency (spectral) structure of individual songs, the kind of data visualized in the typical sonogram, individual syllables of the juvenile bird are indeed quite unstable in structure. However, analysis of the distribution of syllable features across several minutes of singing reveals stable organized structures (e.g., clusters) even in the early song. In other words, while juvenile song structure appears messy at the millisecond time scale (looking at sonograms), if we expand our time scale to examine feature distributions spanning several minutes we will see significant structure and stability in juvenile songs. Using an appropriate temporal resolution (e.g., in zebra finch development it is hours) the vocal changes leading to the final production of an individualized species-typical song can be studied in real time thus providing an overview of the entire process of developmental learning.

The distribution of syllable features evolves smoothly over time (Tchernuchvski et al. 2004), allowing us to identify distinct vocal changes, and to track their progression in real time. Across birds, we see in distribution plot two categories of vocal change are tracked: the emergence of syllable types and the trajectories of changes within each syllable type. Altogether these vocal changes reduce the difference (error) between the juvenile song and the target song model, but a great deal of the specific vocal changes observed is not related in any simple manner to error reduction. For example, vocal changes that occur during the day, while the bird is "practicing", tend to add structure to the developing song syllables, but vocal changes are also induced by sleep, and those changes tend to deteriorate song structure. Together they cause oscillations in the developmental trajectories, and the magnitude of those oscillations is a good indication of the eventual accuracy of the imitation (Deregnaucourt et al., 2005).

Song development databases

Until recently, data on song development was obtained from birds exposed to either live male tutors or recorded songs under testing conditions which could vary from lab to lab. Singing behavior was sampled sparsely, generating a relatively modest number of samples and inferences about developmental changes based upon inspection of sound spectrograms. However, sonograms of short duration present only a static representation of the dynamic developmental process. They can only capture short-term changes in sound (over time scales of milliseconds), whereas song development is a process that spans multiple time scales ranging from minutes to weeks (Saar et al. 2009).

The development of methods that would facilitate analysis of the distribution of an array of song features over extended periods, require the recording of singing behavior continuously under standardized conditions over the entire course of song development, and require the analysis of the resulting comprehensive, longitudinal datasets. There is a need to:

(a) Record and store large amounts of vocal acoustic data,
(b) Automate analysis of that data,
(c) Display the results of that analysis as visual representations that highlight key features of the development process as they occur over time,
(d) Reduce the high dimensionality of the spectrogram to tractable number of degrees of freedom.

This can be done through the introduction of a small set of features that are extracted from the spectrogram.

To facilitate the acquisition and analysis of the continuous recording of song learning of individual birds, Ofer Tchernichovski and Partha Mitra developed an open source software program with an extension to Matlab that automates much of the data acquisition, feature calculation and database handling.

Each bird vocalization is monitored continuously, and each song bout is automatically identified and recorded. The software then performs spectral analysis of the bout and calculates a set of song features such as pitch, frequency modulation, etc. (Tchernichovski, et al. 2000). The features summarize the acoustic structure of the sound. They chose those features to describe the song because they relate to mechanisms of song production (articulation). Song is generated by vibration of sound producing membranes (the medial tympaniform membrane and the medial labium of the syrinx). Those vibrations have basic characteristics such as: amplitude, period (pitch), regularity. They defined spectral features of the sound that quantify these characteristics, namely: sound amplitude, pitch, and Wiener entropy, Figure 1.6. The change in pitch over time is called frequency modulation (Tchernichovski, et al. 2000).

The next step in song analysis is segmentation of the sound into syllable units. In general, sound segmentation is a difficult task, but quite often segmentation by a simple amplitude threshold gives robust syllable units during development (Tchernichovski et al. 2004). Once syllable segments are detected, they can be summarized by an array of acoustic features. For each of the continuous feature measurements mentioned above, the mean and the variance are computed across the syllable. Together, the mean and the variance of the feature values summarize the structure of each syllable in a simple and biologically intuitive manner (e.g., duration, mean pitch, variance of pitch, etc.).

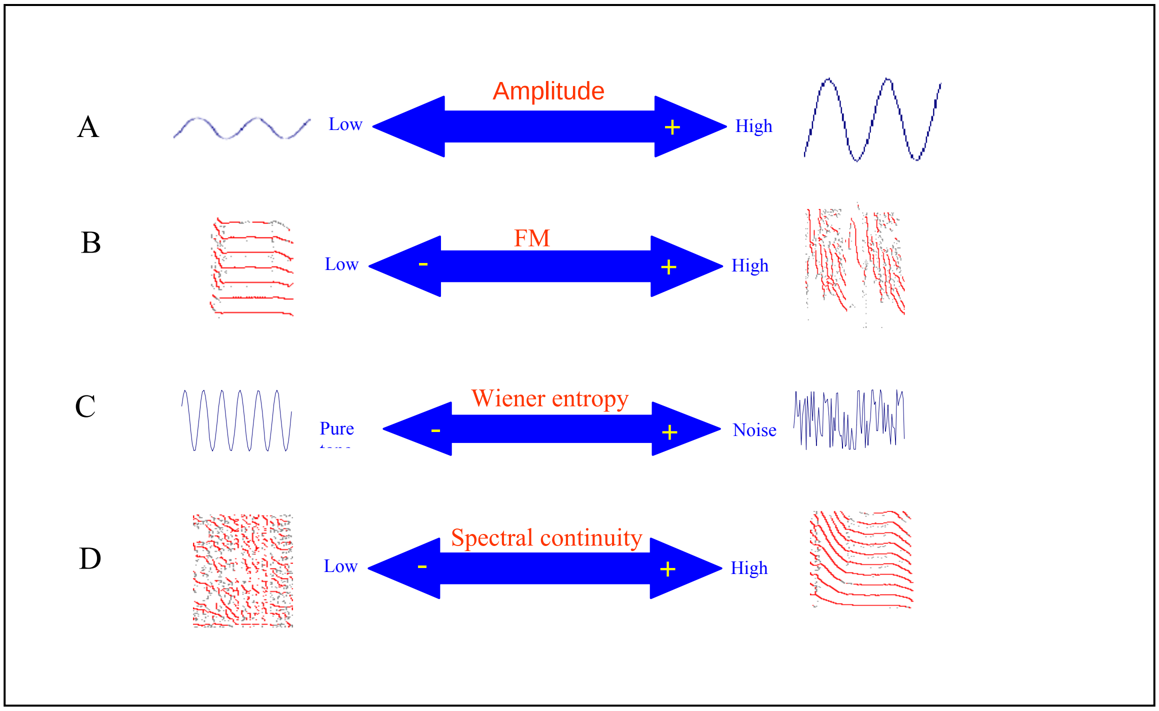


Figure 1.6 Song features. (a) Pitch is a measure of the period of the sound and its value is high when the period is short and low when the period is long. (b) Frequency modulation is a measure of the mean slope of frequency contours. (c) Wiener entropy (a measure of randomness) is high when the waveform is random and low when the waveform is of pure tone. (d) The spectral continuity value is high when the contours are long and low when the contours are short.

Dynamic Vocal Development (DVD) maps:

With the entire vocal ontogeny of a bird on file, tracing vocal changes over time becomes straightforward because one can visualize these changes in a single image that captures changes in features distribution over an entire vocal ontogeny. In this dissertation, I focus on one dimensional developmental histograms based on changes in a single feature, but we often plot two and three dimensional developmental maps. Distribution maps are called “Dynamic Vocal Development (DVD) maps” (Deregnaucourt et al. 2004) because like other maps they represent the distribution of features. However, unlike standard maps which represent the distribution of geographical features in space, DVD maps represent the distribution of acoustic features over time, i.e., they trace the way in which vocal features change over the course of development. DVD maps are composed of a series of single frames each of which represents a cross-section of the status of a feature (such as song duration, or song frequency range) at a specific time during the course of song development (e.g. Day 43). For this reason, such DVD maps are most informative when viewed as movies since the static single frames are transformed into dynamic representations of vocal changes over time.

Tests for song similarity

Because young males learn their songs from older males, a natural phenotypic measure is the extent to which a pupil's song is similar to that of the tutor. In early studies this similarity comparison was done visually. Following segmentation of the song, the best matched note of the tutor's song was identified for each of the pupil's song syllables, and a numerical similarity score was assigned for the match (Scharff & Nottebohm 1991; Tchernichovski & Nottebohm 1998). Such a measure was quite idiosyncratic (albeit accurate), and did not facilitate cross-comparison between results obtained by different laboratories. The automation of feature extraction from spectrograms described above allows doing a more quantitative test for similarity.

Consider the *short timescale similarity matrix* of two songs, defined by taking pair-wise Euclidean distances between features corresponding to different time slices in the songs. The hypothesis of a match between two windows is accepted when a critical similarity threshold is met. The similarity score can be obtained by calculating the probability that the goodness of the match would have occurred by chance. Thus a well defined metric for song comparison is obtained, in contrast to visual inspection of song spectrograms. In this dissertation we will use the measurement % similarity which quantifies what percent of the tutor's sounds included in the pupil's song.

Dissertation outline

When we listen to the zebra finch song it sounds very rhythmic and periodic because of its repeating nature. However, it is not known yet what mechanisms generate the rhythm of the zebra finch song. There are additional uncertainties which brain regions are responsible for the timing of motifs, syllables, and sub-syllabic structure. Furthermore, throughout the bird's development its rhythm changes, we do not know how is the new rhythm acquired, nor if the transition from one rhythm to another is random or constrained in some way.

In order to study rhythm development in songbirds we had to introduce a new concept to bird song research, the quantification of rhythm and periodicity in song structure. By developing a new technique that gives us insights into the song rhythm we have studied how song structure changes at each stage of life.

During the course of a bird's lifespan, zebra finch has four distinct stages of song production:

1. Young juvenile birds (23-90 PHD) are acquiring their song (memorization phase).

2. Young acoustical and socially isolated birds (60-120PHD) at the end of their song's learning period (Postponement of full crystallization).
3. Middle aged birds (120 PHD-4 years) are well beyond their sensitive period for learning, they are sexually mature and sing a stereotyped song (Immelmann 1969).
4. Aged birds (5 years +) are nearing the end of their life.

In the following chapter we utilized rhythm analysis to discover new insights about song and rhythm development in song birds:

Chapter 2: Given the need to understand song rhythms, we developed a technique that measures those rhythms. We present in chapter 2 a description of this method.

Chapter 3: Here we use the dynamic spectrum rhythm analysis on time series of song features to study the development of rhythm in juvenile zebra finches. In juvenile zebra finches we observed two types of rhythm transformations: the first type is a smooth down-modulation of rhythm during several days of song development. The second type is an abrupt transition from one rhythm to a different rhythm. Abrupt and smooth changes in rhythm could relate to different types of changes in neuronal activity which drives those rhythms. Smooth transitions might be caused by a slow shift in the timing of neuronal firing, while abrupt transitions might be caused by reorganization of the rhythm generator exhibited for example by an insertion of a syllable to the repeating unit.(possibly in HVC or in brainstem vocal control centers). Preliminary analysis suggests that the new rhythm is nevertheless related to the previous rhythm. If true, it suggests that only syllables with a specific duration can be integrated into the song, i.e. the zebra finch rhythm is not a side effect (epiphenomenon) syntax development.

Chapter 4: Here we use the dynamic spectrum rhythm analysis to study the development of rhythm in zebra finches at the end of their sensitive period. Zebra finches that are isolated until the end of their sensitive period and then trained do not imitate well. We found that those finches can change the millisecond distribution of their song but those changes do not necessarily improve their imitation. They can modify their syllables and eliminate the production of other syllables. We discovered that their imitation difficulties are caused by their inability to add new syllables to the songs. Zebra finches that sing a long motif before the onset of training, that is, they have more raw materials to work with, were able to imitate better than finches with a short motif. This indicates that at the end of the sensitive period there are developmental constraints on the level of song rhythm.

Chapter 5: We know that in humans there are some speech changes that occur with normal aging and with Parkinson's disease (Calhoun & Eibling 2006, Linville 2001). Carolyn Pytte (Pytte 2007) observed that zebra finches changes are not striking large. However, bengalese finches change their song at late age more than zebra finches. It is important to find an animal model for speech changes at old age so we used the animal model bengalese finch. Changes in song structure with old age have not been explored and compared to those that occur in speech. Therefore, we utilized the rhythm analysis technique to examine songs of bengalese finches that were at least 6 years old, an age approaching the mean life expectancy for this species, and compared their song to middle aged birds (>2 years old). We found that compared to middle aged birds, old bengalese finches have slower song rhythms. This is caused by an increase in the durations of the gaps between the syllables. Also, the ratio of syllable amplitude to call amplitude decreases with age. Unlike the learned song that is produced by the song system (RA, HVC, LMAN), calls are unlearned and produced in the sub-cortical area dorsomedial intercollicular (DM). The decrease in old age of song amplitude compared to call amplitude suggests there is a loss of cortical inputs which are driving the song, i.e. a deterioration of components of the song system at old age.

Chapter 6: We present in this chapter an overall discussion and emphasize the implication of this dissertation.

Chapter 2. A Technique for Characterizing the Development of Rhythms in Bird Song

Introduction

Previous work has shown that song has structure that spans many time scales (Deregnaucourt et al. 2005; Tchernichovski et al. 2004; Deregnaucourt et al. 2004; Saar et al. 2009). Spectral analysis has proven to be a useful tool in analyzing song temporal structure from milliseconds to several seconds. For example, song spectrograms are the basic tool used to characterize the time-frequency structure of individual songs. Timescales that span several minutes can be analyzed by examining the distribution of syllable features. These distributions reveal stable organized structures (e.g., clusters) even in the early song, where the individual spectrograms appear unstructured. Visual examination of spectrograms and syllable clusters across developmental timescales show the existence of longer time scale structures which have been relatively difficult to quantify.

1. Analysis of timescales that span several minutes (syllable level) reveal that song learning is somewhat guided by over-expression which is followed by selection (Immelmann 1969). Furthermore, Immelmann and others (Immelmann 1969, Liu et al. 2004) observed that during the plastic song phase, syllables might appear to be similar to the model syllables, but they are not sung in the right order (this will impact the rhythm of the song). Indeed, we perceive that the rhythm of the juvenile song is different than that of the adult. Since there is no accepted method to measure song rhythms in adult song, let alone juvenile song, which appears unstructured and

unstable, we need to define a metric that will enable us to explore rhythm development.

I show in this chapter that song rhythm can be extracted by computing spectrograms of time series composed of song features, and that the “rhythm spectrogram” provides a useful tool to characterize and visualize song development over the entire ontogenetic trajectory. The strengths of this approach are:

1. Spectral analysis is a systematic way in which periodic or quasi-periodic behavior can be quantified. Zebra finch song will often include additional syllables that are not part of the motif consistently. Rhythm analysis identifies the global periodicity of the song.
2. There is no need for segmentation or identification of syllable prototypes. This is an important advantage because segmentation and syllable prototype are not robust in early song. There are a few factors that add to our inability to segment well. Adult song can be segmented fairly well using an amplitude threshold (Saar et al. 2009). However, the juvenile bird’s song has lower amplitude than the adult bird’s song so we can not segment the songs well with a constant amplitude threshold. There is a need for an adaptive amplitude threshold algorithm to improve the quality of segmentation. However, an adaptive segmentation algorithm is a hard computational problem that was not solved yet. Another factor which makes segmentation at a young age difficult is that the juvenile bird’s song is variable and silence gaps are not consistent. Therefore, we often see merging or truncation of syllables at those ages.

3. Developmental data is abundant; it is so abundant that we are often faced with a problem where we can't see the forest for the trees. Rhythm analysis enables us to summarize an entire song development data into two easy to interpret values; i.e., rhythm analysis reduces the dimensionality of the data. It tells us the duration of the repeating unit in the song (the motif) and how stereotyped that repeating unit is. Furthermore, it helps us identify the important but rare days when the bird modified its song. Therefore, in order to understand song ontogeny one can focus its efforts on those specific significant days when most of the song changes occur.
4. Rhythm analysis is not sensitive to phase shifts in the data of a motif or a bout. Let’s say there is a periodic structure (syllable or note) that is "jittered" with respect to syllable onset, syllable interval analysis will be affected. Spectra analysis is more robust to such jitter. Such "onset-unlocked" periodic structure is likely to be present in the song during development while song rhythm has not crystallized.

There is a pleasing symmetry between the rhythm spectrogram and the song spectrogram, although the latter exhibits the dynamics of the syringeal apparatus and the song system, while the former exhibits developmental dynamics. The methods described here are available in the form of MATLAB code distributed as part of the freely available Sound Analysis software packages http://ofer.sci.ccny.cuny.edu:2001/html/sound_analysis.html and Chronux http://www.chronux.org/ .

Software and databases

All the analysis was performed using Matlab 7 and MYSQL.

Glossary of Terms and Units of Analysis

Previously we defined a motif as a regularly repeated and individually stereotyped sequence of distinct syllables and intervals. In this chapter we will refer the **motif duration** as combined duration of all syllables and silences in a motif, including the silent interval before the first syllable as measured in a song with more than one motif (Figure 2.1)

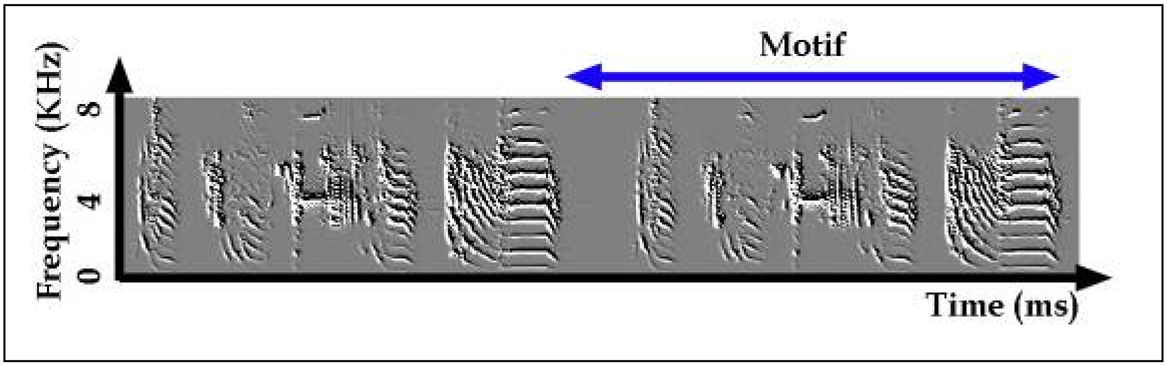


Figure 2.1 An example of a bout with two motifs

Spectral analysis

Fourier transformation

We use Fourier transformation to transform a sound signal from the time domain to the frequency domain. The temporal signal is deciphered into its repeating elements. The cycle of the repeating elements corresponds to its frequency with an inverse relationship. Since we work with digital signals, we use a discrete version of the Fourier transformation called Discrete Fourier transformation (DFT). There are several algorithms to calculate DFT, one of the algorithms is the Fast Fourier transform (FFT). FFT reduces the number of computations needed for a signal with N points from $2N^2$ to $2N\log_2 N$ (Arfken 1985; Percival & Walden 1993). Since the input to the Discrete Fourier transformation is a finite signal, the spectrum will have spectral leakage artifacts. Spectral leakage is an effect in the frequency analysis of finite-length signals where it appears as if some energy has "leaked" out of the original signal spectrum into other frequencies. To reduce the spectral leakage, we can use a window function (also known as a tapering function). The window function is shaped to reduce the spectral leakage and it has a zero-value outside of some chosen interval. We multiply the signal by the window function before applying the DFT to reduce spectral leakage. A sliding window algorithm tapers a small portion of the data and than shifted (usually with overlap) and another segment of the vector is tapered.

Short Term Fourier transformation

Short-Term Fourier transform (STFT) is used to examine the spectral properties of a signal that changes over time. This is done by using a sliding window. Each column of the STFT is the spectrum of one sliding window. The next column is the spectrum of the subsequent sliding window. The result is a graph where the horizontal axis represents time, the vertical axis is frequency; a third dimension indicating the amplitude of a particular frequency at a particular time is represented by the intensity or color of each point in the image (Percival & Walden 1993). Song spectrograms are calculated using STFT.

Thompson's Multitaper Method (MTM)

We make use of Thompson's multi-taper framework of spectral analysis (Thomson 1982; Percival & Walden 1993). MTM uses a bank of windows (Slepian sequences) to compute the spectrum. Those windows are optimal in reducing the spectral leakage.

Rhythm analysis

The song structure may be summarized using a set of song features such as the amplitude, pitch, mean frequency, amplitude modulation, frequency modulation, continuity in time, and continuity in frequency (see chapter 1). The features summarize the acoustic structure of the song. The spectrogram, i.e., the short-time spectrum computed with a sliding window, has proven in the past to be a good way of looking at the fine temporal structure of songs (Thernichovski et al., 2000). The duration of the short-time sliding window is on the order of 10msec; hence it reveals temporal structure at the millisecond timescale. When we compute the spectrum of that short-time sliding window the spectrum shows power for up to several kHz (Figure 2.2 A). Analysis of song features has shown temporal structure dynamics in the song over longer timescales, including circadian oscillations and developmental song dynamics (Deregnaucourt et al. 2005).

To look at longer time scales, we use a nested spectral analysis method. First, song feature time series are estimated. The feature values at a given time point depend on the spectrogram's value at that time point. The feature time series are subjected to a second spectral analysis, and the result is a "rhythm" spectrogram (Figure 2.2 B). In the rhythm spectrogram, the fundamental frequency (that was defined as pitch in a normal spectrogram) is in the range of a few Hz instead of kHz in the song spectrogram.

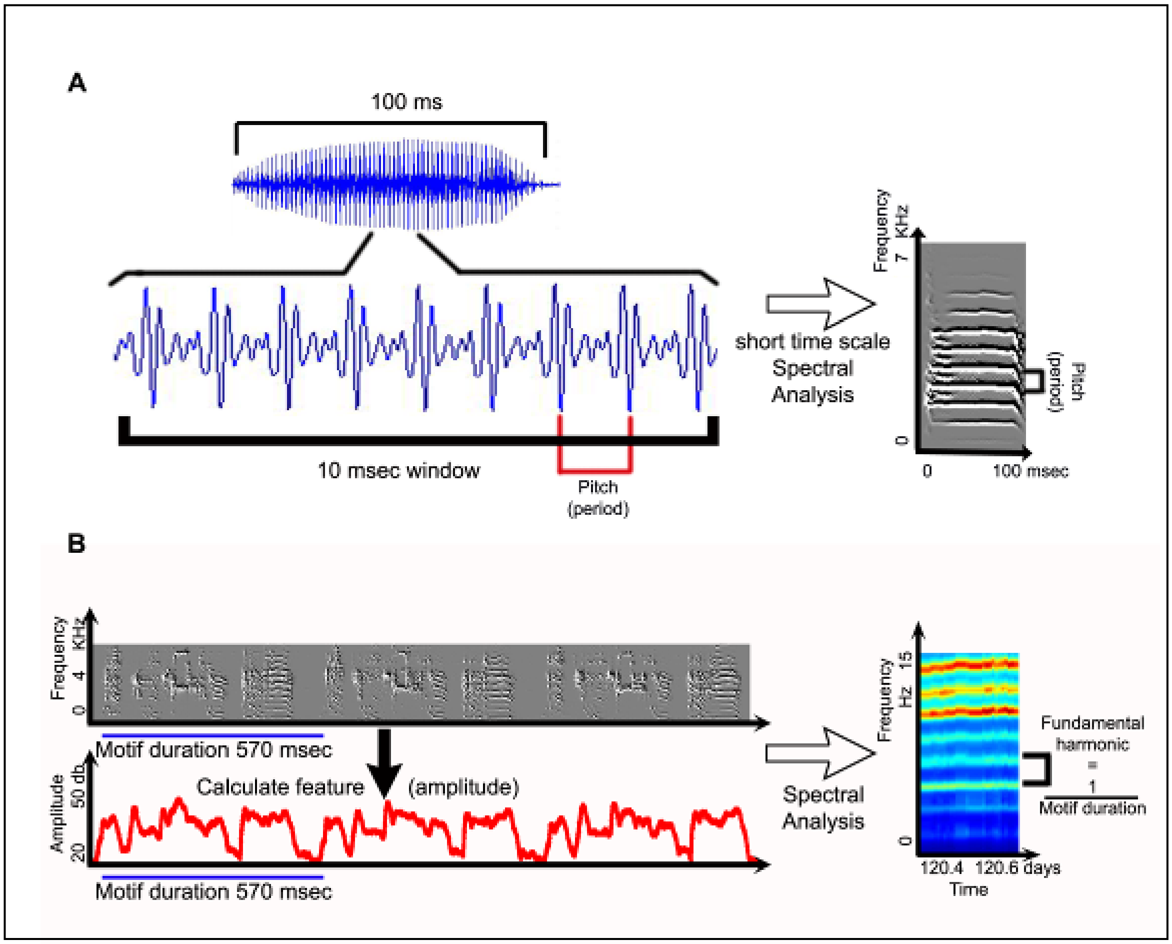


Figure 2.2 Rhythm spectrograms. A. A regular song spectrogram using a 10msec sliding window, shows power up to several kHz. B. Rhythm spectrograms display longer time scales. They are computed by estimating the dynamic spectrum of an appropriate song feature (amplitude in the above example). Each column of the rhythm spectrogram represents the average spectrum of song features sung during a sliding window interval of 2 hours. There is an overlap of one hour between the windows.

We were interested in long time scales on the order of an hour because that a long enough time to look at sufficient amount of time but still gives us resolution to look at diurnal effects, e.g. each column in the rhythm spectrogram would correspond to an hour of singing. A time interval of an hour has many bouts of song followed by silent intervals. The analysis is carried out by first cleaning an interval of song data (i.e. an hour of singing) from background noise. To clean the data interval we used a very low amplitude threshold that was just above noise level. The threshold levels were chosen manually according to the recording quality. Song bouts were identified by long gaps of silences. We subtracted the mean of the data to reduce the DC (the power at f=0) which can be high but does not convey meaningful information. We then performed spectral analysis on the feature time series which contained a constant number of song bouts. All the spectra obtained for a single data interval (e.g. an hour) were then averaged to reduce spectral noise. In this dissertation we used sliding windows. The sliding window data interval was two hours and there was an overlap of one hour. In addition to the plain FFT of the data we often looked at the high pass to see the spectrum in higher frequencies. For example, we passed the time series through a simple diff function (x(n+1)-x(n)) which produces a coarse high pass filter in the frequency domain.

From the rhythm spectrogram, we can derive second order features such as rhythm pitch and entropy (Figure 1.6). In the zebra finch, since the main repeating unit is the motif, the fundamental of the rhythm spectrum is expected to relate to the motif duration. The degree of periodicity of the rhythm may be assessed in the same way as for the regular song spectrum, using the amplitude and width of the corresponding spectral peaks and the Wiener entropy.

A flowchart of the procedure is shown in Figure 2.3. The spectrum of the song waveform x(t) is computed to get the song spectrogram S(f,t), or a derivative of that spectrogram (Tchernichovski et al. 2001). A feature time series F(t) is derived from the spectrogram to get a coarser time scale representation of the song and subjected to a second round of spectral analysis. The result is a “Rhythm” spectrogram $S_R(f,t)$ which shows the temporal structure on longer time scales. Second level features may be derived from the “Rhythm” spectrograms (e.g. the song rhythm as defined by the fundamental frequency if the spectrogram shows a harmonic structure or rhythm entropy (calculated just like regular entropy, Figure 1.6) which quantifies the stereotypy of the repeating unit). Those features reduce the dimensionality of the rhythm spectrograms.

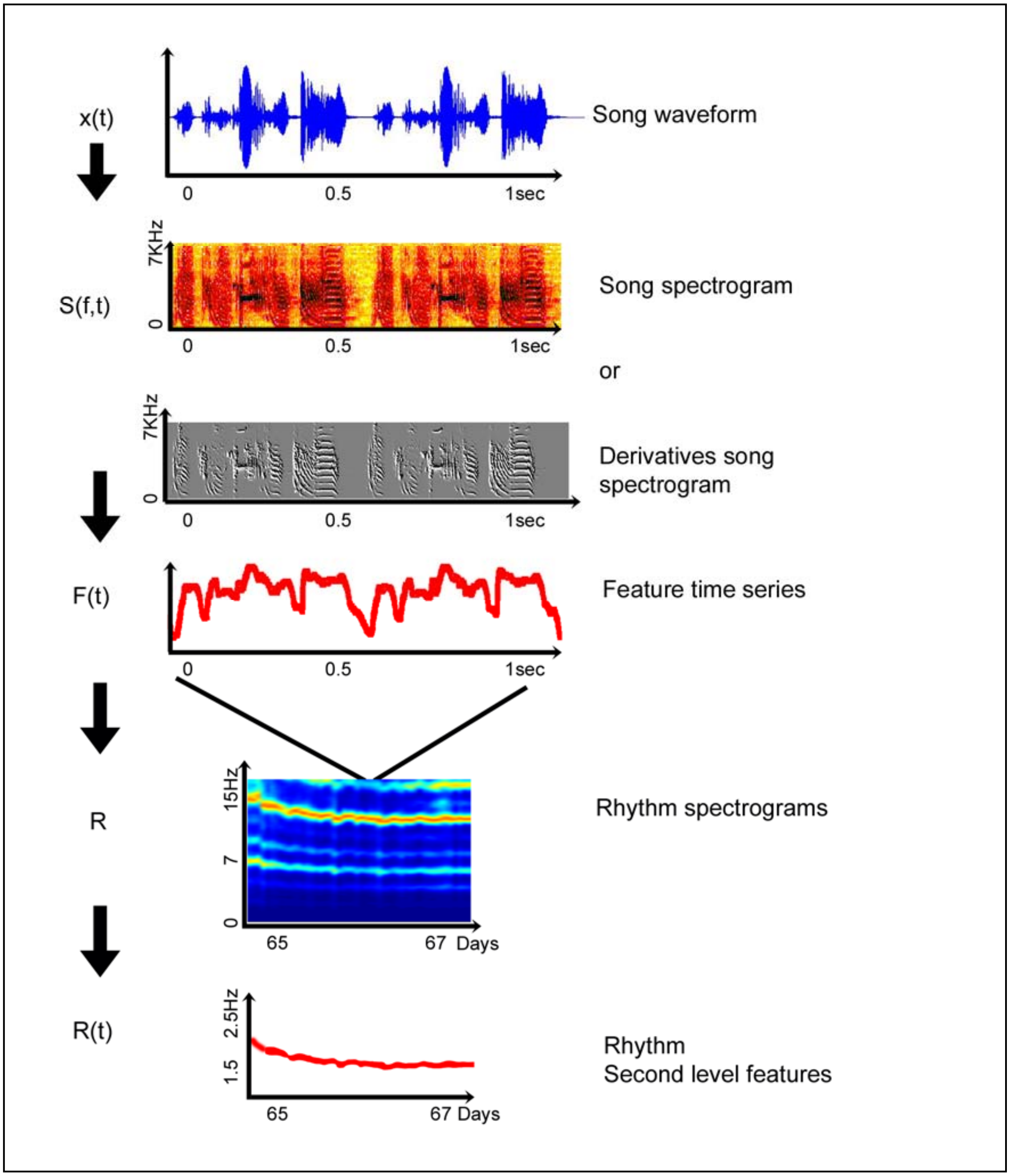


Figure 2.3 A flowchart of the nested spectral analysis. The spectrum of the song waveform x(t) is computed to get the song spectrogram S(f,t), or a derivative of that spectrogram (Tchernichovski et al. 2001). A feature time series F(t) is derived from the spectrogram to get a coarser time scale representation of the song and subjected to a second round of spectral analysis. The result is a "Rhythm" spectrogram $S_R(f,t)$ which shows the temporal structure on longer time scales. Second level features may be derived from the "Rhythm" spectrograms (e.g. the song rhythm as defined by the fundamental frequency if the spectrogram shows a harmonic structure or rhythm entropy which quantifies the stereotypy of the repeating unit). Those features reduce the dimensionality of the rhythm spectrograms.

We developed a semi automated technique to extract the fundamental frequency of the harmonic structure (rhythm) from the rhythm maps. The fundamental frequency corresponds to the duration of the repeating unit, i.e. the song motif. The method is based on:

A. Because song features are not perfect sinusoid they will form a harmonic,

B. The observation that except for a limited number of abrupt transitions during development the fundamental frequency is continuous during development, within the diurnal oscillation boundaries

C. The assumption that the number of harmonics is limited to 2 dimensions. I.e., there are fewer than 2 different repeating units. This is a reasonable assumption since we did not observe more than two possible repeating appearing together in an hour worth of singing. In song ontogeny this corresponds to out observation of up to two repeating unit types of motives. This occurs when a new syllable is added. One repeating unit is the original motif and the other is the new longer motif

For each data interval window (two hours of singing) we used a sampling function (a set of equally spaced impulses, impulse train or a Dirac comb**)**, Figure 2.4. We summed the energy of the samples, and normalized the sum by the number of harmonics. We calculated the Dirac comb for sampling intervals from close to zero HZ (DC) to 30Hz.

Since there could be a number of harmonics, which means there are a number of repeating units, we used an n dimensional matrix (n can be defined by the user, in zebra finches we observe up to 2 harmonics. This makes since because we observed only up to two repeating unit types of motives. This occurs when a new syllable is added. One repeating unit is the original motif and the other is the new longer motif. Hence we used n=2) that includes all the combination of harmonic sets. A user visually inspects the adult song spectrum and the multidimensional matrix. He chooses a possible frequency interval for the harmonic sets. Within those boundaries, the algorithm finds the combination of harmonics with the highest energy. Then the algorithm continuously tracks the maximum power in preceding rhythm windows. If the change in the harmonic frequencies is large or if the entropy of the rhythm is high, the program stops and wait for the user to chose another harmonic frequency boundary. This technique only works when rhythm entropy is sufficiently low. Therefore we ran it only when in low entropy cases.

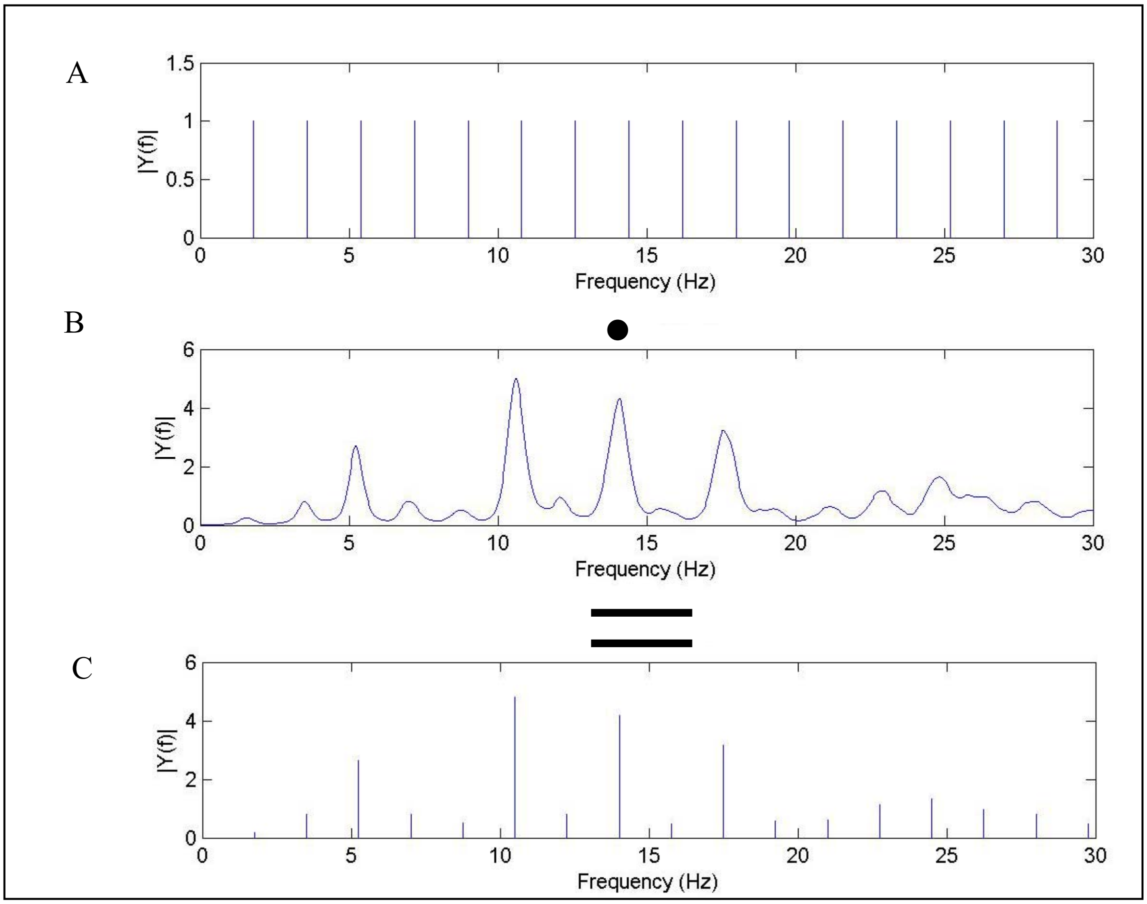


Figure 2.4 Calculation of the energy in a 1.75Hz harmonic. A. We used a sampling comb with an interval of 1.75 Hz B. Rhythm spectrum. C. The result of the sampling comb.

Simulations of rhythms in time and frequency domain

To better illustrate our results we would like to show simulations of different periodic structures and their (rhythms) transformation to the frequency domain[1]. Signals that are fully localized in time (e.g., a spike) spread all over the frequency domain (becoming white). Signals that are fully localized at the frequency domain (a frequency peak in the spectrum) spread over time to infinity (an infinite sine wave). Therefore, some signals can be better understood when observed in frequency domain and others in time domain and so it makes sense to combine the two representations – which is what we do when we plot a sonogram.

In the case of a pure sinusoid, all the energy is centered in one frequency and we see a sharp peak in the power spectrum (Figure 2.5). An infinite white noise signal in the time domain transforms to a flat power spectral density in the frequency domain. However, since our vector is not infinite it will add white noise to the spectrum. Figure 2.6 A shows the frequency transformation of a uniformly distributed noise with 100K samples. Figure 2.6 B shows the frequency transformation of a uniformly distributed noise with 10K samples. The average power spectrum of the noisy vector with 10K samples is $5\ 10^{-3}$. In comparison, the average power spectrum of the noisy vector with 100K samples is only $5\ 10^{-4}$.

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[1] Note, that the presented signals are a snapshot of a much longer signal used for computing spectra and that the time series average was set to zero (the DC was removed) prior to spectral analysis.

The zebra finch bout is composed of motives with a silence gap in between them. If we embed gaps of silence with durations uniformly distributed and placed in random locations throughout the noisy 10K samples vector the result is still a noisy spectrum (Figure 2.6 C). Since the gaps of silence do not have any energy, the average power of the spectral density is lower than the white noise signals without gaps ($4\ 10^{-4}$). Therefore, random white noise in the time domain adds white noise across the frequencies of the spectrum (Figure 2.7). Note, that in Figure 2.7, the signal is composed of two sine waves with different frequencies and white noise. The signal's spectrum has two clear peaks (corresponding to the two sine waves) and a spectral noise of much lower power than the temporal noise.

When we embed gaps of silence with random durations in random locations throughout a pure sinusoid vector, there is a peak in the spectrum which corresponds to the frequency of the sinusoid, however this peak is broader and nosier than the case in Figure 2.5 because it's energy is lower (Figure 2.8). When the peak is broad and noisy the entropy of the spectrum is high.

% silence gap	Spectrum's entropy
0%	-1.23
10%	-0.6
80%	-0.4

Table 2.1 When we embed gaps of silence with random durations in random locations throughout a pure sinusoid vector, the spectral peaks becomes broader and nosier.

The square wave signal transforms into a harmonic stack in the frequency domain (Figure 2.9). The duration of a square wave cycle corresponds to the inverse of the distance between harmonics ($\frac{1}{\text{frequency difference between harmonics}}$) or $\frac{1}{\text{fundamental frequency}}$. A square wave signal contains sharp transitions in the time domain. Those time domain sharp transitions translate in the frequency domain to a series of sinusoids with higher frequencies than the fundamental (a harmonic series). Since the square wave signal is periodic, the sinusoids share that periodicity and are a multiplication of the fundamental cycle.

Harmonics indicate a periodic waveform that is not sinusoidal. The more fine structure the temporal signal has (i.e., fast changes relative to the period length such as in a square wave signal) the stronger the harmonic components. Fewer higher harmonics therefore indicate less fine structure in the presence of a steady period (rhythm). A square wave signal is a gross representation of song features since song features are non-zero during production of syllables and zero otherwise.

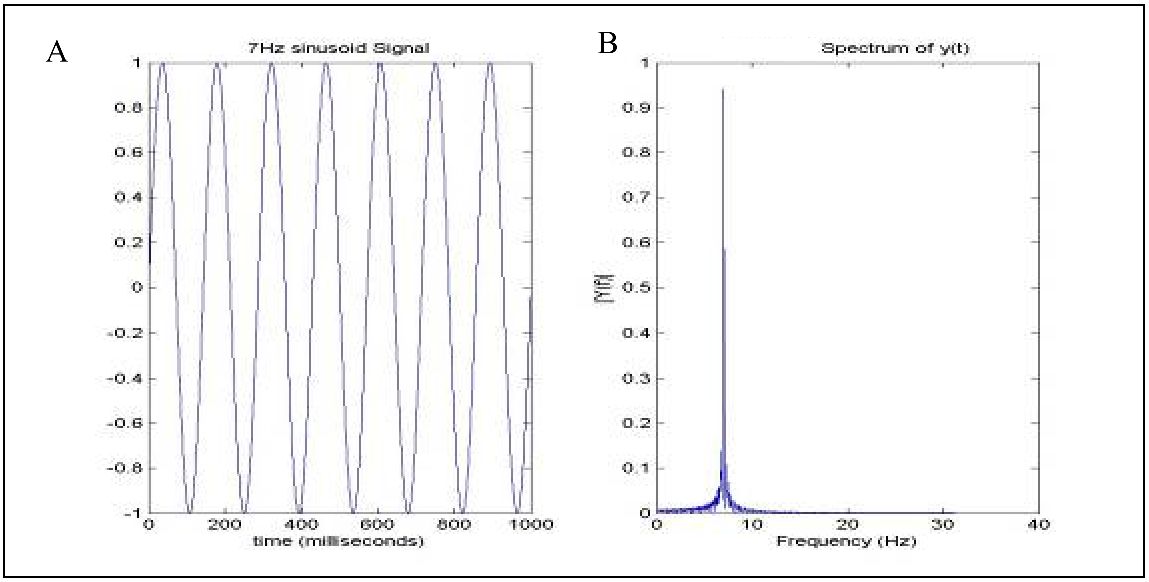


Figure 2.5 A sinusoid in the time domain transforms to a peak in the frequency domain. The 7Hz peak in the frequency domain corresponds to the cycle of the sinusoid.

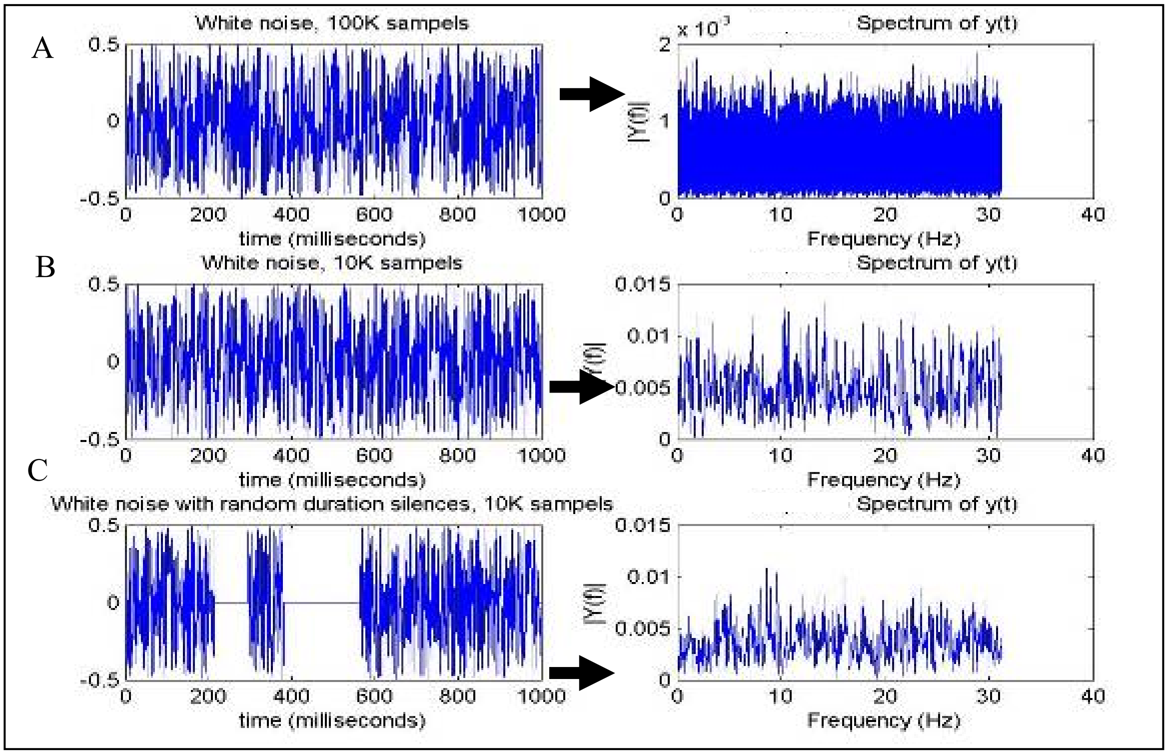


Figure 2.6 An infinite white noise in the time domain transforms to a broad and flat power spectral density in the frequency domain. However, since our signal is not infinite, it adds fluctuations to the spectrum. A. shows the frequency transformation of a uniformly distributed random noise with 100,000 samples. B. shows the frequency transformation of a uniformly distributed random noise with 10,000 samples. The range in values, and the average of the frequency transformation of the 10,000 samples noisy vector is larger than the 100,000 samples noisy vector. C. If we embed gaps of silence with durations uniformly distributed and placed in random locations throughout the noisy 10K samples vector the result is still a noisy spectrum. Since the gaps of silence do not have any energy, the average power of the spectral density is lower than in the previous case.

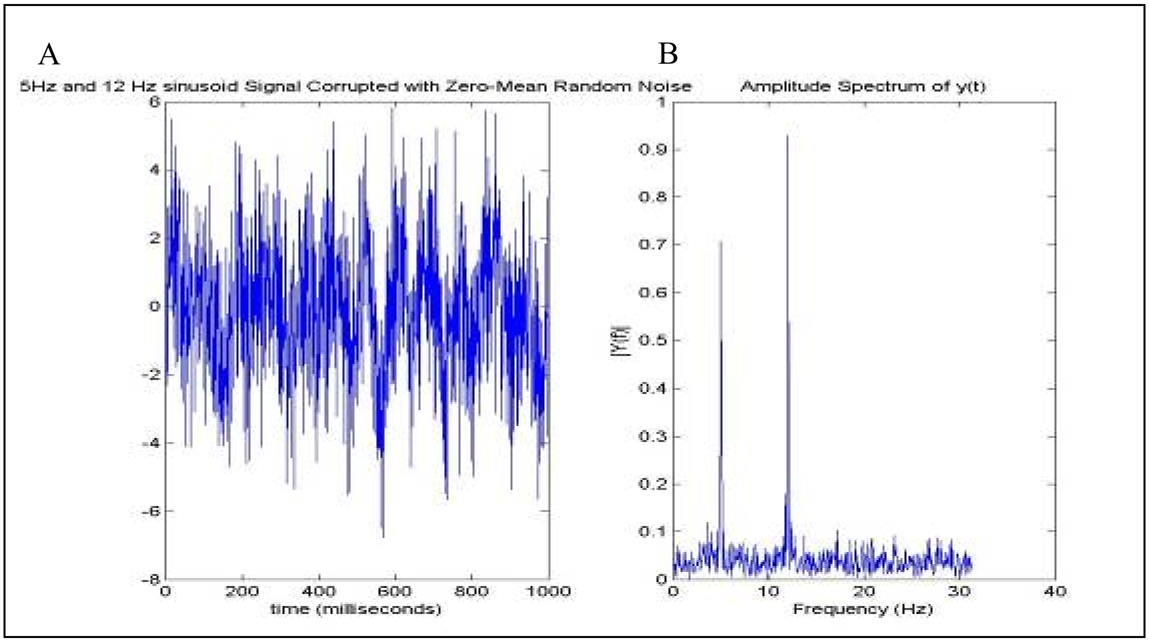


Figure 2.7 Two sinusoids with an added uniformly distributed random noise in the time domain transform to two peaks in the frequency domain. The temporal noise adds frequency noise across the spectrum. Since the energy of the pure sinusoid is centered in one frequency, the peak of the sinusoid is clearly seen in the spectrum. The location of the peaks corresponds to the cycles of the sinusoids (5Hz and 12Hz).

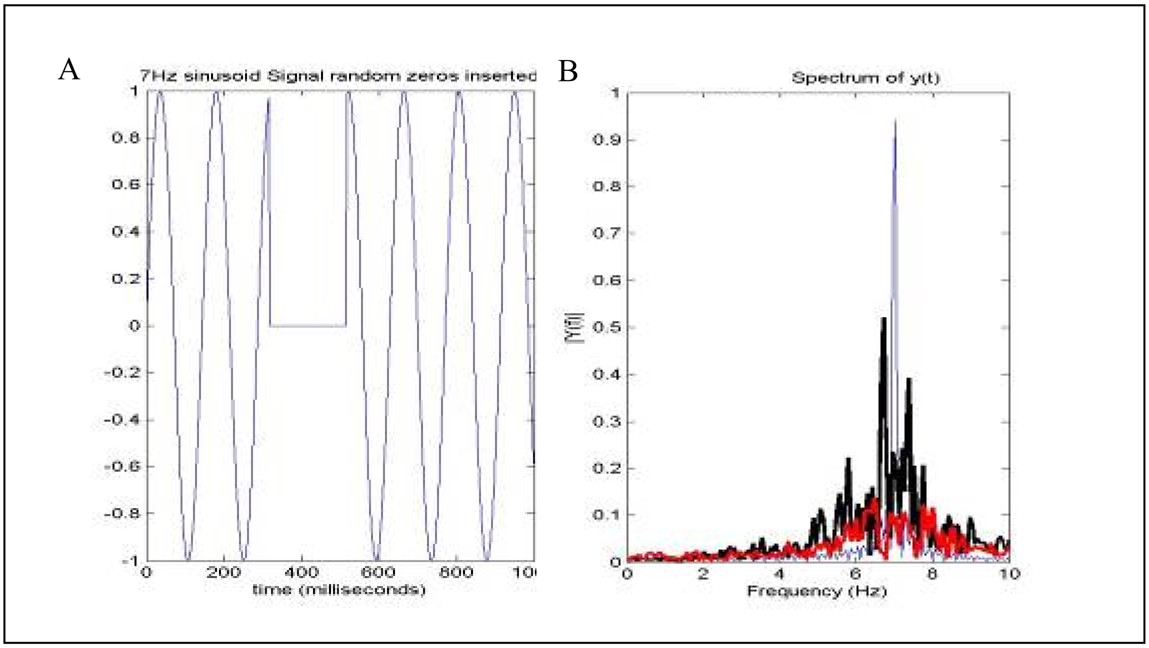


Figure 2.8 Insertion of gaps of silence with random durations and in random locations throughout a pure sinusoid broadens the spectrum peak in the frequency domain and reduces its energy. B. The 7Hz peak in the frequency domain corresponds to the cycle of the sinusoid. Blue spectrum curve corresponds to 0% silence gap (spectrum's entropy= -1.23), black spectrum curve corresponds to 10% silence gap (spectrum's entropy= -0.6) and the red curve corresponds to 80% silence gap (spectrum's entropy= -0.4).

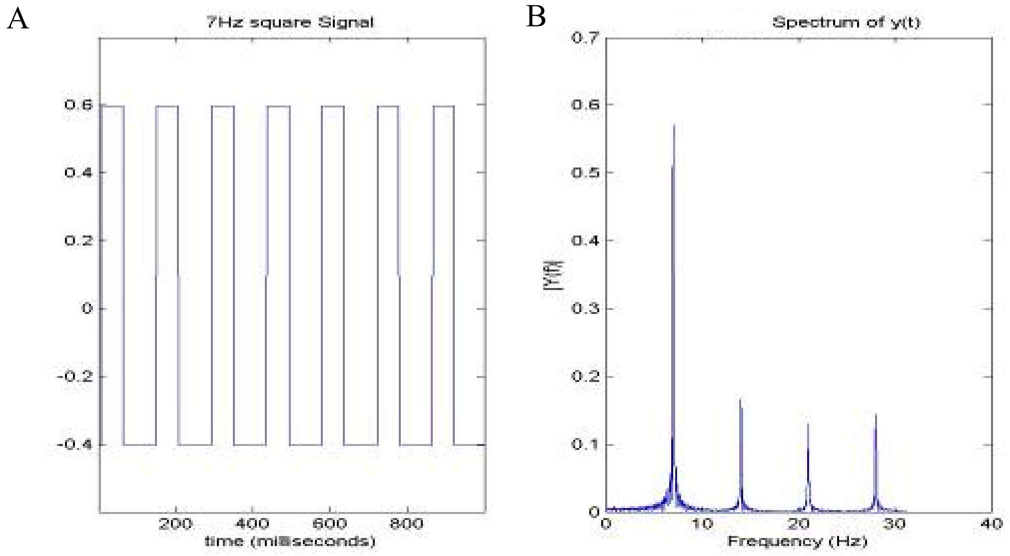


Figure 2.9 A square wave signal is a gross representation of song features since song features are non-zero during production of syllables and zero otherwise. The square wave signal transforms into a harmonic stack in the frequency domain. The 7Hz peak in the frequency domain and the 7Hz gap between the harmonics corresponds to the cycle of the square wave signal.

Can we infer from a harmonic peak in the rhythm map a syllable duration? I will show here that we can not be done. For the sake of simplicity, we generated a repeating complex but stereotyped motif which is composed of uniformly distributed random features and a constant silence gap (Figure 2.10). The frequency transformation of this signal is a harmonic stack, where the distance between the harmonics is equal to the cycle of the motif plus the silence gap. I would like to show a few simulations that explain better the nature of the harmonic stacks that we see in rhythm analysis. When a sinusoid signal, say of 7Hz, that has a different cycle than the motif and the gap is added to the motif, then in the frequency domain the harmonics that are close to the sinusoid cycle (7Hz) increase in power (Figure 2.11). **No additional harmonics are added** and the energy at 7Hz will remain zero. This is the case only when the 7Hz is **“locked to the**

motif". If we add a continuous 7Hz wave to the signal – we will see energy (a peak in the spectrum) in 7Hz (Figure 2.12). Therefore, all the periodic structures that are time-locked to the motif will end up – in Fourier Analysis – as energies distributed within the harmonic frequencies which stem from the motif period. The simplest possible motif, and the most complex possible motif will, in typical cases, have similar number of peaks in the power spectrum, all harmonics of the motif period. Since different song syllables are locked to the motif (the have lower energy than the motif because they are only a part of the entire motif), we saw here that we can infer from a peak in the harmonic the exact duration of the syllable.

If we add to a song that contains the repeating complex but stereotyped motif a continuous sinusoid, say of 2Hz, that is **not** time locked to the motif, even if we keep the song's silence intervals silent, we will see additional peaks in the spectrum. In addition to the 5Hz harmonic stack that originates from the repeating motif units, a new 2Hz harmonic stack will appear in the spectrum. Because the silences are cut out from otherwise continuous sinusoid, this causes sharp transitions in the combined motif-sinusoid signal. Therefore there is a harmonic stack instead of a single peak (Figure 2.13).

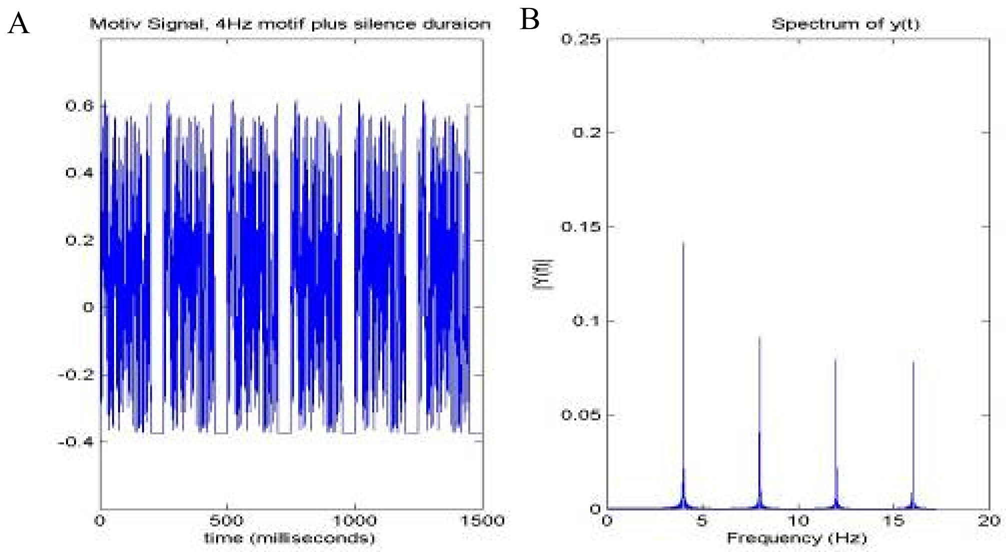


Figure 2.10 A simulated motif which is composed of repeating complex but stereotyped motifs. The stereotyped motif is composed of uniformly distributed random features and a constant silence gap. The frequency transformation of this signal is a harmonic stack, where the distance between the harmonics equals to the cycle of the motif plus the silence gap. Note that all the “noise” (see figure 2.5) is gone – and the complex structure within repeating motifs is fully captured in the harmonics of the motif duration. This caused by the systematic inclusion of the silences in the motif.

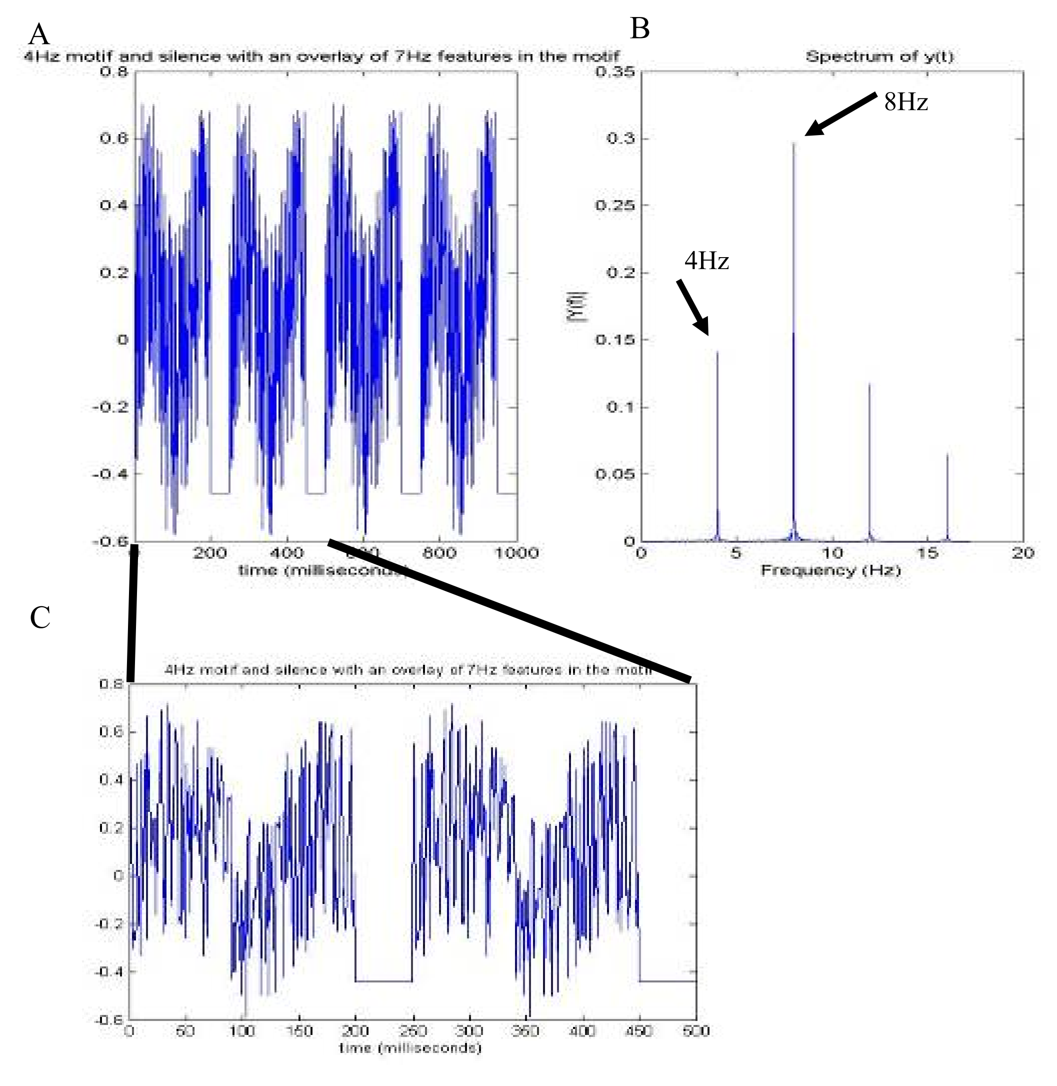


Figure 2.11 A. A sinusoid signal, with a cycle of 7Hz, is time locked to the motif. B. In the frequency domain, the 4Hz and 8Hz harmonics, which are close to the 7 Hz sinusoid cycle, increase their power. No additional harmonics are added. C. A zooms in on the first 500 ms.

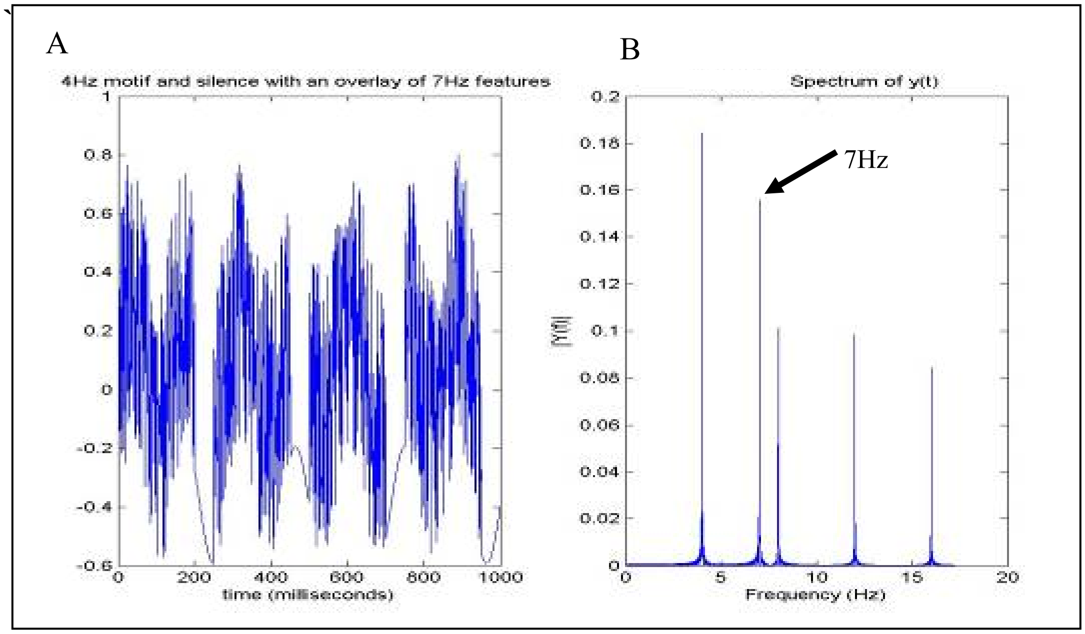


Figure 2.12 A. We added a 7Hz sinusoid signal that is not time locked to the motif (which is composed of uniformly distributed random features and a constant silence gap) B. In addition to the 5Hz harmonic stack there is a 7Hz peak.

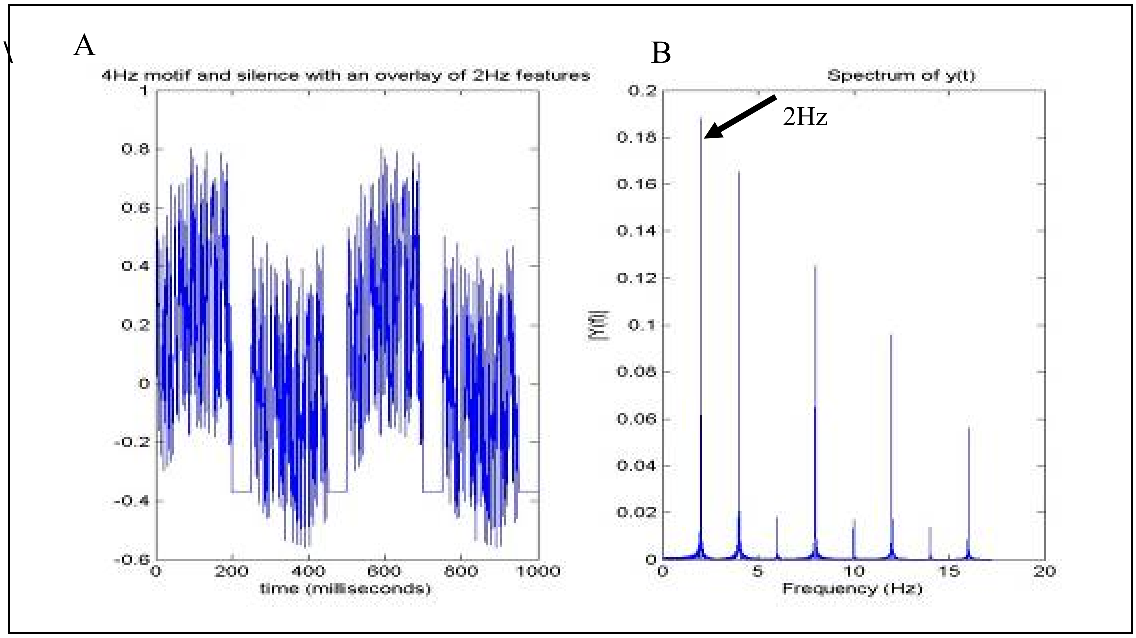


Figure 2.13 If we add to a song that includes a repeating motif of 250ms a continues sinusoid, say of 2Hz, that is not time locked to the motif, and keep the song's silence intervals silent, there will be additional peaks in the spectrum. In addition to the 5Hz harmonic stack that originates from the repeating motif units, there is a 2Hz harmonic stack. The additional harmonic stack is caused by the imperfection of the sinusoid.

When the number of motifs in a bout is random (uniformly distributed between 1-10), the silence between the motifs is random (normally distributed) and the gap between the bouts is constant, then the spectrum will show a strong harmonic for the motif frequency, and a wide low peak for the variable bout duration. Figure 2.14 and 2.15 show this example. The motif frequency (4Hz) is clearly seen in the spectrum. The frequency of the peaks is: 3.9649 8.1053 12.0351 16.0351 Hz. The bout's average period is 2818ms (0.35 Hz) with a std of 1442 and the first wide peak in the spectrum is at 0.43 Hz.

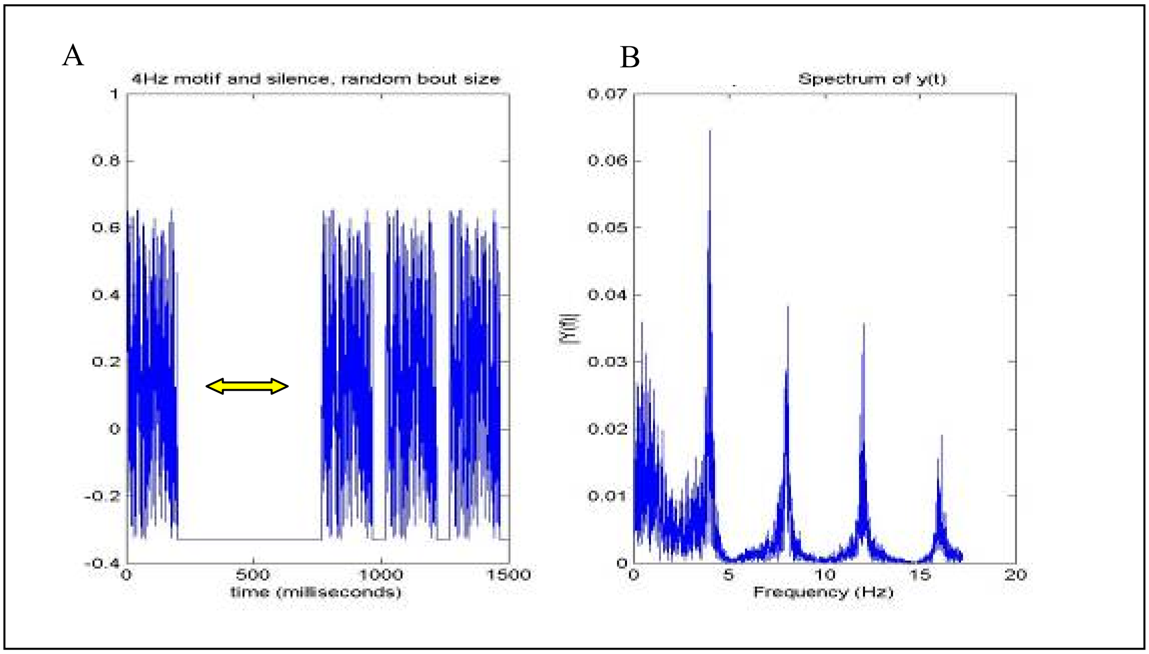


Figure 2.14 When the number of motifs in a bout is random (uniformly distributed), the silence between the motifs is constant, and the gap between the bouts is variable in its duration, with a normal distribution, then the spectrum will show a strong harmonic for the motif frequency, and a strong peak for the average bout duration. B. The motif frequency (4Hz) is clearly seen. The bout's average period is 2818ms (0.35 Hz) with a std of 1442. The first wide peak in the spectrum is at 0.43 Hz. A. The yellow arrow indicates the variable bout's gap.

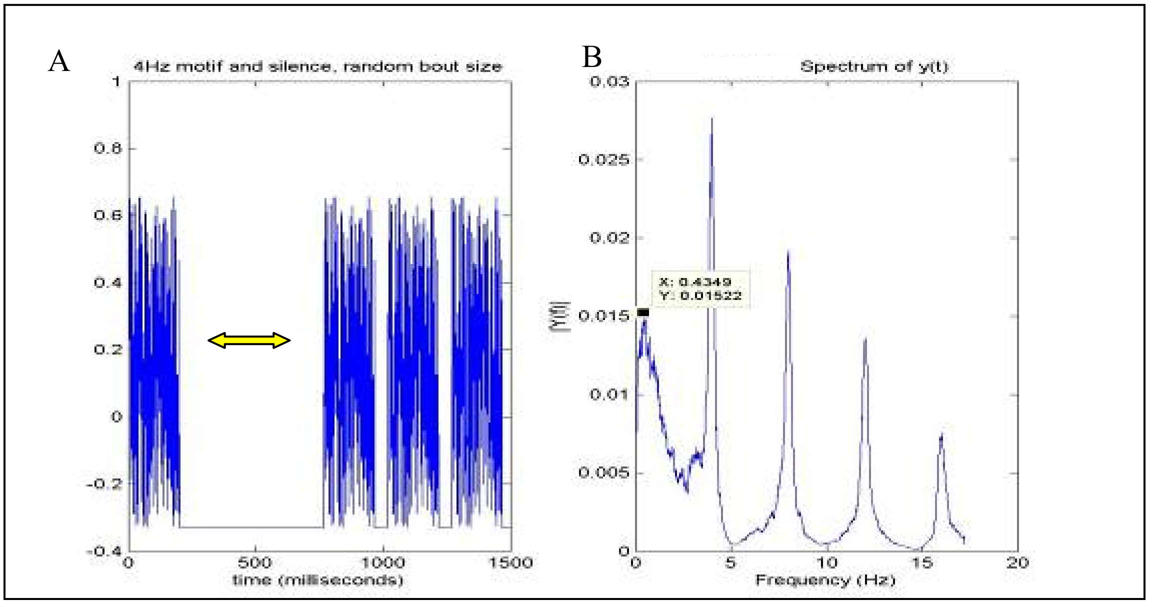


Figure 2.15 Smoothed spectrum (30ms running window). When the number of motifs in a bout is random (uniformly distributed), the silence between the motifs is constant, and the gap between the bouts is variable in its duration, with a normal distribution, then the spectrum will show a strong harmonic for the motif frequency, and a strong peak for the average bout duration. B. The motif frequency (4Hz) is clearly seen. The bout's average period is 2818ms (0.35 Hz) with a std of 1442. The first wide peak in the spectrum is at 0.43 Hz. A. The yellow arrow indicates the variable bout's gap.

In order to better understand the effect of the bout's variance we simulated vectors with different bout variances (the number of motifs in the bout is distributed uniformly; the silence duration is distributed normally). The simulation shows that when the variance is low the dominating harmonic is the bout's harmonics, not the motif's. The energy of the motif's frequencies is distributed among the bout's harmonics, Figure 2.16 A, 2.17 A. When the bout's variance is high, the motif's harmonics are dominating, and there is a low frequency wide peak that corresponds to the variable bout's duration, Figure 2.16 D, 2.17 D.

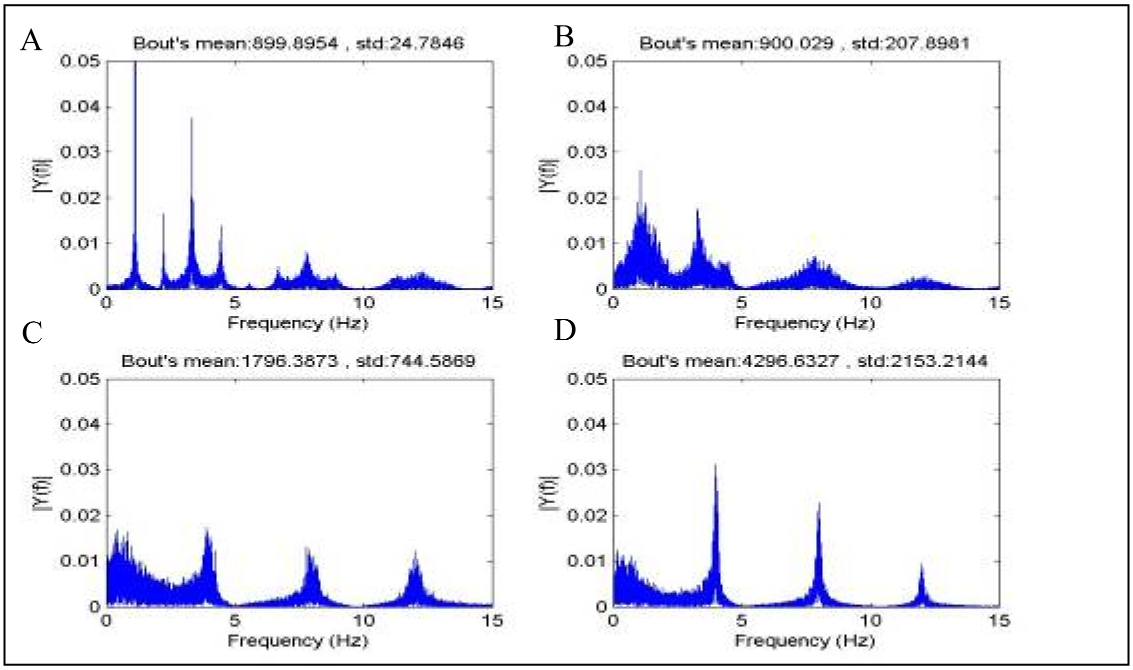


Figure 2.16 A simulation of the effect of the variance and the average duration of the bout. The motif period is 250ms. The number of motifs in the bout is distributed uniformly; the silence duration is distributed normally. A. We see that when the variance is low the dominating harmonic is the bout's harmonics, not the motif's. The peak's frequencies are 1.1014 2.2203 3.3392 4.4580 5.5420 6.6608 7.8147 Hz which correspond to the average bout's duration of 900ms. The energy of the motif's frequencies is distributed among the bout's harmonics. D. When the bout's variance is high, the motif's harmonics are dominating, and there is a low frequency wide peak that corresponds to the bout's duration. The peak's frequencies are: 0.3329 (bout's frequencies) 4.0129 (motif fundamental frequency) 8.0082 12.0386 Hz.

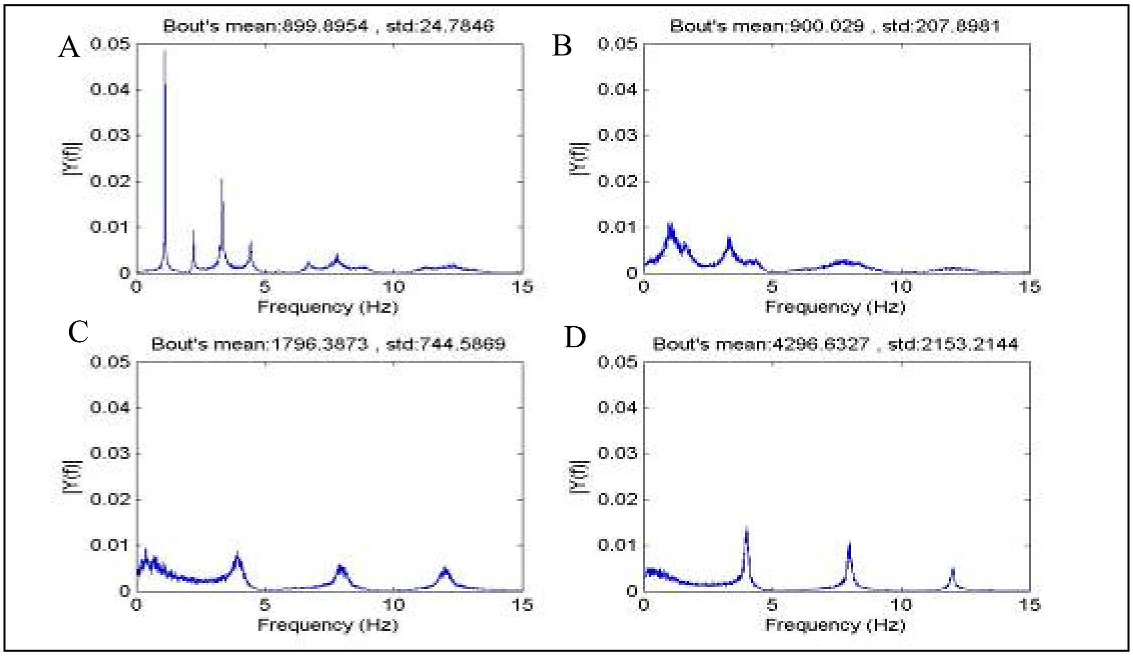


Figure 2.17 A simulation of the effect of the variance and the average duration of the bout. Smoothed spectrum (20ms running window). The motif period is 250ms. The number of motifs in the

bout is distributed uniformly; the silence duration is distributed normally. A. We see that when the variance is low the dominating harmonic is the bout's harmonics, not the motif's. The peak's frequencies are 1.1014 2.2203 3.3392 4.4580 5.5420 6.6608 7.8147 Hz which correspond to the average bout's duration of 900ms. The energy of the motif's frequencies is distributed among the bout's harmonics. D. When the bout's variance is high, the motif's harmonics are dominating, and there is a low frequency wide peak that corresponds to the bout's duration. The peak's frequencies are: 0.3329 (bout's frequencies) 4.0129 (motif fundamental frequency) 8.0082 12.0386 Hz.

In the following examples we will analyze the rhythm-gram of the amplitude feature taken from a real song. The song we use is shown in Figure 2.18. We build a simulated vector with 200 motifs. The duration of the motif plus a constant silence gap is 550ms. The frequency transformation of this signal is a harmonic stack, where the distance between the harmonics equals to the cycle of the motif plus the silence gap (1.82Hz), Figure 2.19.

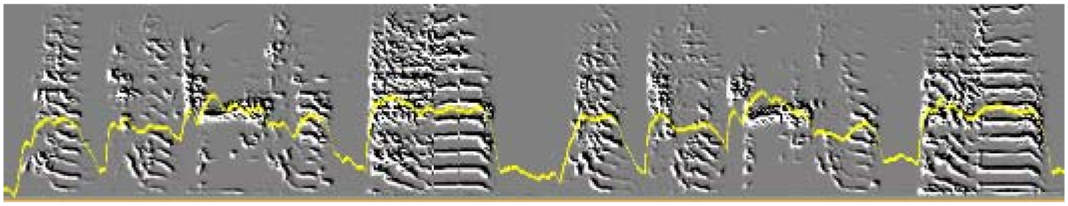


Figure 2.18 An example of a real song bout with two motifs.

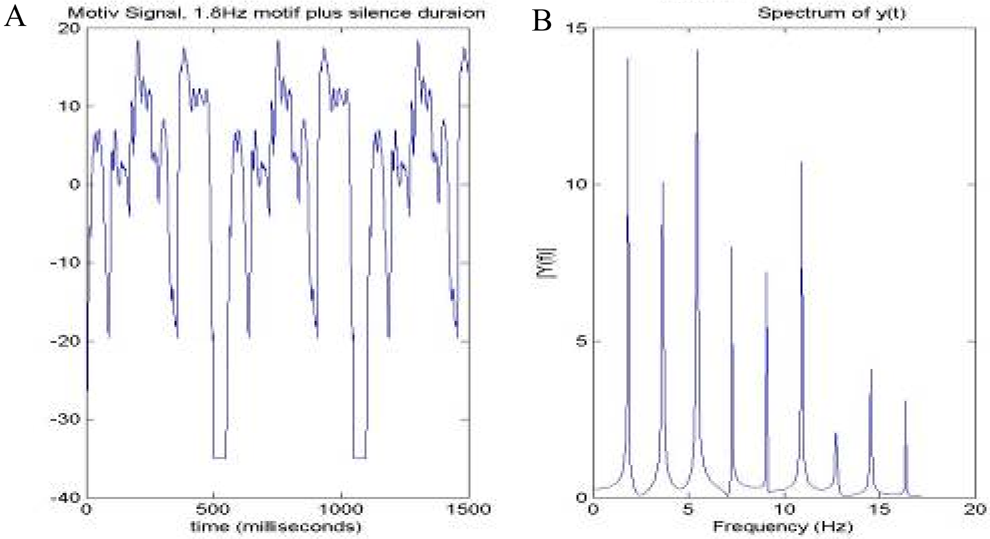


Figure 2.19 An actual feature (amplitude) of a real motif with a constant silence gap (period of 550 ms). The frequency transformation of this signal is a harmonic stack, where the distance between the harmonics equals to the cycle of the motif plus the silence gap (1.82Hz)

We created a simulated bout where the number of motifs in the bout is random (uniformly distributed between 1-10) and the gap between the bouts is random (normal distribution). There is a strong harmonic in the spectrum with a fundamental of 1.8Hz (which corresponds to the 550ms period of the motif). The energy in the low frequencies is relatively high because of the random bout duration (figure 2.20, 2.21)

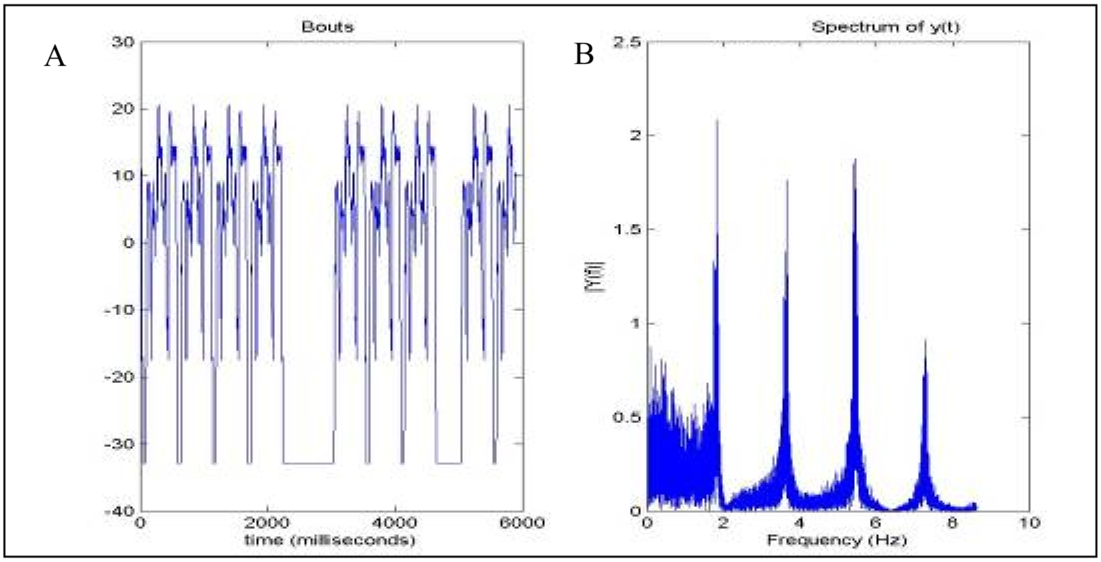


Figure 2.20 A simulation of bouts and its spectrum. A. a simulation of a bout where the number of motifs in a bout is random (uniformly distributed between 1-10) and the gap between the bouts is random (normal distribution). B. There is a strong harmonic in the spectrum with a fundamental of 1.8Hz (which corresponds to the 550ms period of the motif). The energy in the low frequencies is relatively high because of the random bout duration.

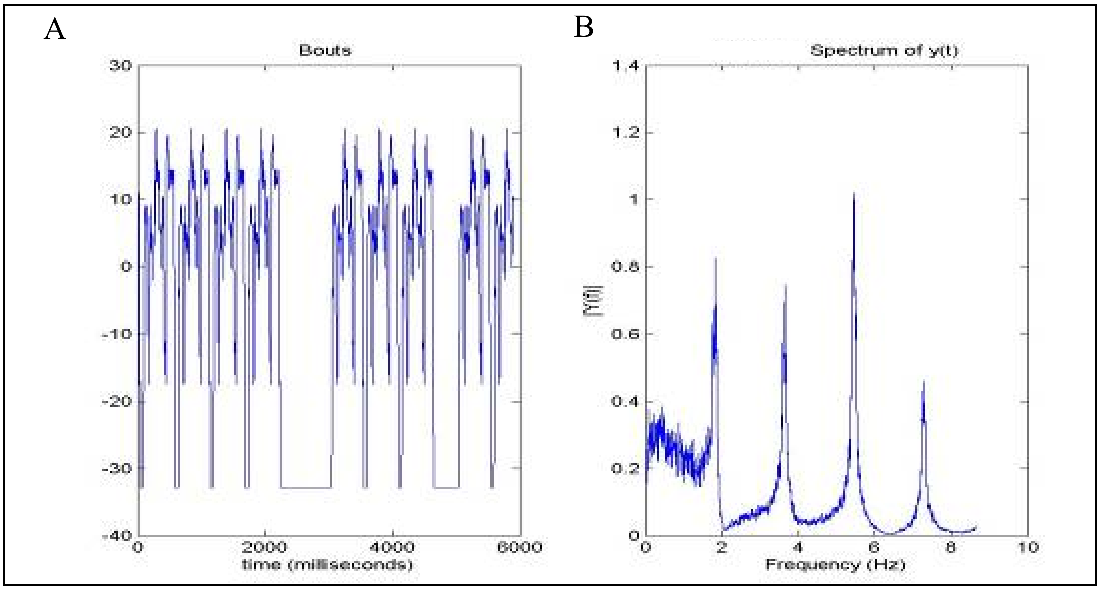


Figure 2.21 A simulation of bouts and its smoothed spectrum (20ms running window). A. a simulation of a bout where the number of motifs in a bout is random (uniformly distributed between 1-10) and the gap between the bouts is random (normal distribution). B. There is a strong harmonic in the spectrum with a fundamental of 1.8Hz (which corresponds to the 550ms period of the motif). The energy in the low frequencies is relatively high because of the random bout duration.

In the next example, the bout's random gap (normal distribution, mean 800ms, std 206) has a 50% chance to include a long call (duration of 158ms) in a random location (uniformly distributed in the gap). We simulated the long calls using real calls. The spectrogram does not show the frequency of the call (6.3Hz), but the energy in the low frequencies increases, Figure 2.22, 2.23.

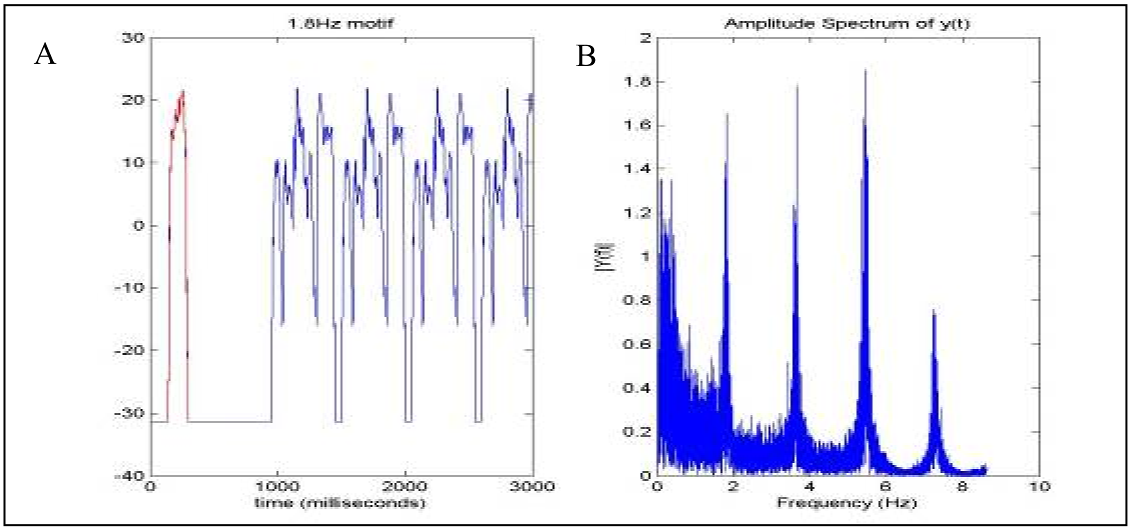


Figure 2.22 A simulation of a bout with random call embedded in the bout's gap . A. The bout's random gap (normal distribution , mean 800ms , std 206) has a 50% chance to include a long call (duration of 158ms, colored in red) in a random location (uniformly distributed in the gap). B. The spectrogram does not show the frequency of the call (6.3Hz), but the energy in the low frequencies increases.

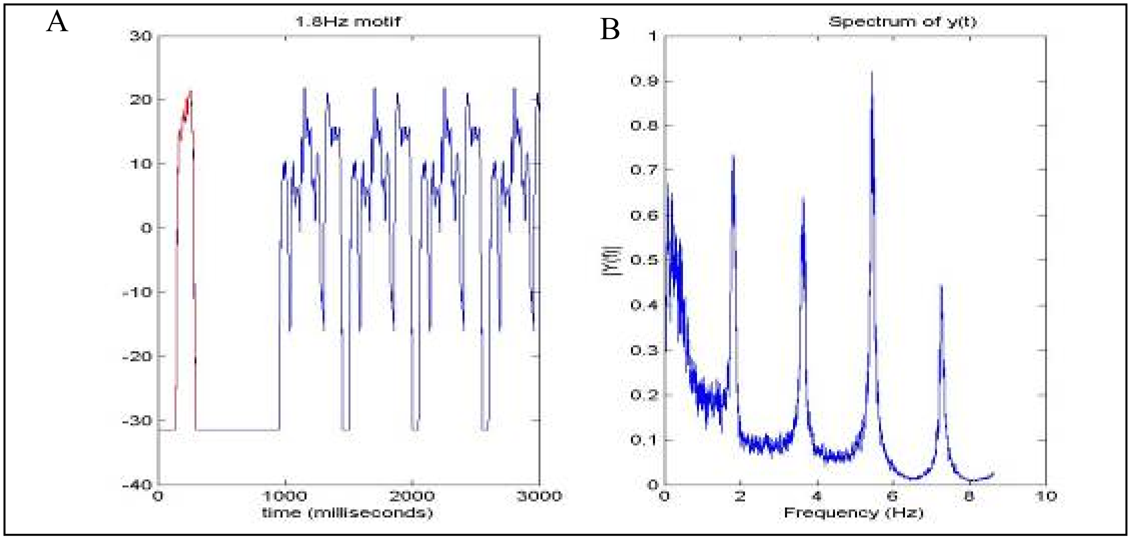


Figure 2.23 A simulation of a bout with random call embedded in the bout's gap and its smoothed spectrum (20ms running window). A. The bout's random gap (normal distribution , mean 800ms , std 206) has a 50% chance to include a long call (duration of 158ms, colored in red) in a random location (uniformly distributed in the gap). B. The spectrogram does not show the frequency of the call (6.3Hz), but the energy in the low frequencies increases.

Another common case in the zebra finch song is introductory notes preceding the bout. We simulated introductory notes using real introductory notes. The duration of the introductory notes is 90ms and a 30ms silence follows it. The number of introductory note repetitions is uniformly distributed between 1-10. The spectrum (Figure 2.24, 2.25) has a distinct 1.8Hz harmonic that corresponds to the motif duration. There is a peak at 8.3Hz which corresponded to the note's period (and its gap), and a hint of its harmonic at 16.6Hz. In addition, the low frequencies have high energy. Note, in actual rhythms analysis from real song data of many bouts during 2 hours of singing we do not see the removal of a peak when we remove introductory calls (Figure 2.26). This is phenomena is probably caused due to a relationship between the call's and the motif's rhythm. The relationship of calls and song motifs should be explored more thoroughly in the future.

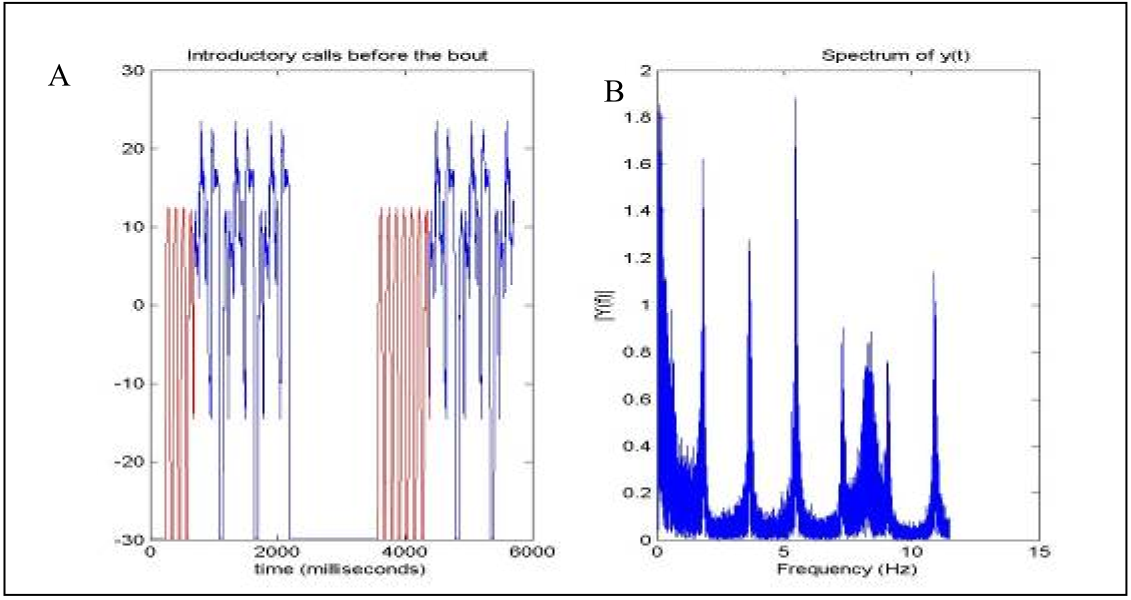


Figure 2.24 A simulation of a bout with introductory notes. A. The duration of the note is 90ms and a 30ms silence follows it. The number of note repetition is uniformly distributed between 1-10. B. The spectrum has a distinct 1.8Hz harmonic that corresponds to the motif duration. There is a peak at 8.3Hz which corresponded to the introductory notes's period (and its gap), and a hint of its harmonic at 16.6Hz. In addition, the low frequencies have high energy.

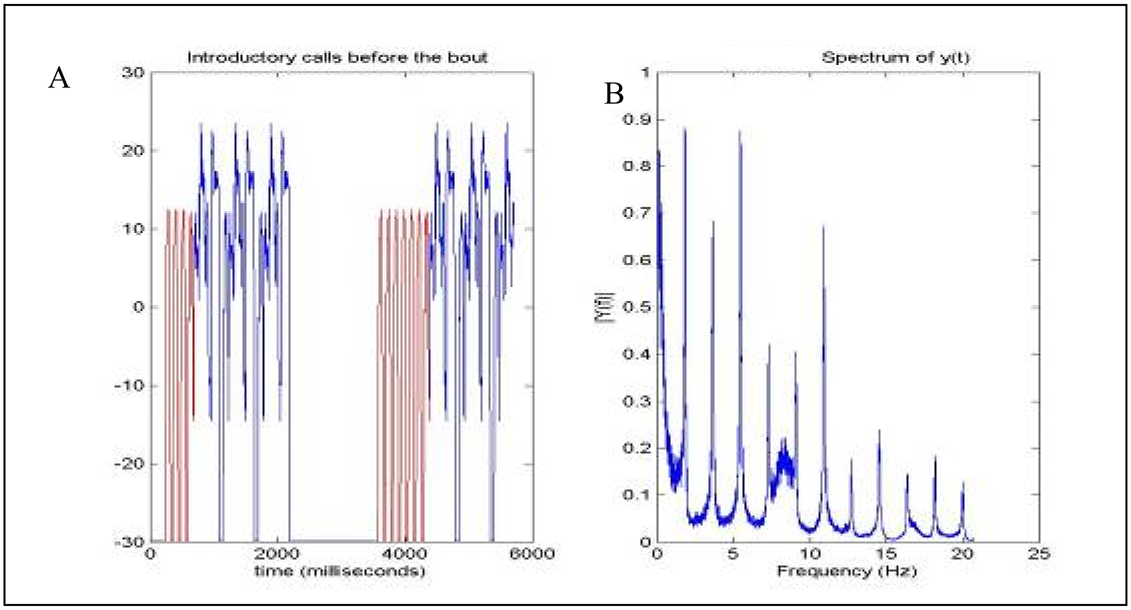


Figure 2.25 A simulation of a bout with introductory notes and its smoothed spectrum (20ms running window). A. The duration of the note is 90ms and a 30 ms silence follows it. The number of introductory notes repetition is uniformly distributed between 1-10. B. The spectrum has a distinct 1.8Hz harmonic that corresponds to the motif duration. There is a peak at 8.3Hz which corresponded to the note's period (and its gap), and a hint of its harmonic at 16.6Hz. In addition, the low frequencies have high energy.

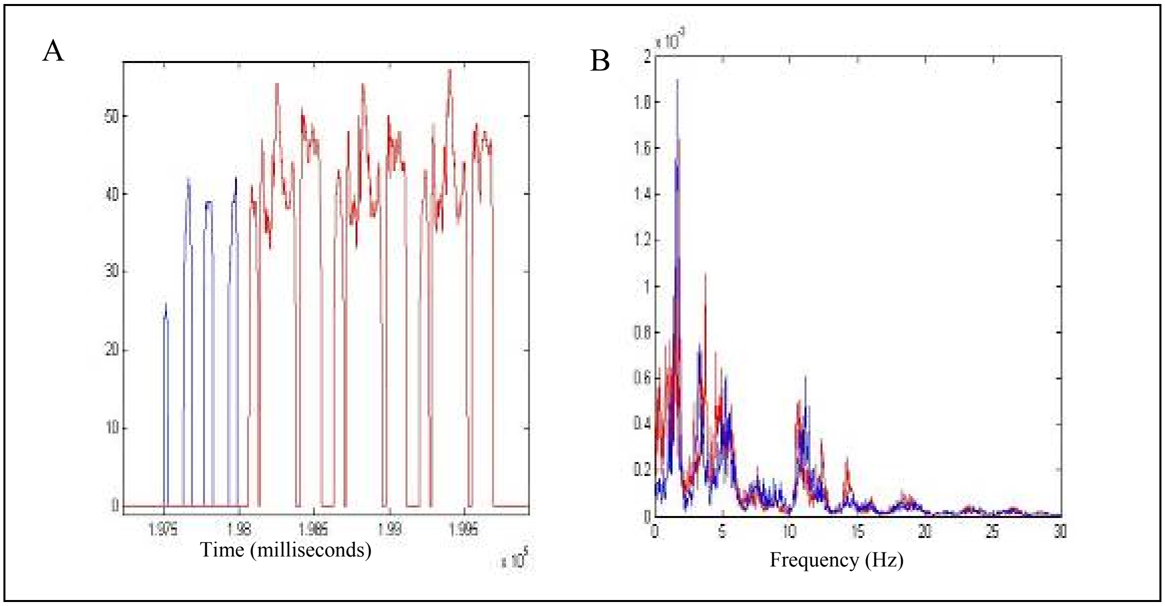


Figure 2.26 In actual rhythms analysis from real song data of many bouts during 2 hours of singing we do not see an additional peak when we remove introductory calls. A. example of song amplitude features, blue original song red song after removal of calls. B. song spectrum- the peaks remain similar. blue original rhythm spectrum, red spectrum after removal of calls

The power of using the Fourier transformation is that complicated periodic signals such as the zebra finch song is decomposed into the sum of simple waves. The zebra finch song is composed of repeating elements (motif). The number of motif repetitions in a bout is usually not stereotypical, more so, the silence gap between bouts is not stereotypical (in the rhythm algorithm described next we make sure the gap between bouts is random). Therefore, all the periodic structures that are time-locked to the motif will end up, in Fourier analysis, as energies distributed within the harmonic frequencies which stem from the motif period.

Developmental analysis of rhythms

The adult zebra finch song is composed of a few renditions of the song motif. Each motif has a number of syllables. The rhythm spectrogram shows this repeating structure in the frequency domain, with the fundamental frequency corresponding to the motif duration. In order to verify that this is true, we checked on 20 adult birds, and found that indeed, the fundamental of the rhythm spectrograms corresponds to the motif durations. During development and in some adult birds, there are instances where two types of motifs with two motif durations are sung in one bout, or in different bouts but at the same hour. In those cases, there would be two harmonic trains with different fundamentals. The structure of the harmonics in the rhythm spectrogram, i.e. the energy distribution across the harmonics for one column, is explained by the syllabic structure.

In figure 2.27 A shows a rhythm spectrum at the developmental stage where a syllable was added to the motif, leading to a change in the song rhythm. The added syllable started to appear on day 49. For a number of days the bird sang both types of motifs, with and without the new syllable. By day 60 the new syllable appeared in all the motifs. The fundamental started at 2.5Hz, and after the syllable was inserted the fundamental harmonic was 1.666Hz. This corresponded to a change in the motif duration from 400msec to 610msec (Figure 2.27 B). On the days where both types of motif were sung, two superposed harmonic stacks are visible in the rhythm spectrogram. Note that the Matlab color bar shows dark blue as low energies, yellow as intermediate power, and red as high power.

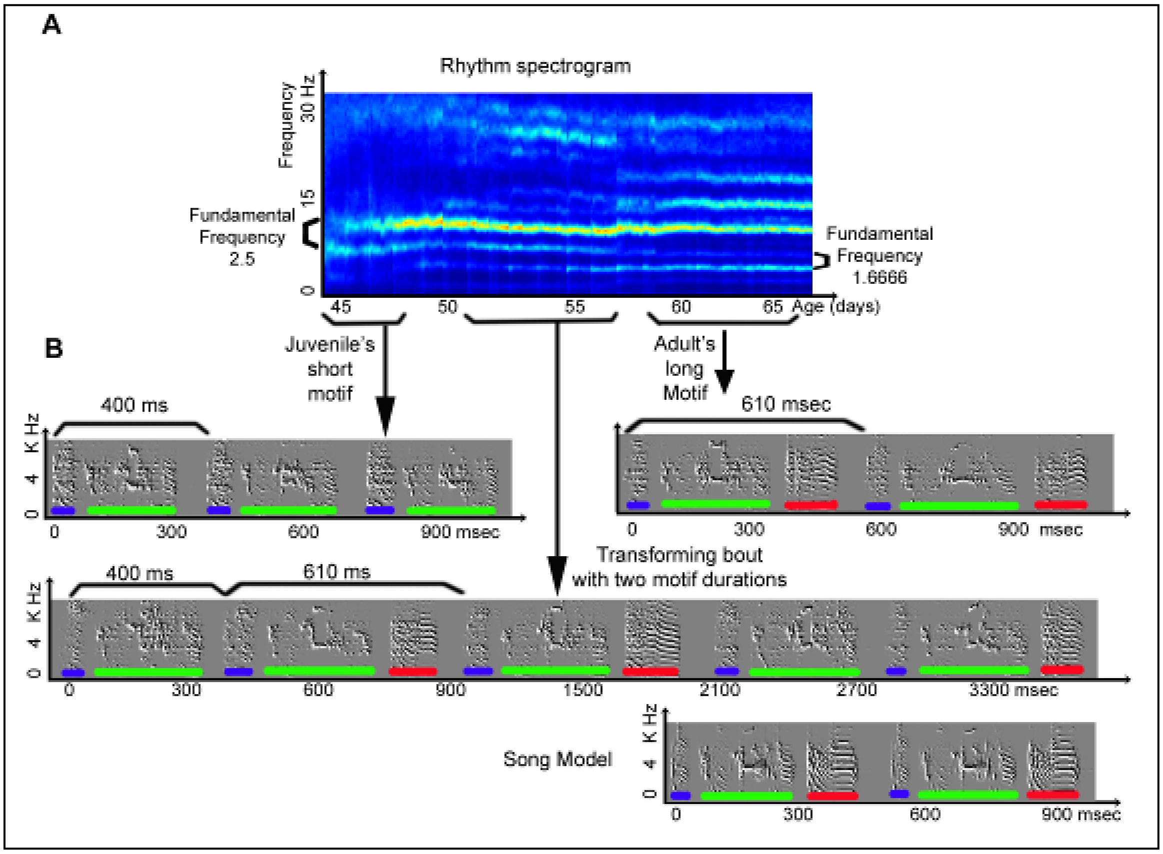


Figure 2.27 Changes in the motif duration show up as changes in the fundamental frequency of the rhythm spectrogram. A. shows a rhythm spectrum at the developmental stage where a syllable was added to the motif, leading to a change in the song rhythm. B. The added syllable started to appear on day 49. For a number of days the bird sang both types of motifs, with and without the new syllable. By day 60 the new syllable appeared in all the motifs. In panel A. we see that the fundamental started at 2.5Hz, and after the syllable was inserted the fundamental harmonic was 1.666Hz. This corresponded to a change in the motif duration from 400msec to 610msec. On the days where both types of motif were sung, two superposed harmonic stacks are visible in the rhythm spectrogram. Note that the Matlab color bar shows dark blue as low energies, yellow as intermediate power, and red as high power.

The juvenile's song structure is highly variable, not only in its notes and syllables, but also in its motif composition. It is often hard to visually identify a motif, or any repeating unit in the juvenile's song spectrogram. The rhythm spectrogram has proven to be a useful tool in identifying repeated units even in these relatively unstructured songs. Figure 2.28 A shows the rhythm spectrogram for a juvenile bird, age 47-48 days, using the amplitude feature. A strong spectral peak is visible in the rhythm spectrogram at 1.35Hz. Figure 2.28 B shows a sample of songs from the same days. It is hard to identify by eye any repeating unit in the song spectrogram, but a periodicity of 740msec (corresponding to 1.35Hz) may be found in the onsets of song syllables (highlighted by the black lines- Figure 2.28 B).

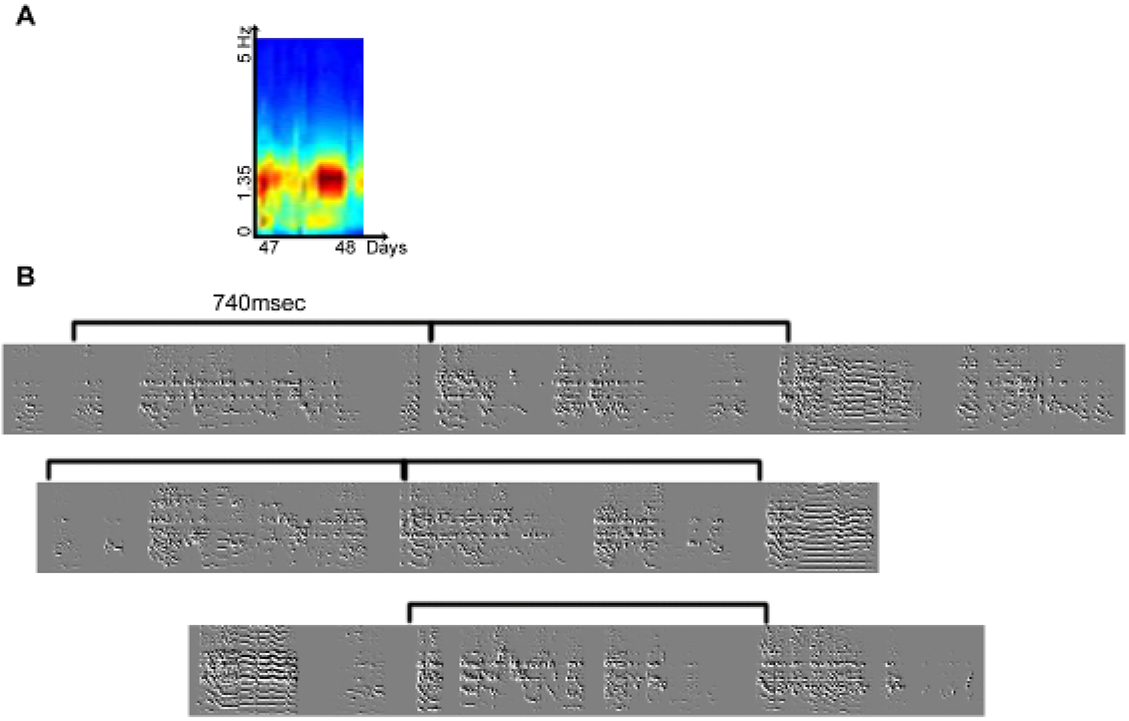


Figure 2.28 The rhythm of juvenile song can be identified early during development. A. shows the rhythm spectrogram for a juvenile bird, age 47-48 days, using the amplitude feature. A strong spectral peak is visible in the rhythm spectrogram at 1.35Hz. B. shows a sample of songs from the same days. It is hard to identify by eye any repeating unit in the song spectrogram, but a periodicity of 740msec (corresponding to 1.35Hz) may be found in the onsets of song syllables (highlighted by the black lines).

Discussion

In this chapter we have presented a method that nests spectral analysis across timescales to study longer time scale structure in birdsong development. This technique can detect rhythm early in the zebra finch song development, in contrast to previous characterizations of such song as unstructured. It can detect the variability of rhythms across different renditions of a song and track the transition from the juvenile rhythms to the adult rhythms which correspond to the song motif. The study of rhythm development should provide a different perspective from the one where attention is paid to template matching at the level of the spectral frame (the song rhythm) (Deregnaucourt 2004; Saar et al. 2009).

In chapters 3 and 4 we will utilize rhythm analysis to study rhythm development in birds trained from a juvenile age and in birds that are socially and acoustically isolated until the end of their critical period and then trained. In chapter 5 we use rhythm analysis to study rhythmic changes that occur with aging, toward the end of the bird's life.

Chapter 3. The development of rhythm

Introduction

Vocal ontogeny in songbirds provides a good model for studying how a complex set of learned vocal signals is acquired (Thorpe 1958; Marler 1970; Nottebohm 1972; Tchernichovski et al. 2001). The young bird, exposed to a song model at the age of 43 days (at the beginning of the sensory-motor stage) , must solve the problem of changing its unstructured subsong to imitate the song model. We exposed the bird on day 43 because at this age the bird is at the beginning of its motor-sensory sensitive period. There could be different strategies involved. For example, the young bird could have an innate motor program that can generate a large repertoire of species-typical sounds. In that case, imitation would be a process of selection and reorganization (syntax changes) of those sounds (Marler & Pickert 1984, Nelson & Marler 1994, Marler 1997). Another option is starting from an arbitrary starting point of the subsong, the juvenile bird first develops a coarse approximation to the whole song motif of his tutor (Liu et al. 2004) and later on refines it. In this case, song imitation is a generative process, where sounds are shaped and transformed, rather then selected. An example of such trajectory is the path of increasing acoustic mismatch until an abrupt correction occurs by period doubling (Tchernichovski et al. 2001). Looking at song development, there is evidence to both generation and selection.

Rhythm analysis gives us information about longer time scale structure in birdsong development. Because no segmentation and classification of sounds is necessary, it is relatively easy to examine rhythm structure even in very early stages of the zebra finch song development, and track the transition from juvenile rhythms to adult rhythms (Saar 2008).

Zebra finch song consists of a single motif which repeats over and over. The simple fact that the motif repeats itself generates a rhythm; the juvenile bird transforms its motif, and hence generates another rhythm. Are the old rhythm and new rhythm related to one another in some way or is the zebra finch rhythm an epiphenomenon? If the song's rhythm was an epiphenomenon, i.e. the bird was not concerned with the song's rhythm, but with the motif syllable structure, then it will randomly form rhythms and developmental transitions from one rhythm to an unrelated rhythm. However, if rhythm is a salient feature of song development we might observed an evolution of the rhythm over development, namely, new rhythms will be related to the previous rhythm, and we might observe some constraints in rhythm transition over development. Song development might be constrained by its rhythm history because we are looking at the evolution and development of a rhythm generator, as opposed to the process of unconstrained syllable assembly (Tchernichovski et al. 2001, Liu et al. 2004).

We will consider four postulated rhythm development scenarios (Figure 3.1); A gradual appearance of end-point rhythms during song development with no significant transitory rhythms (Figure 3.1 A); Stochastic developmental transitions between unrelated rhythms until final rhythm stabilize (Figure 3.1 B); Smooth modulation of rhythm (Figure 3.1 C); Abrupt, yet constrained modulation of rhythms, where the old rhythm is related to the new rhythm, such as bifurcations or transitions with a ration of 2:3 or 3:4 (Figure 3.1 D)

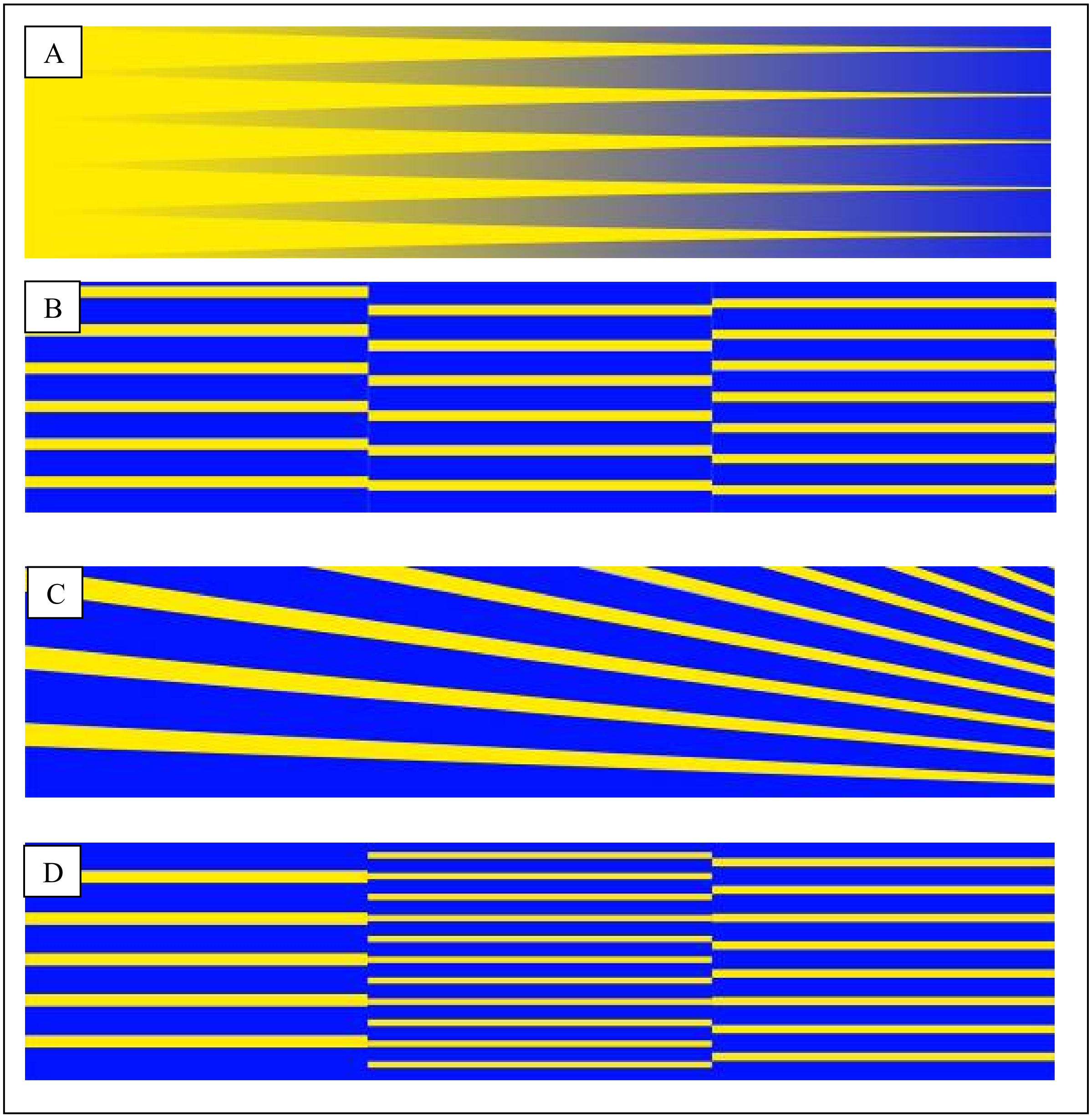


Figure 3.1 There are four postulated rhythm development scenarios. A. A gradual appearance of rhythm. B. Transition from one rhythm to an unrelated rhythm. C. Smooth modulation of rhythm. D. Abrupt, yet constrained modulation of rhythms.

- A gradual appearance of rhythm (Figure 3.1 A) will happen if the juvenile bird is producing a coarse approximation to the whole motif.
- A transition from one rhythm to an unrelated rhythm (Figure 3.1 B) will happen if song development is a relatively unconstrained assembly of syllables. For example, in the case of selection from a hidden repertoire of sounds, imitation is a simple process of selection and assembly of unrelated song parts.
- Smooth modulation of rhythm (Figure 3.1 C) will occur when the juvenile bird has a motif prototype that it stretches or contracts so that it can modify its features until a close match with the model is achieved.
- Nonlinear modulation of rhythms such as bifurcation (Figure 3.1 D) will happen when developmental trajectories of prototypes follows paths of increasing acoustic differentiation that leads to period multiplication. Or when a new song segment with duration that is related to the original motif, is inserted to the song while conserving much of the previous rhythm structure.

Method

Subjects & training

We used 23 zebra finches (Taeniopygia guttata) from the City College of New York breeding colony. Sébastien Derégnaucourt and Ofer Tchernichovski trained and recorded the birds. All birds were kept in isolation from day 30 to day 120 post hatching day (PHD). Birds were raised from hatching under an artificial photoperiod of 12 h : 12 h LD (Deregnaucourt et al. 2005).

Training procedure

Birds were trained with one of three song model playbacks (Tchernichovski et al. 2001), starting from day 43 after hatching. We used three song models so that we can get a large enough number of birds trained on each song mode, and still have the option to look at developmental learning with different training end points. The day the training started, two keys were inserted into the training box (Adret, 1993). Key pecking triggered a song playback from a small speaker housed within the plastic male model. Each playback consisted of two identical repetitions of a single motif recorded from an adult bird. Each day consisted of two training sessions (one in the morning and one in the afternoon). In each session, the first 10 key pecks were reinforced with a song playback.

Song models

We used three song models that differ in complexity at both levels: phonetic and syntactic. Each bird was trained with a single song model. It enables us to use the complete variety of syllables produced by zebra finch males such as different harmonic stacks and high pitch note (see figure 1.6).

Song recording and analysis

All songs were recorded digitally (16 bits, 44.1 kHz) from each bird, continuously from day 35 to day 120. A song recognition procedure detects and saves the recorded songs, discarding isolated calls and cage noises. After lights-off (when the bird is quiet), the system switches into analysis mode. First, each recorded song bout is partitioned into syllables, then each syllable is analyzed and its time-frequency structure summarized by a set of simple features, such as: duration, mean pitch, frequency modulation, etc. Most zebra finches produce 1-2 million song syllables during song development (between days 35-90), the features of which are saved to a single database file. With the entire vocal ontogeny of a bird on file, tracing vocal changes becomes straightforward and we can visualize the raw data of an entire vocal ontogeny in a single image.

The spectrum interval we used for the rhythm analysis is 0-30Hz. We used this upper bound because we expect song rhythm (i.e, repeating elements in the song) to be less than 15 Hz. Since the energy of the harmonics decreases with frequency, we visually and analytically identified the 0-30Hz spectrum interval to contain most of the harmonics for all birds and yet it is still not as affected by noise artifacts as higher frequencies intervals.

Data analysis

All the analysis was performed using Matlab 7 and MYSQL, except for feature calculations, which were done using Sound Analysis Pro 2 (SAP II).

Results

We calculated developmental rhythm maps for 23 birds that are trained from day 43. In this chapter we used all of the 23 birds in the correlation analysis presented at the end of the chapter. But before presenting figures that summarize developmental process (Figures 3.5-3.21) across birds we would like to start by showing examples of 3 rhythm maps of birds trained with each song model, and later on detailed examples of six representative birds. Across birds, we see that rhythm transitions come about in two forms: smooth downward modulation and abrupt transition in rhythms where the new rhythm and the old rhythm seem to be related. As shown in the detailed examples, smooth down-modulations take place when the bird is time-warping specific syllables that are already included in the motif or the entire motif. For example smooth modulations could be due to altered fine structure of a syllable, or else the temporal stretching or extending of the syllable. In contrast, abrupt transitions occur when the bird inserts a new syllable into its song, or modifies an existing syllable.

Rhythm maps:

Figures 3.2-3.4 present examples of developmental rhythm maps of nine birds that were trained with one of three song models. Rhythm maps differ in detail across birds, but in all birds examined so far we observed a few consistent similarities: peaks in rhythm spectrum emerge a short time after the onset of training, diurnal oscillations are observed, and there are smooth and abrupt rhythm transitions.

It seems that stable rhythms appear early on, those rhythms change but the number of abrupt changes is limited (we observed less than 5 peaks in the 0-30Hz rhythm band). We observed two types of rhythm change, abrupt and smooth transitions. Smooth transitions often proceed monotonically (without changing direction) over several days. Abrupt rhythm transitions,which represent rhythm abrupt transition such as an appearance of a new syllable or reorganization of syllables, tart with some ridges (localized high power) disappearing, a short period of instability, and then an emergence of new ridges. Within a bird, abrupt rhythm transitions are rare events (occurring only a few times during an entire development). Therefore, during most of the developmental time, vocal changes occur within the framework of a stable (static or slowly changing) rhythm.

Note, since some of the data is missing (due to technical difficulties in recording), the missing data is interpolated in Matlab for display purposes only.

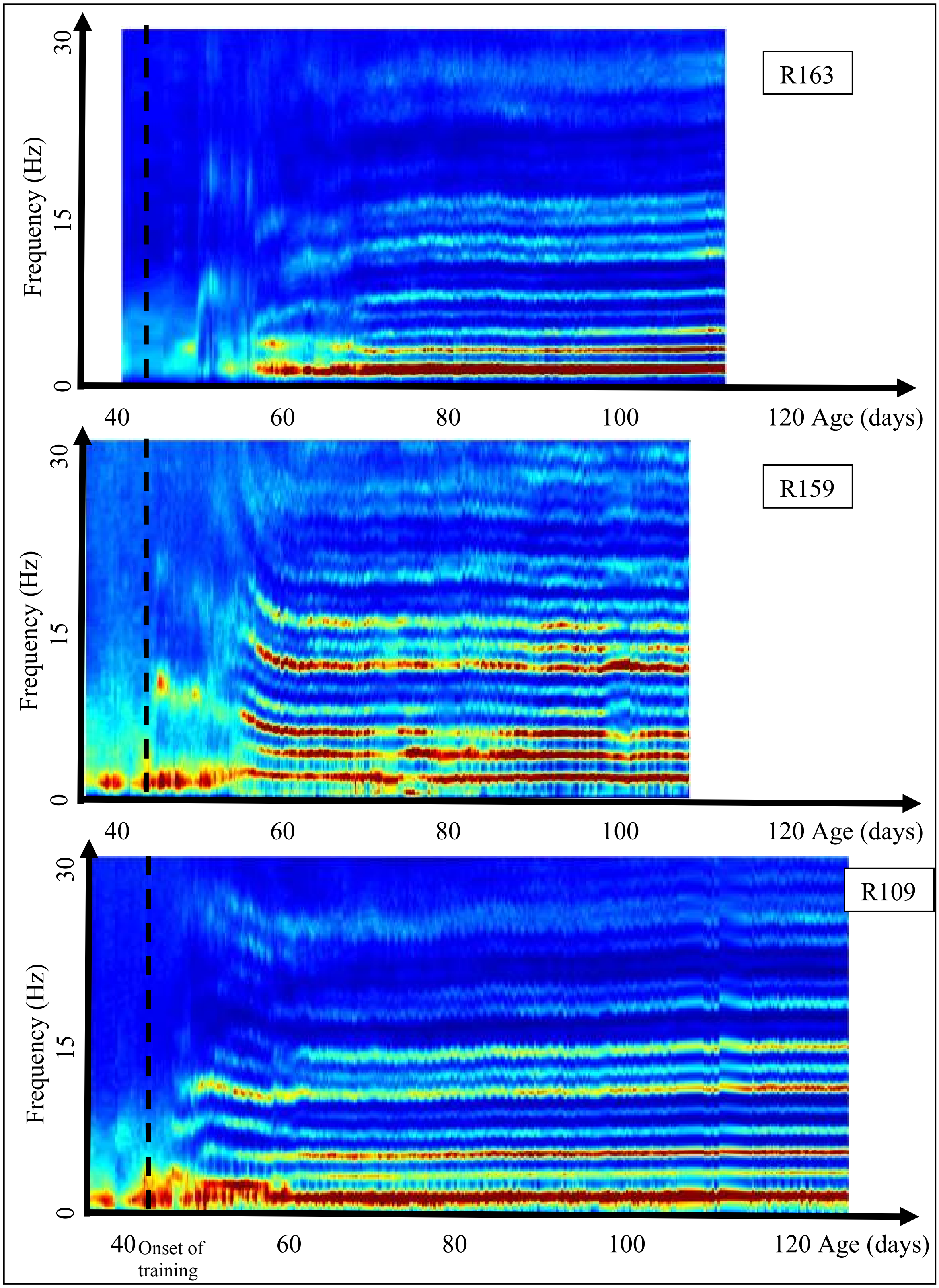


Figure 3.2 Example of rhythm maps of birds trained from day 43 with song model A. We named this song model A as “Simple” (this song model is relatively simple with few simple syllables).

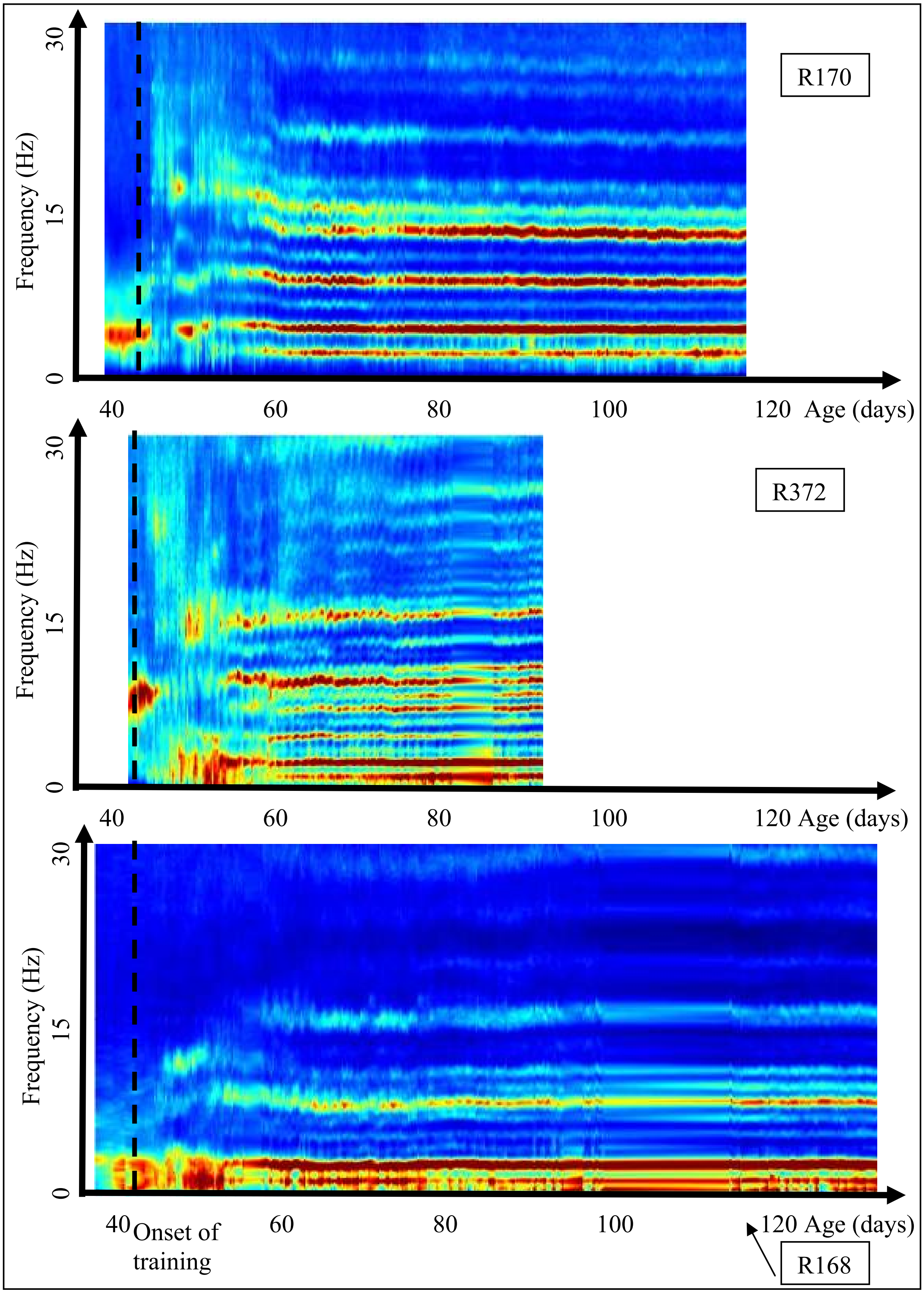


Figure 3.3 Examples of rhythm maps of birds trained from day 43 with song model B. We named this song model B as "Samba" (this song model complexity is middle range).

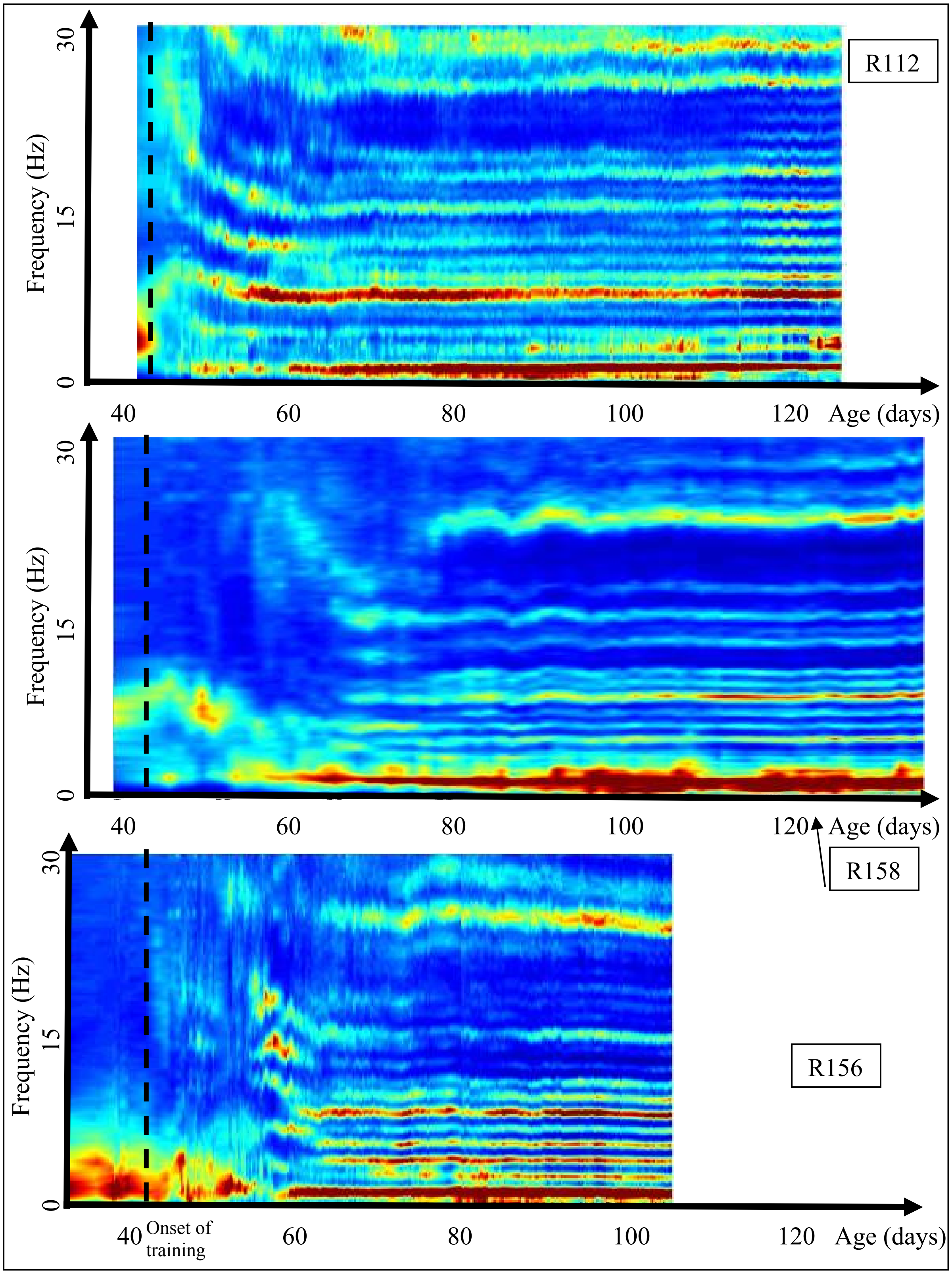


Figure 3.4 Examples of rhythm maps of birds trained from day 43 with song model C. We named this song model C "Bells". This song model is relatively complicated with many short syllables.

We would like to present a few detailed examples of rhythm development before continuing to cross-bird analysis. The examples showed here are presented to show a principle we observed repeatedly, we did not quantify the phenomena we see here.

***An example of a bird that shows smooth rhythm transitions*:** Birds such as R159 display only smooth modulation of rhythm transition which resembles the principle described in Figure 3.1 C. Those birds have a motif prototype that stretches or contracts modifying its features gradually until a close match with the model is achieved

Bird r159 - song model A (Simple).

Rhythm analysis reveals the same developmental processes as ontogeny analysis does, but it facilitates a much faster discovery process. Imagine going through hundred of song images of ontogeny songs to discover an underlying developmental process, rhythm analysis shortens the work to just looking at rhythm map

Bird r159 shows smooth rhythm transitions between the ages 50 to 70 days (Figure 3.5). At age 50 post hatching day (PHD) the bird has a prototype motif that it stretches and modifies until a close match with the model is achieved (Figure 3.6 A, B).

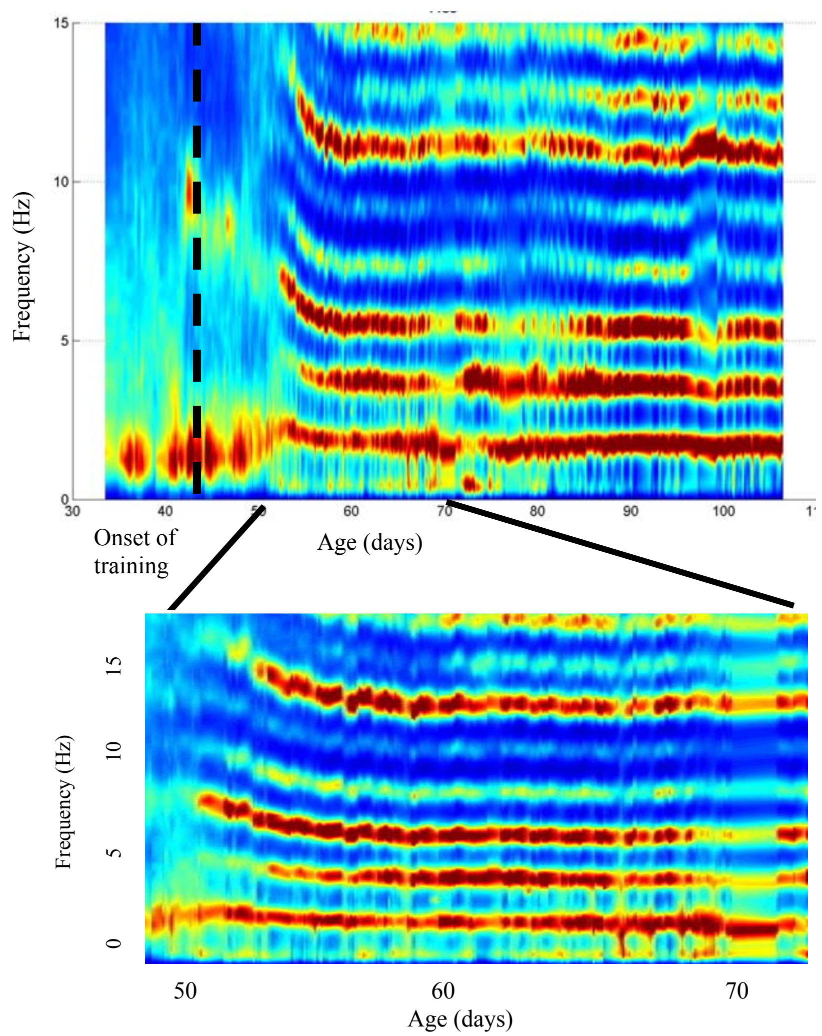


Figure 3.5 Bird 159 shows smooth rhythm transitions between the ages 50 to 70 PHD.

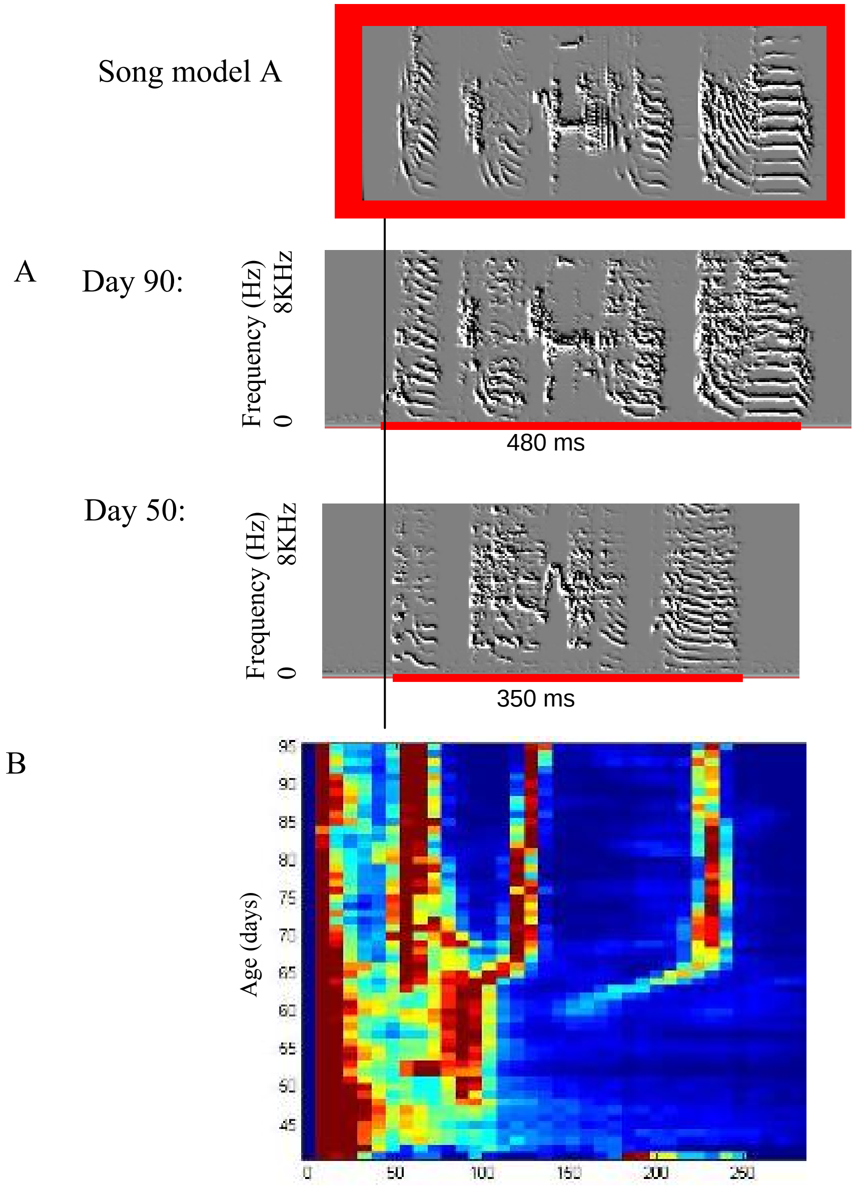


Figure 3.6 At age 50 bird R159 has a prototype motif that it stretches and modifies until a close match with the model is achieved. A. Song ontogeny B. R159 DVD map shows that the peaks (syllables) stretch during development.

***Examples of birds that show abrupt rhythm transitions*:** The following birds have made one or multiple abrupt rhythm transitions. In all the birds we analyzed so far, the new modulated rhythms seem to be related to the old rhythm. This relationship indicates that there are some rhythmic constraints, i.e. rhythm changes are limited to a number of known options (Figure 3.1 D)

Bird B6 – song model C (Bells)

The rhythm map of bird B6 (Figure 3.7) shows two abrupt rhythm transitions, around age 50d and around age 70d. Close inspection of song ontogeny (Figure 3.8) reveals that around day 47 an additional song segment is incorporated in the song. The new song segments are first sung only at the end of the song. It integrates as a part of the song around day 50. The duration of the new segment that is added at age 50 equals the duration of the original segment (period doubling). Around day 70, another new syllable appears. This time, the new syllable first appears at the beginning of the song and then integrates (note that there are non harmonic peaks in the spectrum of age 70). Ontogeny analysis indicates of a possibility that the onset of the new syllable that is added at age 70 aligns on the onset of a syllable that already exists (Figure 3.9) (further statistical analysis should be done to prove this finding). The period of the new syllable is 110ms while the period of the old motif is 308ms. Therefore, there is a rhythmic relationship of 4:3 between the old rhythm and the new rhythm.

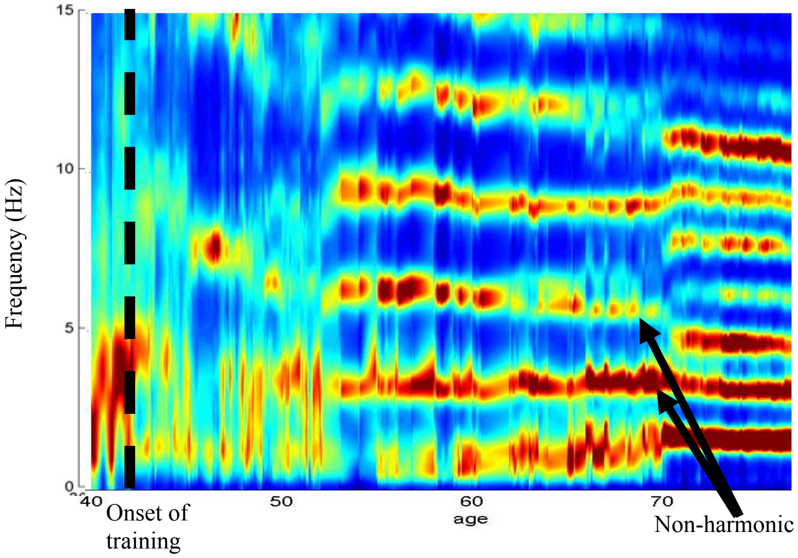


Figure 3.7 The rhythm map of bird B6 shows two abrupt transitions, around age 50 and around age 70.

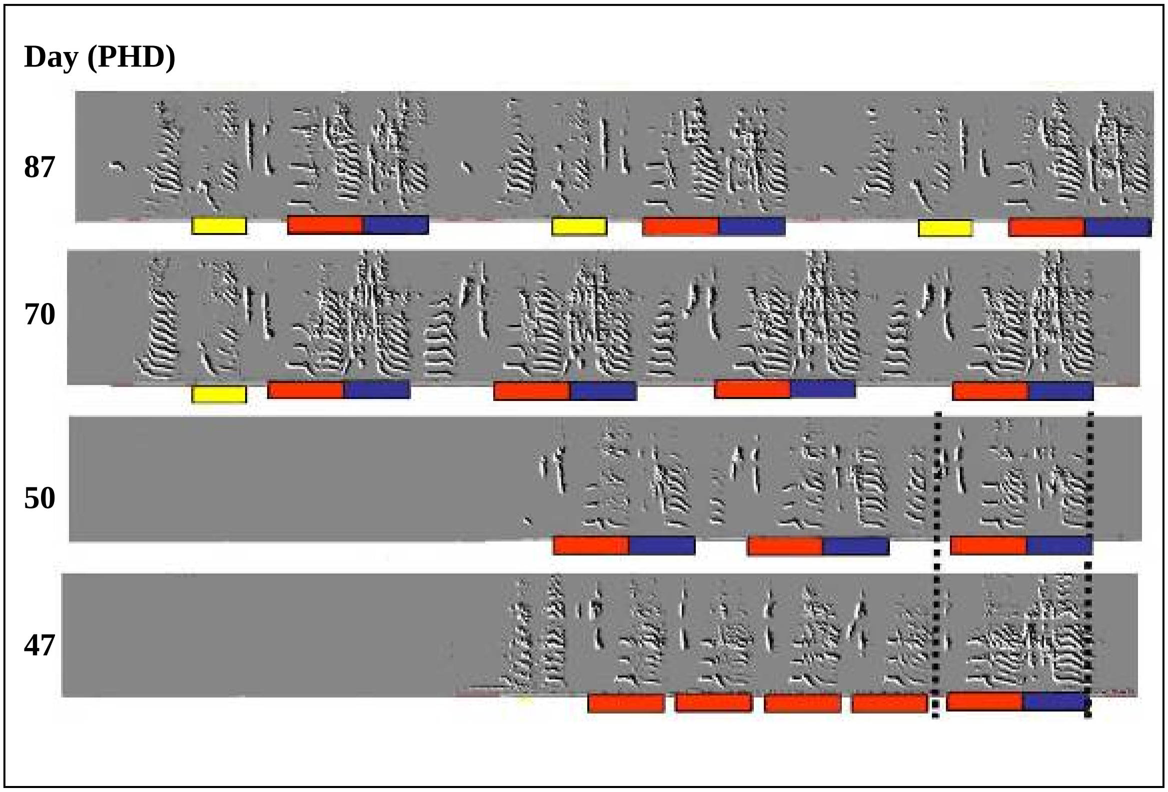


Figure 3.8 Bird B6's song ontogeny reveals that around day 47 an additional song segment is incorporated in the song (marked in blue). The new song segments (marked in blue) first appear only at the end of the song. It integrates as a part of the song around day 50. Around day 70, another new syllable appears (marked in yellow). This time, the new syllable (marked in yellow) appears at the beginning of the song and then integrates.

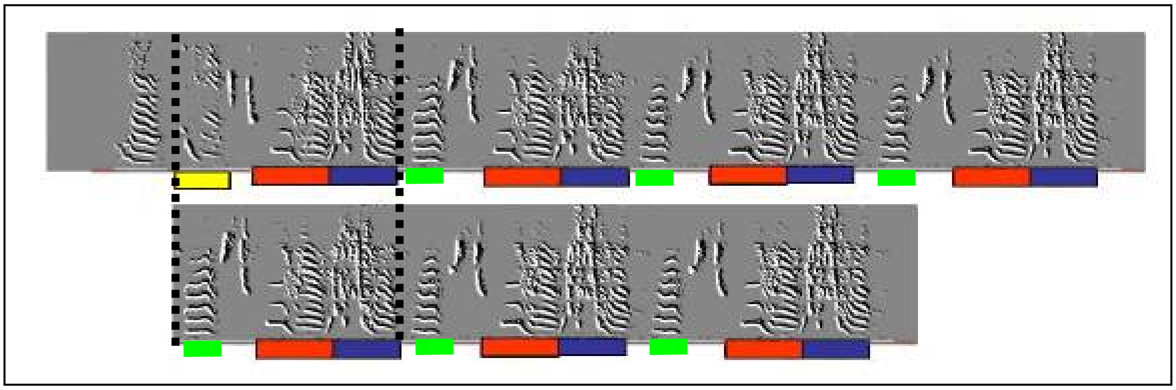


Figure 3.9 Song ontogeny of bird B6 reveals that at age 70 the onset of the new syllable (marked in yellow) aligns on the onset of a syllable that already exists (marked in green).

Bird R155 – song model C (Bells)

The rhythm map of bird R155 shows that between ages 60 to 70 PHD there is an abrupt transition with a ratio of 2:3. The period of the juvenile motif is 333ms while the period of the new motif is 500ms (according to the rhythm map, Figure 3.10). Between ages 85-90 there is another abrupt transition with a ratio of 3:5. The period of the adult's bird motif is 833ms (according to the rhythm map, Figure 3.10).

Song ontogeny shows that before the age of 60 PHD the period of the bird's motif is indeed 333ms, (red and blue syllables in Figure 3.11 A). Between ages 60 to 70 a new segment (marked in green) is introduced (Figure 3.11 A). While the new segment is inserted, the bird sings compounded songs (Figure 3.11 B). The period of the juvenile's motif is 333ms, while the new motif is 500ms (according to song spectrograms). Between ages 85 to 90 another segment (marked in yellow) is added to the song, Figure 3.12. The period of the new motif is 830ms (according to song spectrograms). Bird R155 shows that there could be two abrupt transitions in during development and those abrupt rhythm transitions seem to be constrained.

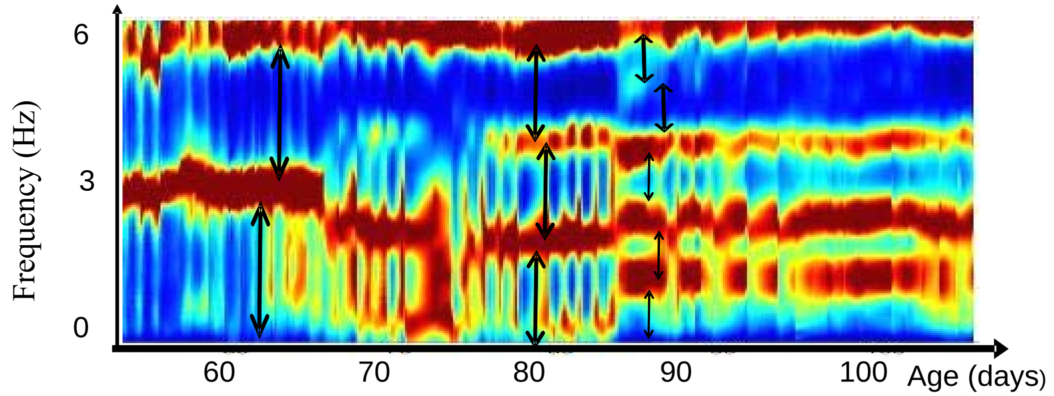


Figure 3.10 The rhythm map of bird R155 shows that between age 55 to 80 there is an abrupt transition. The rhythm bifurcation ratio is 2:3. Between ages 85-90 there is another abrupt transition with a ratio of 3:5.

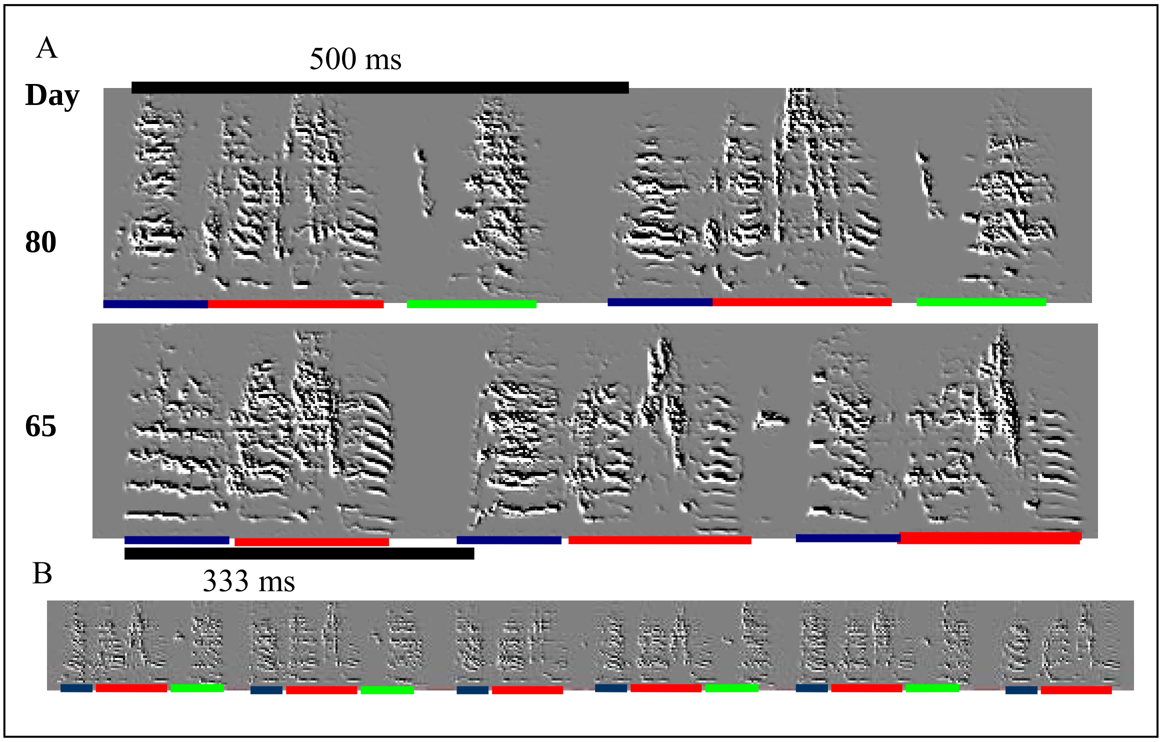


Figure 3.11 Song ontogeny of bird R155 shows that A. between ages 60-70 a new segment (marked in green) is added to the song. B When the new segments are added to the song, the bird sings a compound song.

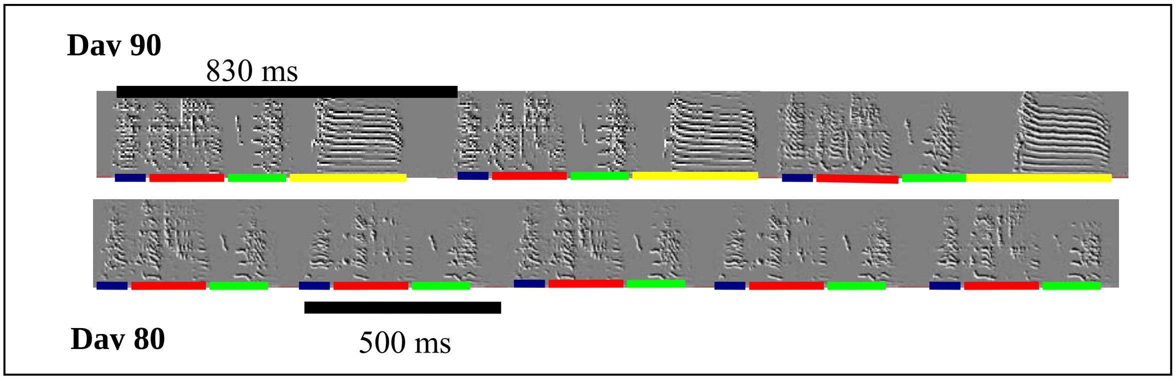


Figure 3.12 Between ages 85 to 90 a new segment (marked in yellow) is added to bird R155 song. The duration of the new motif is 830ms.

***Examples of birds that show smooth and abrupt rhythm transitions*:** Some birds modulate their song rhythms smoothly (stretch or contract syllable prototypes or their entire motif, Figure 3.1 B) before performing an abrupt rhythm transition (Figure 3.1 D). Possibly, those smooth modulations are preparation for the abrupt transitions. Since abrupt transitions seem to transform old rhythm to a new related rhythm, the smooth modulations prior to the abrupt transitions might originate from constraints on abrupt transitions, That is, the bird could not incorporate a new syllable into its motif before a certain rhythm ratio was attained. Only when a certain rhythm was attained can it perform a transformation that keeps the rhythm ratios that we see.

Bird R112 – song model C (Bells)

The rhythm map of bird R112 shows smooth transitions between ages 50 to 60 PHD. At age 55 PHD the period of the motif is 266ms. There is an abrupt transition after age 60 PHD (Figure 3.13). Dong ontogeny shows that the smooth transitions are caused by stretching of the motif. The motif period is 270ms on day 55 (according to song spectrograms, Figure 3.14). Song ontogeny also reveals the abrupt transition is caused by differentiation of prototype (red syllable Figure 3.15) and incorporation of a syllable that used to be sung at the end of the song (blue syllable Figure 3.15). The new blue syllable is half the duration of the prototype (Figure 3.15).

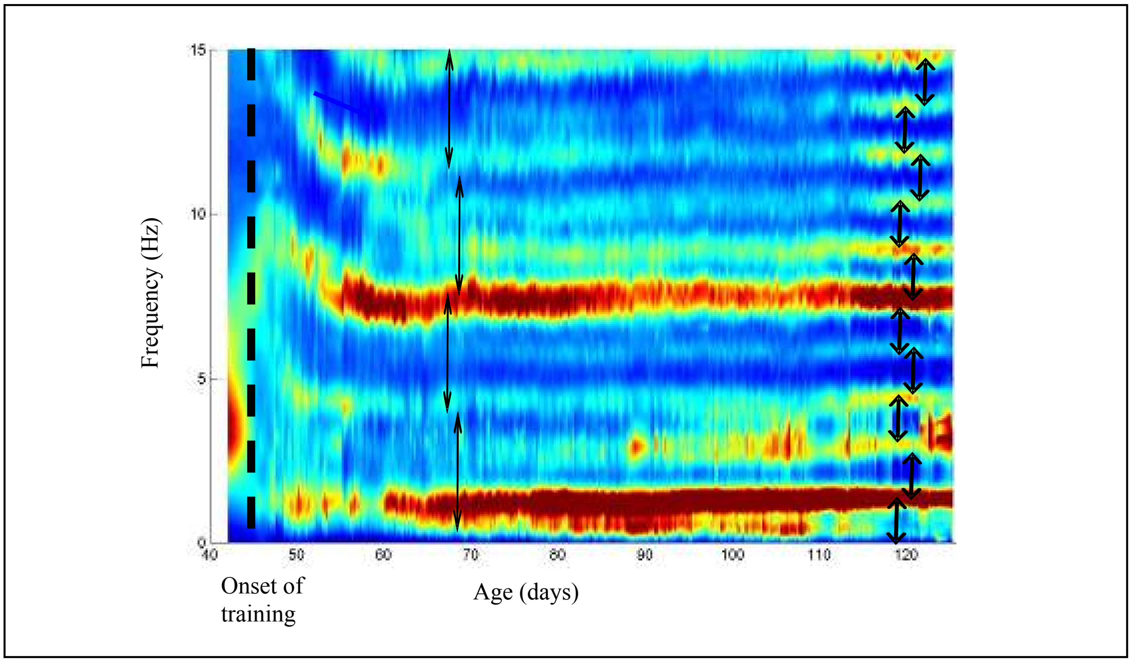


Figure 3.13 The rhythm map of bird R112 shows smooth transitions between ages 50 to 60, followed by period doubling.

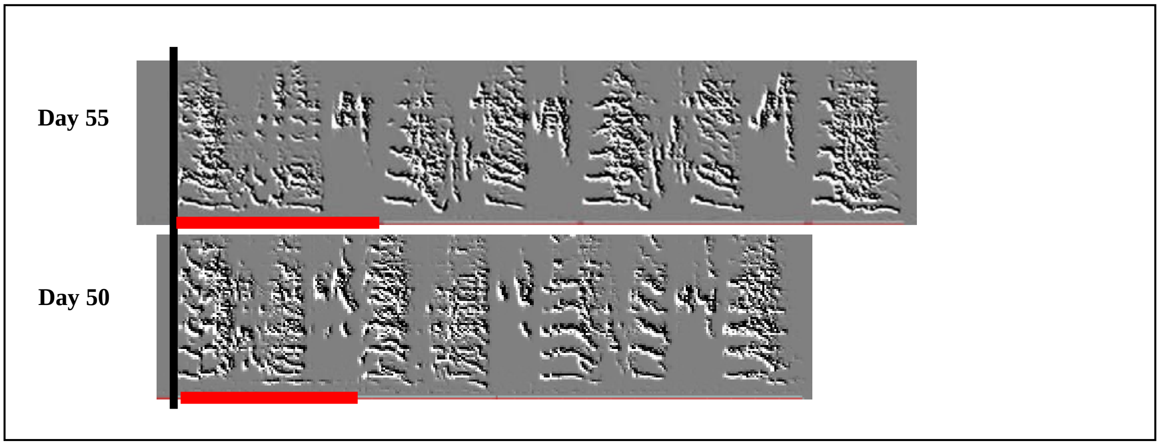


Figure 3.14 Song ontogeny of bird R112shows that the smooth transitions are caused by stretching of the motif.

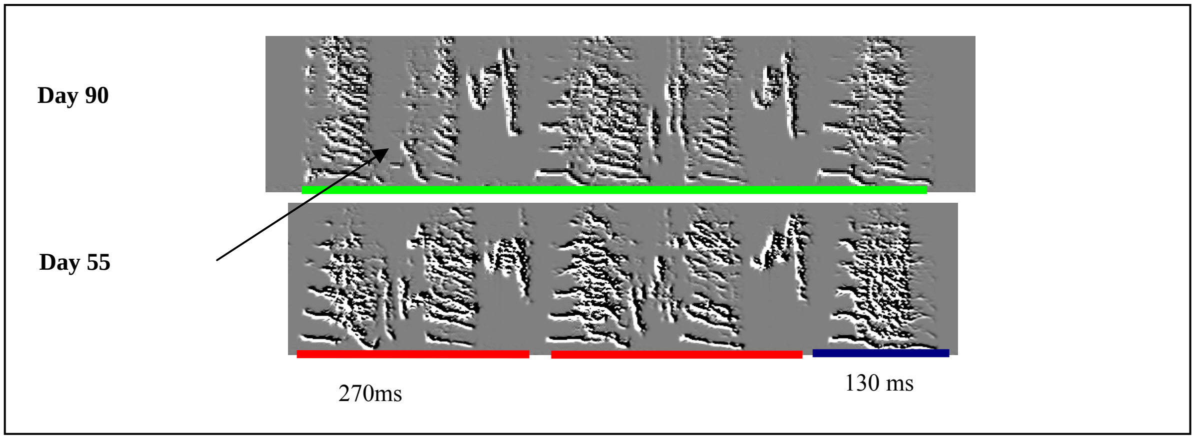


Figure 3.15 Song ontogeny of bird R112 shows that abrupt transitions are caused by differentiation of the prototype (marked in red, day 55 sonogram) and incorporation of a syllable that was at the end of the song (marked in blue, day 55). The new blue syllable is half the duration of the prototype. Note the silence that separates the first syllable of day 55 song. This silence gap distinguishes the two red syllables from one another and makes the song a no repeating element.

Bird r109 - song model A (Simple)

Bird r109 rhythm maps (Figure 3.16) shows smooth transitions between the ages of 50 to 60 PHD. In day 60, the rhythm map shows an abrupt transition in the rhythm. There is a relationship between the old rhythms and the new rhythms. The first and third harmonic (the first and third harmonic of the fundamental frequency) on day 50 transform to be the second and fifth harmonic respectively. This bifurcation has a ratio of 2:3.

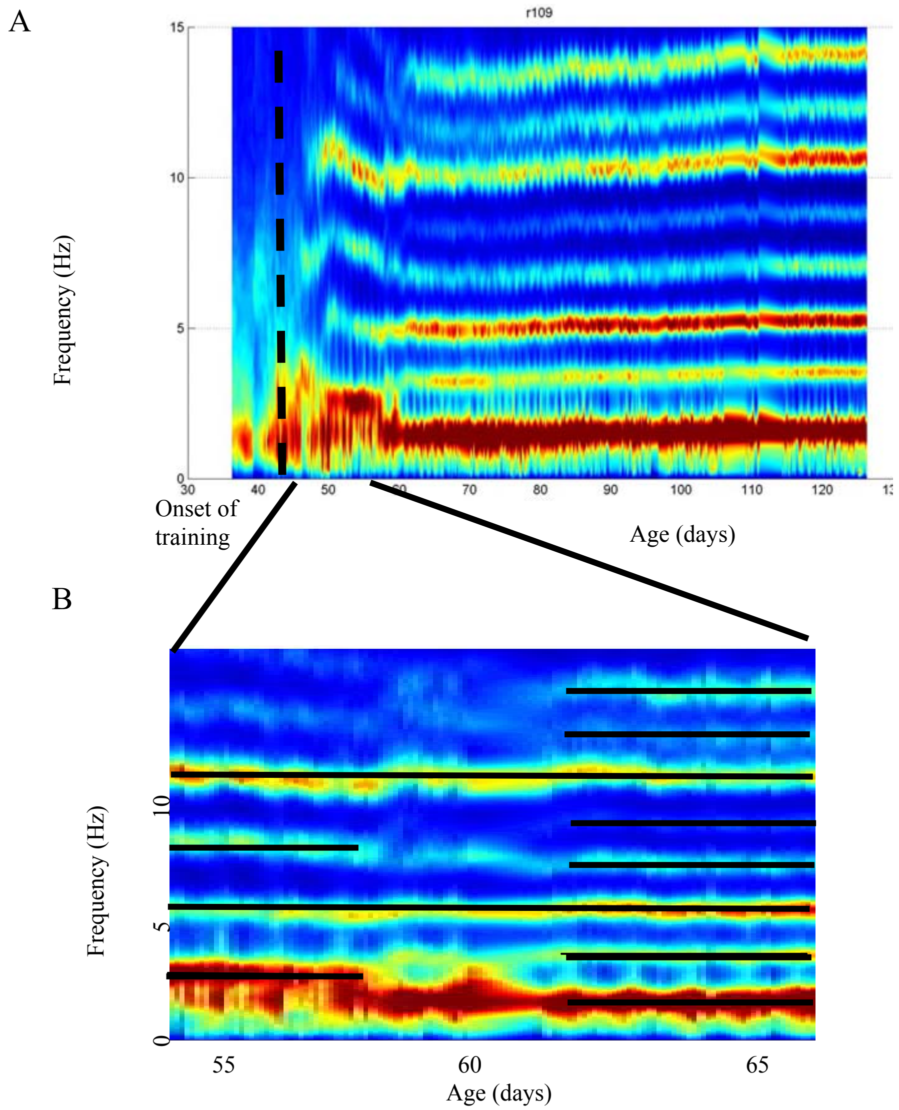


Figure 3.16 Bird r109 rhythm maps show smooth transitions between ages 50 to 60 days. In day 60, there is an abrupt transition in the rhythm. There is an indication that there is a relationship between the old rhythms and the new rhythms. The first and third harmonic (the first and third harmonic of the fundamental) of day 50 transform to be the second and fifth harmonic respectively. The bifurcation ratio is 2:3.

The ontogeny of bird R109 motifs show a transition from two syllables to three syllables (Figure 3.17). The bird stretching its motif between days 50-60, this could be in preparation for the rhythm transition (we can not prove this point). On day 60 a new syllable is integrated into the song (Figure 3.17). This syllable has already appeared in the song on day 53, but it appeared only at the end of the song bout; after finishing a motif that included a new syllable, the bird could not start another motif. On day 57 we see examples of a single motif that follows the new syllable. However, the bird does not integrate yet the new syllable into its entire song. It generates a compound bout, where the first iterations of the motif have two syllables, while the last iterations have three syllables (Figure 3.17).

Temporal alignment of song spectral derivatives on the onset of the first syllable (Figure 3.18) shows that the transition to the new motif happens abruptly (Figure 3.19; Figure 3.20 E). The temporal alignment also shows that the first appearances of the new syllable seem to align on “hot spots” in the old motif. The new syllable starts exactly when the first syllable of the old motif should have started. With further development, the bird slowly shifts the time slot of the new syllable’s onset and shortens the interval between the new syllable and the previous syllable (Figure 3.20 B, D and E).

Figure 3.21 presents the DVD maps of syllable duration for bird R109. The peak of the new syllable (marked with an arrow) starts around day 55, but its energy is low and it is smeared. The peak becomes clear only after day 60. Another insight we learn from this DVD map is that between ages 50-60 the long syllable stretches, while the short syllable does not change so much or even condenses. This means that the motif/syllable stretching could be non-linear across the motif.

DVD maps compliment rhythm maps by showing the existence of a new syllable and explain abrupt transitions in rhythm. They do not provide information about the integration of the syllable into the repeating unit (the motif). As we will see in the next section, it is often the case that bad learners will produce a syllable that they can not integrate into their motif. Rhythm analysis provides us with additional insights into the periodicity of the motif, which interval analysis does not describe (such as when does the syllable integrates into the repeating unit and becomes part of the rhythm)

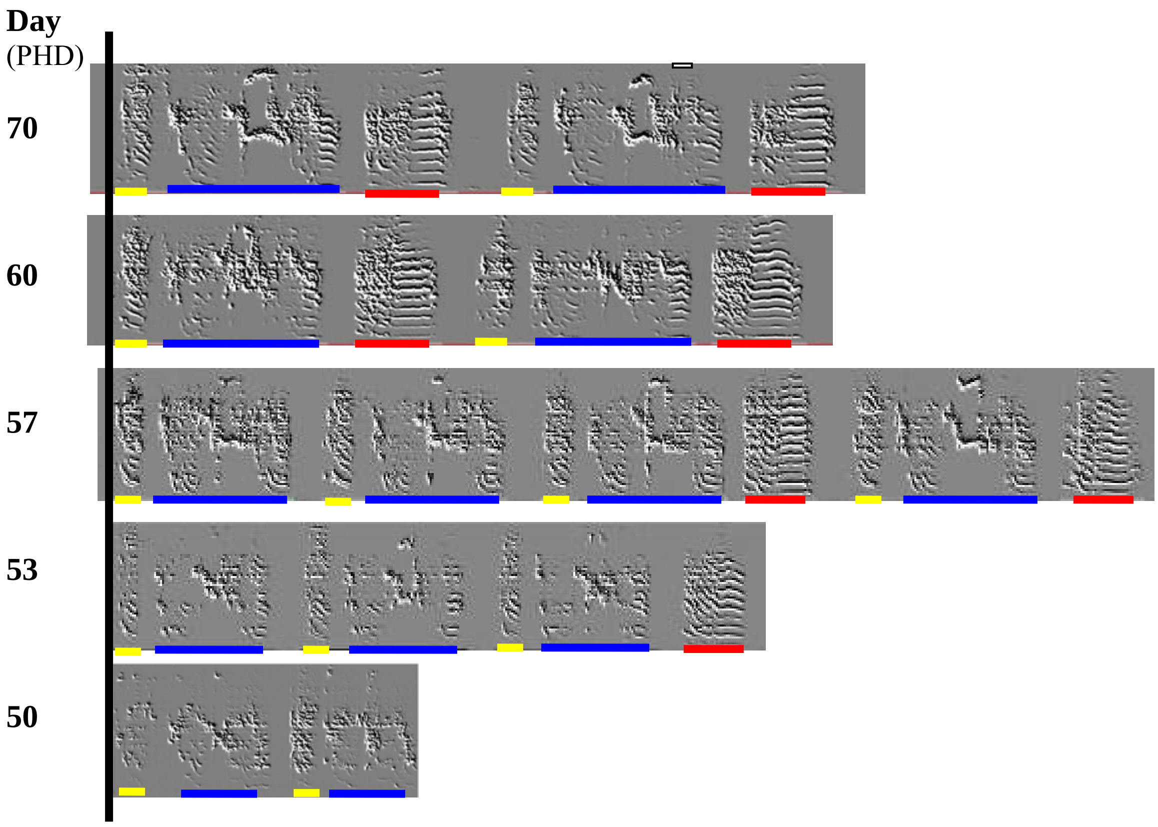


Figure 3.17 The ontogeny of bird R109 motifs shows a transition from two syllables to three syllables. Between days 50-60 there is a trajectory of motif stretching. In day 53 there is a new syllable in the song (marked in red). However, the bird could not start another motif after finishing a motif with the new syllable. At day 57 we see another single motif repetition following the new syllable (marked in red), but the bird does not integrate the new syllable in the song yet. It generates a compound bout, where the first iterations of the motif have two syllables, while the last iterations have three syllables. On day 60 the bird integrate the syllable into its song.

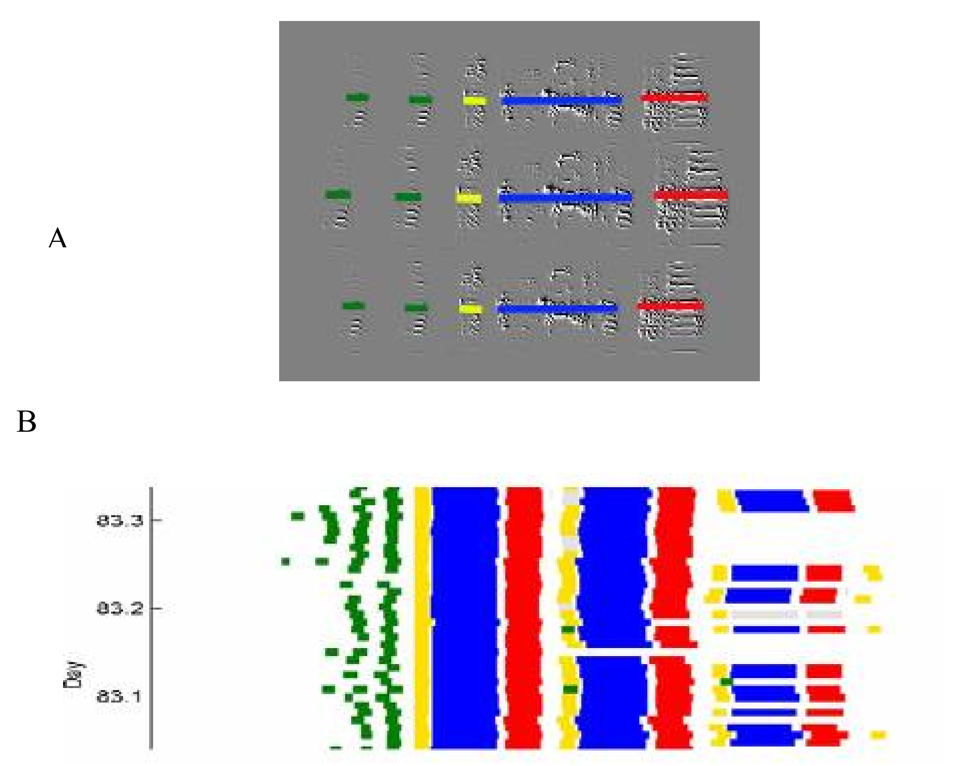


Figure 3.18 Alignment of bird R109 song bouts into raster plots. A. This panel shows an alignment example of the song spectral derivatives at the onset of the first syllable (marked in yellow). Each colored line represents the duration and syllable type (cluster association). B. This panel shows an alignment example of many bouts at the onset of the first syllable.

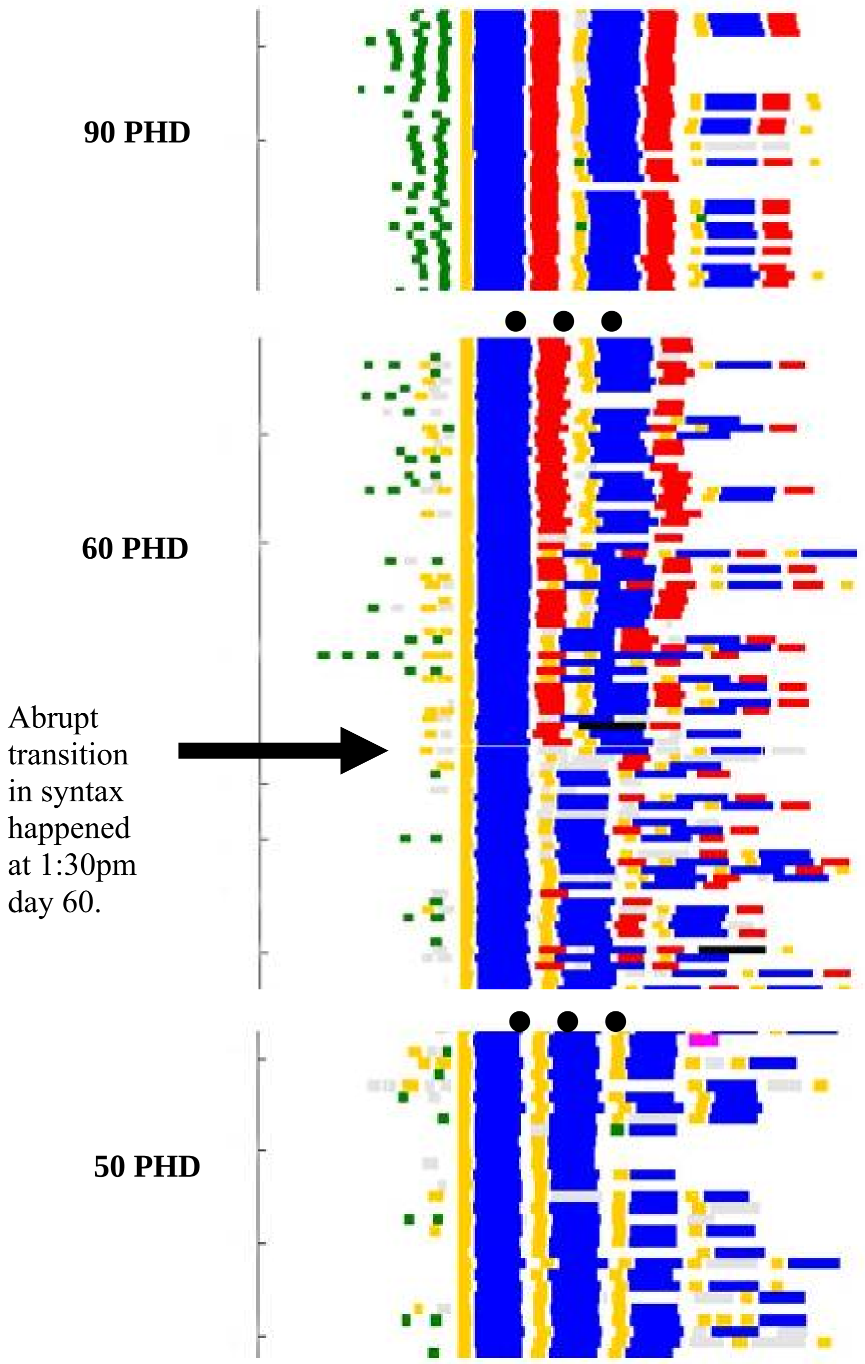


Figure 3.19 The temporal alignment of bird R109 song's spectral derivatives at the onset of the first syllable shows that the transition to the new motif happens abruptly.

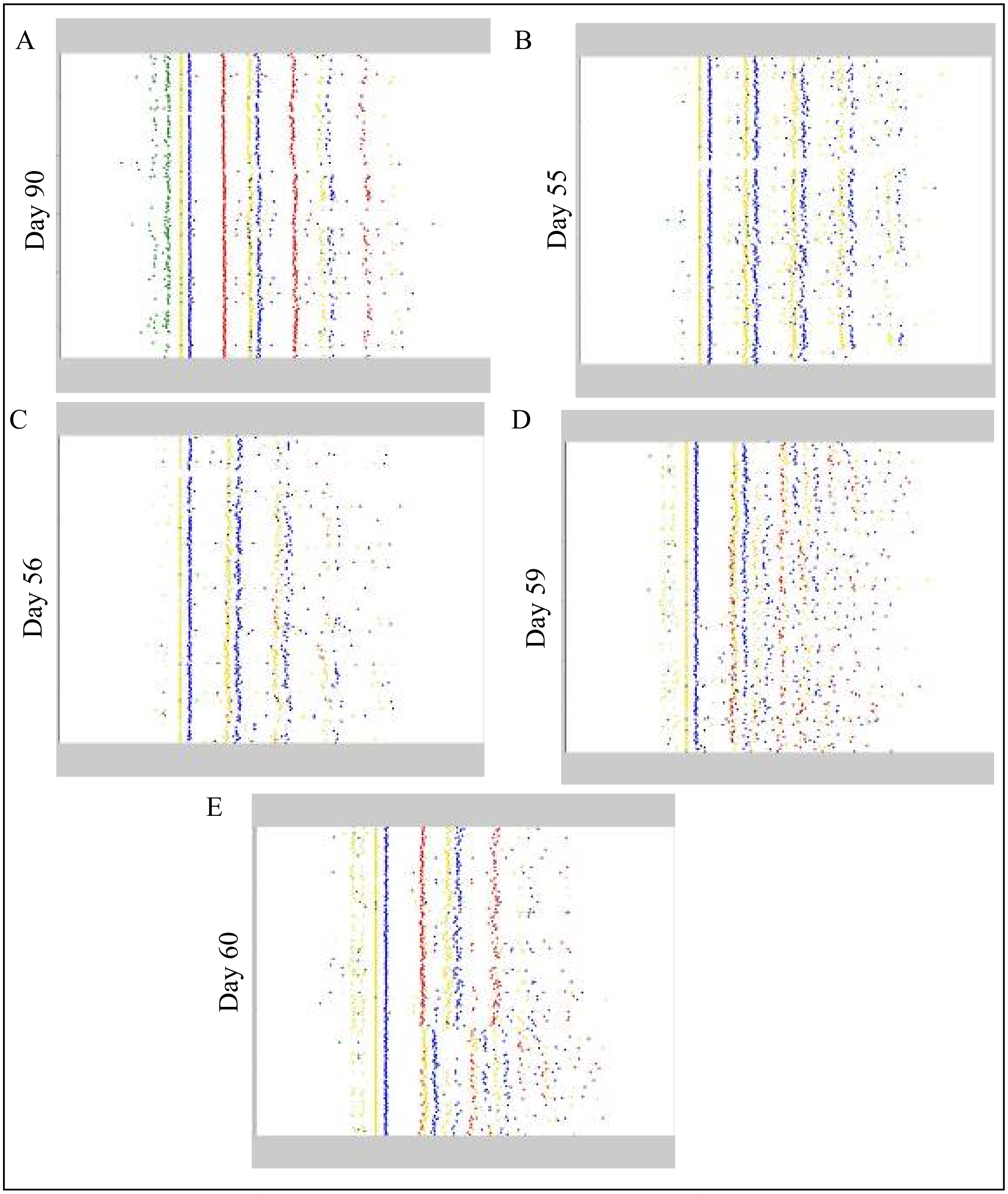


Figure 3.20 Raster plot of onsets colored according to syllable cluster for bird R 109. A. A raster plot of a mature song (90 PHD) where the green dots are onsets of introductory calls, yellow dots are onsets of the short first syllable, blue dots are onsets of the long second syllable and red dots are onsets of the medium length new and third syllable. B A juvenile song's raster plot (55 PHD) – the new syllable (red) appears in the song but it is not part of the song yet. C. We see here the Initial occurrences of the red onsets (56 PHD). The red onset first occurs at the end of the bout, and then spreads to the middle and the beginning. The red onsets begin at the time slot of the yellow onsets. D With development (59 PHD), the bird slowly shifts the time slot of the red syllable's onset and shortens the interval between it and the previous syllable. E. A fast transition between rhythms (60 PHD). The red onset (the new syllable) exists before the transition but not in an orderly manner. On day 60, there is an abrupt transition, where the new syllables are consistently part of the song.

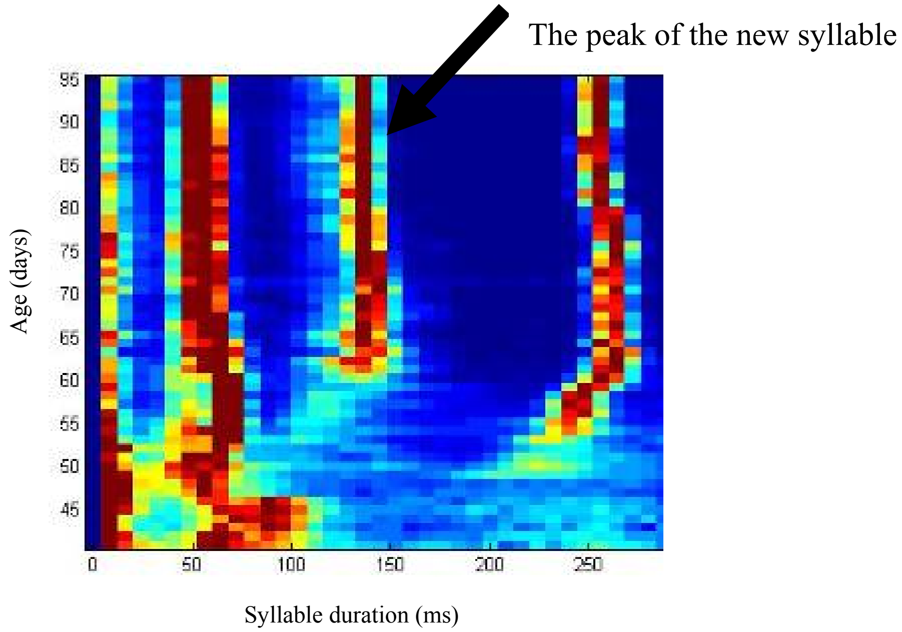


Figure 3.21 DVD maps of syllable duration for bird R109. The peak of the new syllable (marked with an arrow) starts around day 55. However, on day 55 the peak is not clear. It becomes clear only after day 60. DVD maps compliment rhythm maps by showing the existence of a new syllable; they do not provide information about the integration of the syllable into the repeating unit (the motif).

What does the rhythm features tell us about goodness of imitation?

Can we look at an adult bird's rhythm (on day 73) and find out from it how well that bird imitates? Does a bird rhythm tell us how well it imitates? We will show here three features that summarize spectral properties and correlate with imitation abilities: rhythm entropy, fundamental frequency of rhythm harmonics and number of rhythm peaks in the rhythm map. The birds were trained from age 43. We are interested in the learning that occurred during the first 30 days of training, since most of the training is done before day 73 (Figure 3.22).

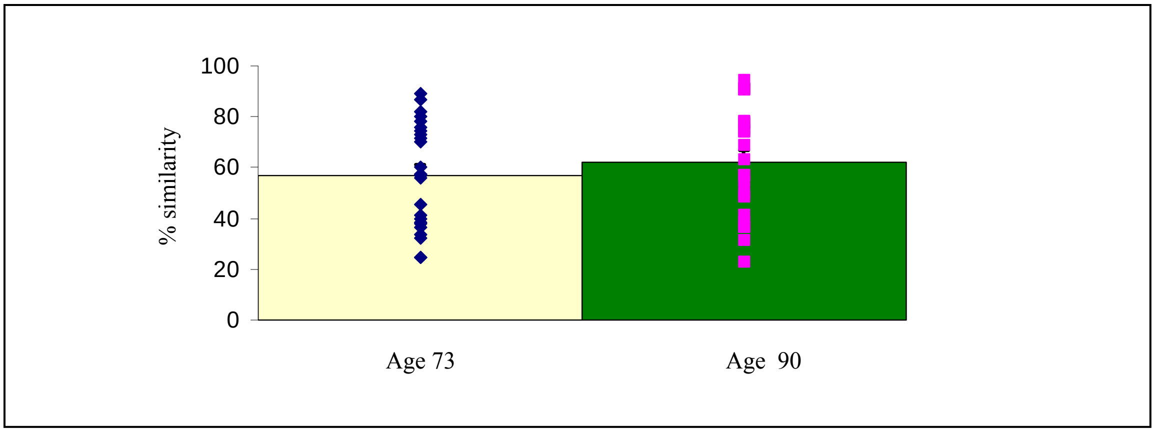


Figure 3.22 There is no significant difference between the average % similarity at age 73 and age 90 for birds trained from age 43. This figure shows data of 23 birds trained from day 43.

Wiener entropy:

Wiener entropy is a measure of the width and uniformity of the power spectrum. A narrow power spectrum (the extreme of this is a pure tone) has a large, negative Wiener entropy value. A broad power spectrum (e.g. white noise) has a Wiener entropy value that approaches zero. Rhythm Wiener entropy is a measurement for the rhythmicity of the song. Arrhythmic birds with a broad rhythm spectrum, such as juvenile birds, have high rhythm entropy. Adult birds have low rhythm entropy (Figure

3.23). We plotted and calculated a linear regression of the entropy of the rhythm spectrum between 0-30HZ against the gain (day 43 to day 73) in % similarity (i.e., what percent of the song is similar to the model, see page 29 for explanation of song similarity) from the onset of training to 30 days after training (day 73). The entropy is correlated to the gain in % similarity with a coefficient of determination ($r=-0.68$, $n=17$, $p<0.01$), Figure 3.24 A. This correlation means that rhythm spectra with low entropy indicates that the bird imitated well.

The fundamental frequency

The fundamental frequency of the rhythm harmonics indicates the duration of the repeating unit. Figure 3.27 (A) shows the fundamental frequency of the rhythm harmonics versus the gain (day 43 to day 73) in % similarity. The fundamental frequency is correlated to the gain in % similarity ($r=-0.8228$, $n=18$, $p<0.001$). This means that birds that achieve a long motif structure (a long repeating unit) also achieve good imitation.

The number of rhythm peaks:

Another descriptive feature is the number of rhythm peaks. Juvenile birds have fewer rhythm peaks than adult birds (Figure 3.25). A few factors influence the number of peaks.

- The duration of the motif determines how many possible harmonics can be in a frequency interval.
- The song becomes more rhythmic with development (Figure 3.23). As the repeating unit stabilizes, we see higher harmonics appearing.

- Finally, the specific shape of the repeated waveform may cause missing harmonics. Missing harmonic des not happen frequently in our data.

We plotted and calculated the linear regression of the number of peaks in the adult rhythm spectrum (0-30Hz) against the gain (day 43 to day 73) in % similarity (i.e., what percent of the song is similar to the model) from the onset of training to 30 days after training. The number of rhythm peaks is correlated to the gain in % similarity ($r=0.442$, $n=22$, $p<0.05$) (Figure 3.26 A). This means that rhythm spectra with many spectral peaks indicate that the bird imitated well. This feature strengthen what we saw earlier, birds that achieve a long motif structure (a long repeating unit) also achieve good imitation.

Intermediate discussion

The correlation of rhythm entropy with similarity to the song model is not a trivial result (Figure 3.24). It means that birds that achieved better imitation have a more rhythmic song than other birds. Why is that? After all, a bird that imitates badly can theoretically be just as stereotyped. This result **could** indicate that bad imitators seek to improve their song by keeping their motif variable, and hence could be stuck with a compound song after the song becomes stereotyped (Figure 3.28). Indeed, rhythm entropy and fundamental frequency seems to be correlated ($r=0.445$, $n=15$, $p<0.1$).

The correlation of the features: number of peaks and fundamental frequency, with imitation is another indication that bad imitators have a compound song (Figure 3.26, 3.28). In bad imitators who sing a compound song, only the stereotyped parts will be captured by the rhythm analysis, i.e. those bad imitators will have a shorter repeating unit. A bird imitating song model A perfectly (we named this song model “Simple”) will have a fundamental frequency of 1.84Hz , song model B (we named this song model

"Samba") 1.25Hz , and song model C (we named this song model "Bells") 1.3Hz. The average fundamental frequency at age 120 PHD was 2.13Hz (table 3.1). This means that many birds have **a repeating unit which is shorter than their song model**. However, those birds often have additional syllables in their song that are not incorporated into the song's rhythm (Figure 3.28).

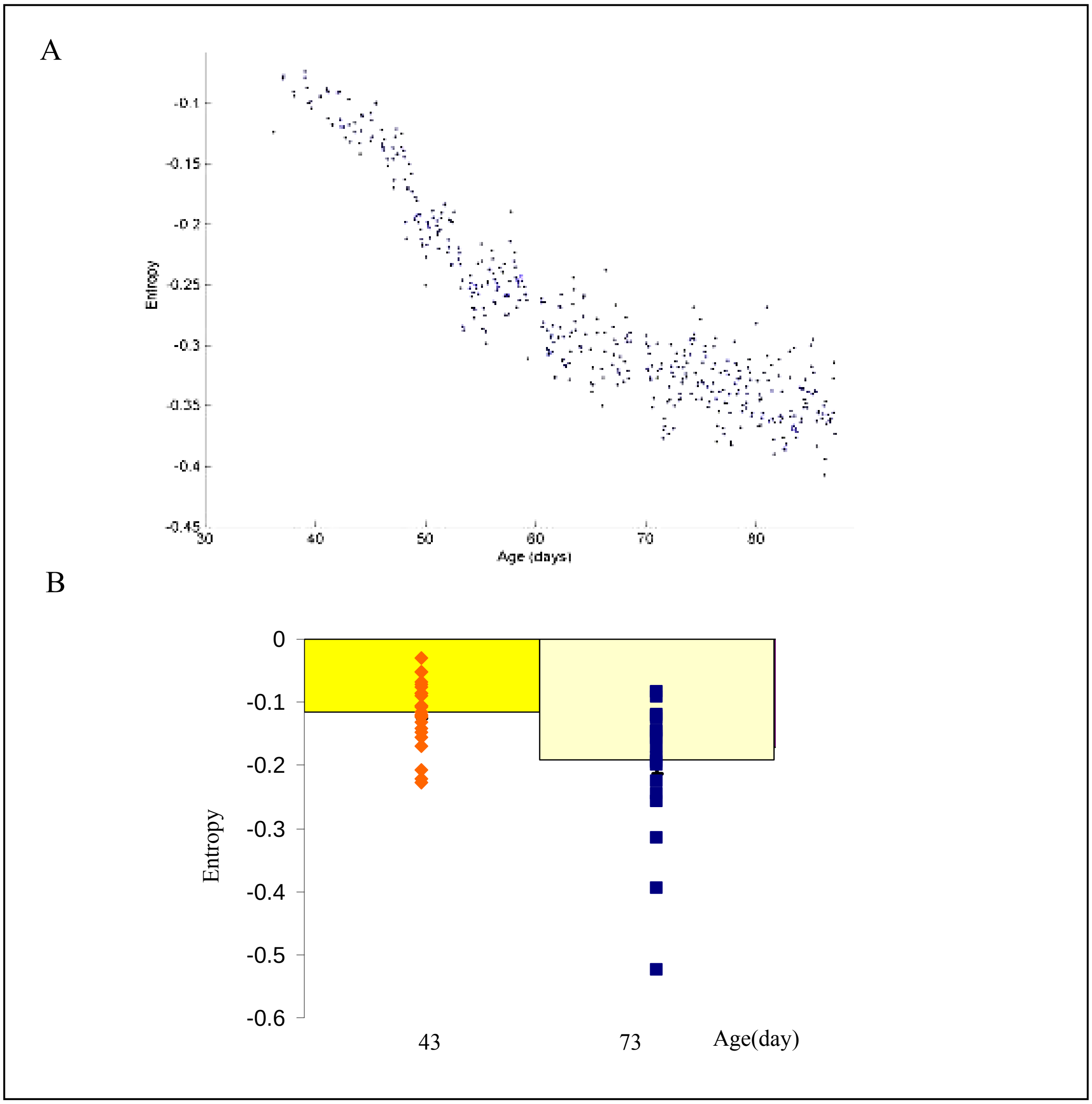


Figure 3.23 Rhythm entropy estimates how structured rhythm is. As shown here, during song development rhythm entropy decreases. A. Developmental rhythm entropy of bird R109. B. Average rhythm map's entropy at the onset of training (age 43) and 30 days after training (age 73) for 22 birds trained from day 43

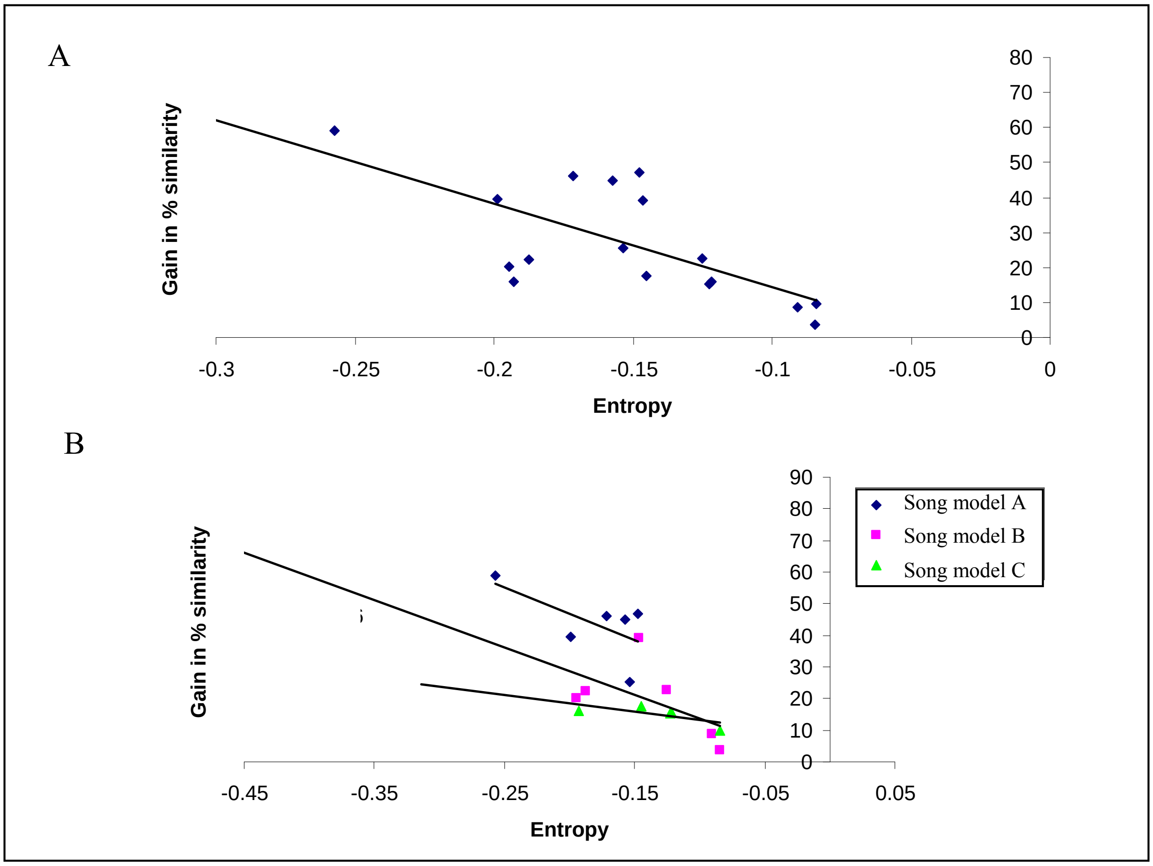


Figure 3.24 The entropy of the rhythm spectrum between 0-30HZ vs. the gain in % similarity (i.e., what percent of the song is similar to the model) from the onset of training to 30 days after training (day 43-73). A. The entropy is correlated to the gain in % similarity (r=-0.68, n=17, p<0.01) B. We group the data according to the training song model. (Song model A: r=-0.63, n=6, p<0,2 B: r=-0.57, n=6, p<0.3 C: r=-0.7, n=5, p<0.2)

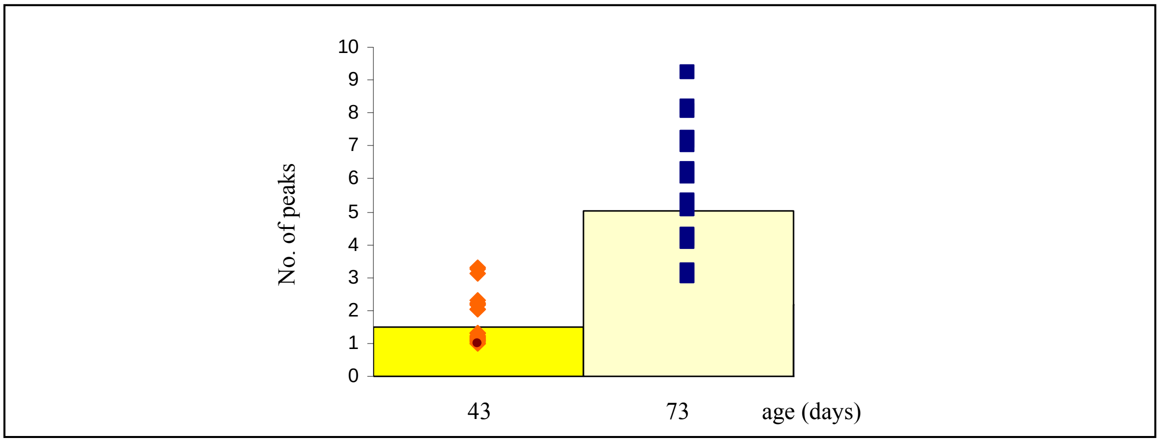


Figure 3.25 Juvenile birds have fewer rhythm peaks than adult birds. This figure shows the average number of rhythm peaks at the onset of training and 30 days after training for 21 birds trained from age 43. The peaks have been jittered a bit for display purposes.

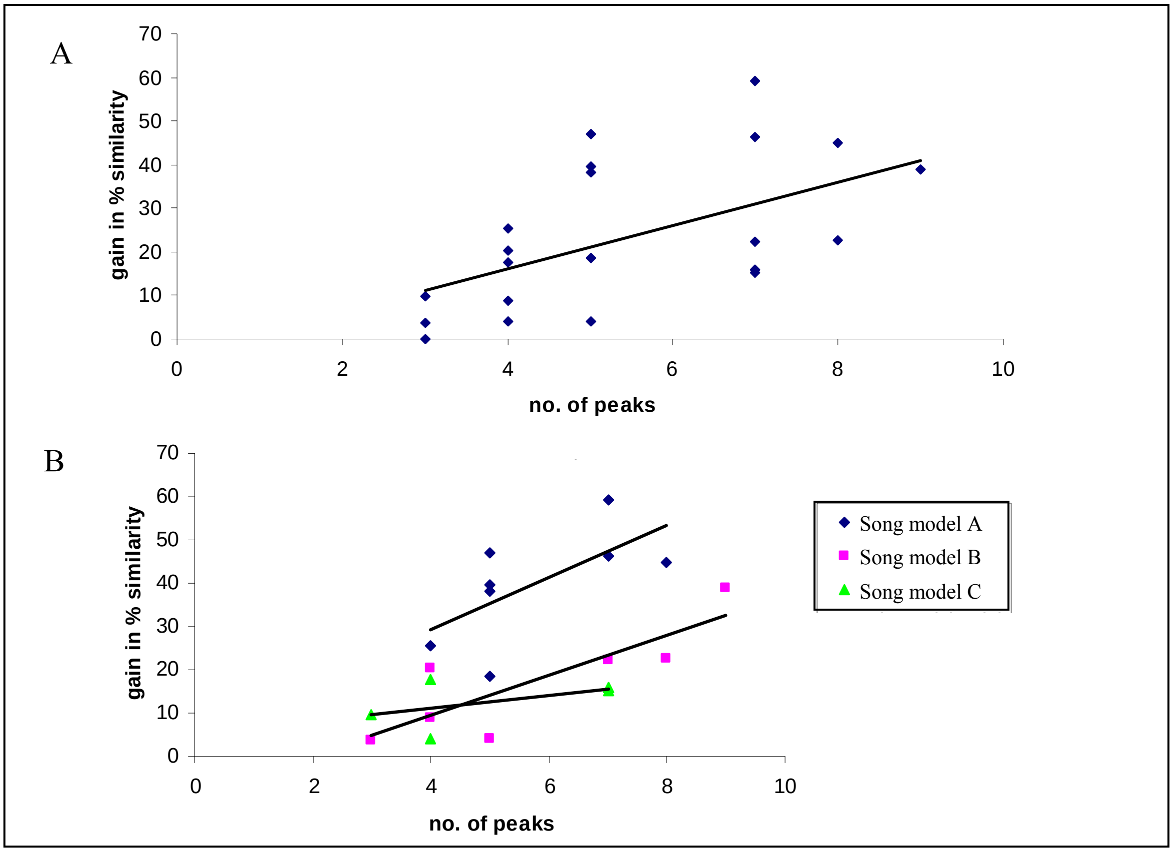


Figure 3.26 The number of peaks in the rhythm spectrum vs. the gain in % similarity (i.e. what percent of the song is similar to the model) from the onset of training to 30 days after training (day 43-73). A. The number of rhythm peaks is correlated to the gain in % similarity (r=0.442, n=22, p<0.05). B. We group the data according to the training song model. (Song model A: r=-0.647, n=8, p<0.09 B: r=0.695, n=7, p<0.09 C: r=0.565, n=7, p<0.2)

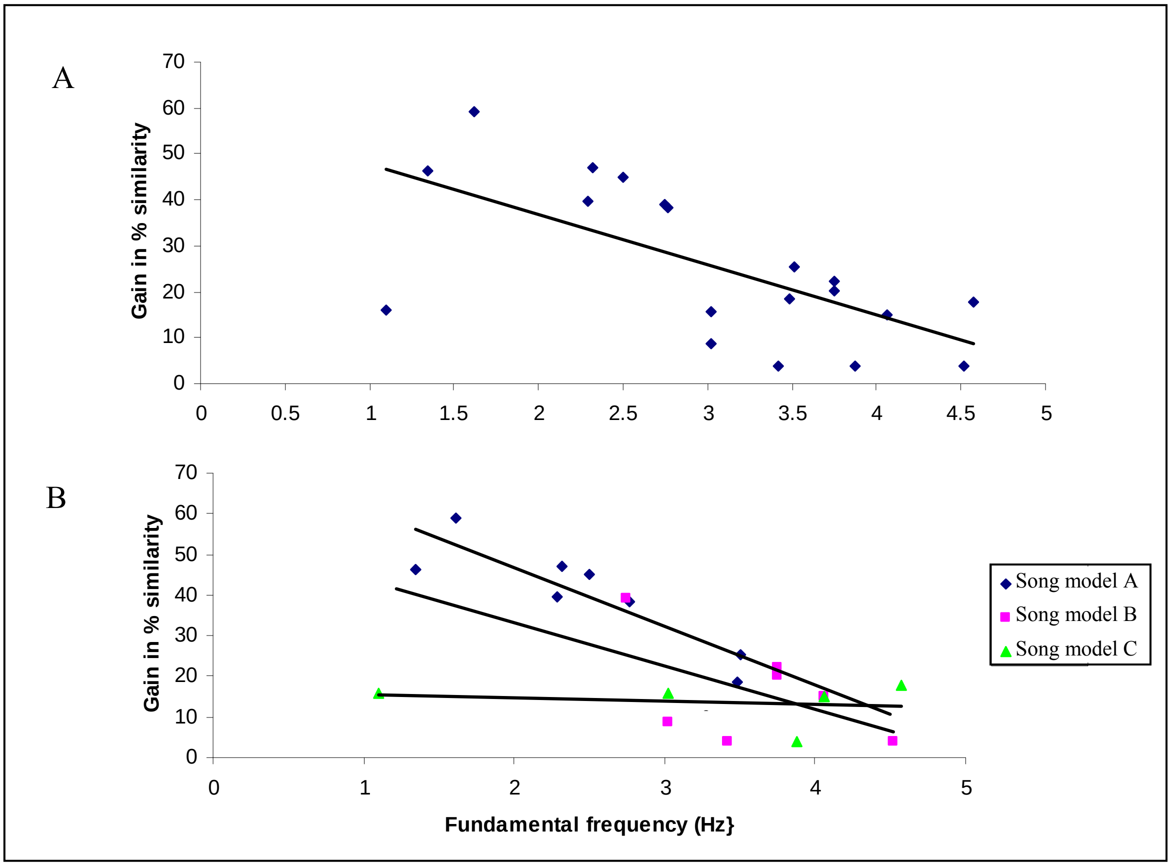


Figure 3.27 The fundamental frequency of the rhythm spectrum (at age 90) vs. the gain in % similarity (i.e., what percent of the song is similar to the model) from the onset of training to 30 days after training (day 43-73). The fundamental frequency is correlated to the gain in % similarity (r=-0.8228, n=18, p<0.001). B. We group the data according to the training song model. . (Song model A: r=-0.88, n=8, p<0.005 B: r=-0.5321, n=6, p<0.3 C: r=0.103, n=4, p<0.9)

Song model	**Perfect imitation fundamental frequency (Hz)**	**Average fundamental frequency (Hz)**
A	1.84	2.47
B	1.25	2.061
C	1.3	1.91

Table 3.1 According to the average fundamental frequency at age 120 PHD per song model, on average birds have a shorter repeating unit than the song model.

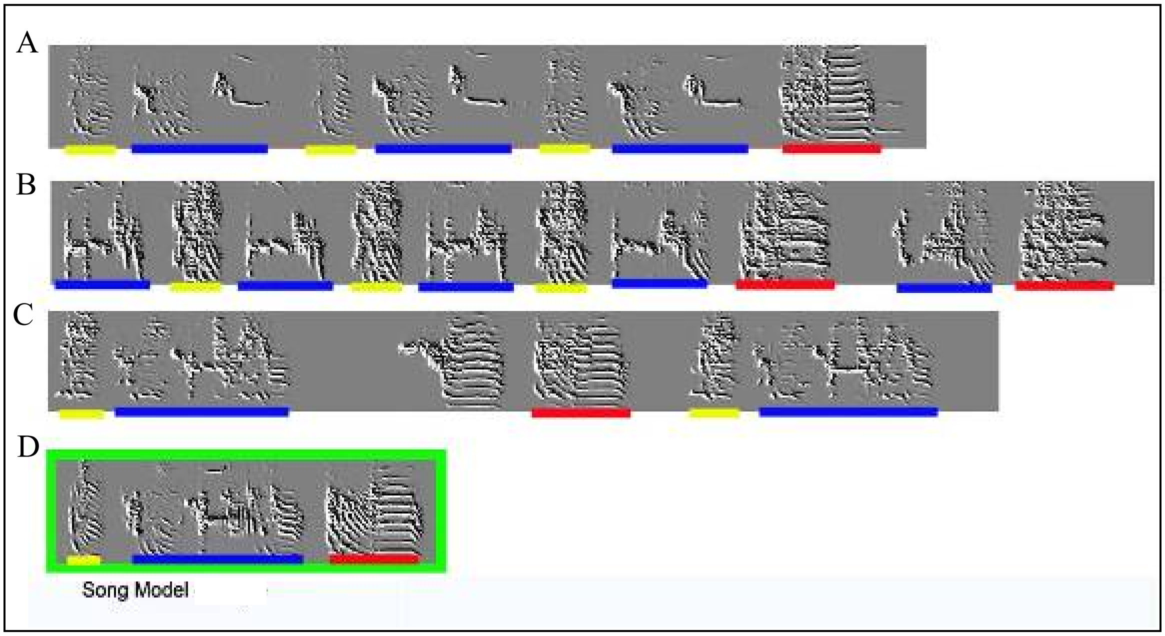


Figure 3.28 Three examples of adult songs of birds that are trained with song model A, and do not integrate one of the syllables in to their rhythms (A-C). D is the song model the birds heard. All the birds are producing an imitation of the three syllables in the motif, but only the first and second syllables (yellow and blue syllables) are incorporated in their rhythm. A. The fundamental frequency of the rhythm of this bird is 3.5Hz. The duration of the yellow and green syllable for that bird is 305ms, which corresponds to its fundamental frequency. B. The fundamental frequency of the rhythm of this bird is 4.5Hz. The duration of the yellow and green syllable for that bird is 250ms, which corresponds to its fundamental frequency. B. The fundamental frequency of the rhythm of this bird is 2.6Hz. The duration of the yellow and green syllable for that bird is 370ms, which corresponds to its fundamental frequency.

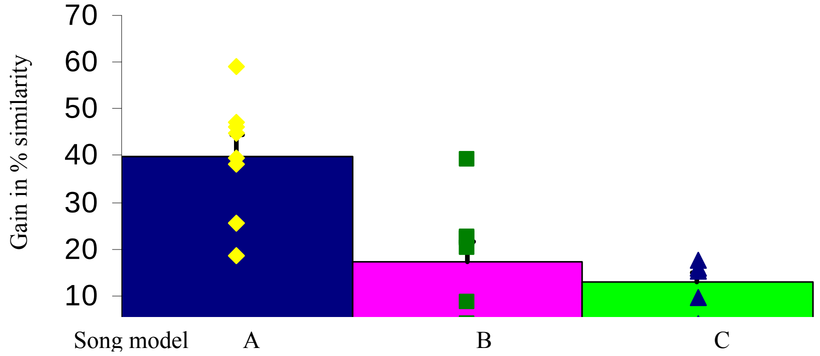


Figure 3.29 It is easier for birds to imitate some song models over others. Birds trained with song model A achieve on average better imitation than birds trained with song models B and C. The data is according to 25 birds trained from day 43.

Discussion

The developmental trajectory of nervous system dynamics shows hierarchical structure on time scales spanning ten orders of magnitude, from milliseconds to years. Analyzing and characterizing this structure poses significant signal processing challenges. In the context of birdsong development, temporal structure on the millisecond timescale is normally captured using a short time Fourier analysis, and structure on the second timescale using song spectrograms. In this chapter, we used a dynamic spectrum on time series of song features to study the development of rhythm in zebra finch.

Across birds, we see that rhythm transitions come about in two forms: smooth downward modulation and abrupt transition in rhythms, where the new frequency and the old frequency seem to be related to the old rhythm. We see smooth down-modulations when the bird is time-warping (usually stretching) specific syllables that are already included in the motif. Abrupt transitions occur when the bird inserts a new syllable into its song, or differentiates a prototype. The abrupt rhythm transitions shown in this chapter convey a relationship between the old rhythm and the new rhythm, which support a strategy of nonlinear modulation of rhythms where the old rhythm relates to the new rhythm (Figure 3.1 D).

Rhythm entropy is correlated with imitation abilities (Figure 3.19). This indicates that birds that imitate well sing a stereotyped song. Bad imitators have a more complex song, with a shorter repeating unit (Figure 3.21, 3.22), and might have additional elements that are not incorporated in to the rhythm (Figure 3.24). The constraint on incorporating those additional elements into the repeating unit exhibited by the relationship between the old rhythm and the new rhythm, and the smooth rhythm transitions some birds do before an abrupt rhythm transition (which could be preparations for an abrupt rhythm transition), are additional indications that rhythm is not an epiphenomena. Rhythm development seems to be a process of nonlinear and smooth modulations of rhythm rather than a gradual appearance of rhythm or a simple transition from one rhythm to an unrelated rhythm.

We would like to review a model that could explain the constraints on rhythm transition that we observe. This model connects our observations of abrupt rhythm transitions to the participation of respiratory nuclei in the control of the rhythm generator. We know of two forebrain nuclei that have been implicated in the control of the temporal structure of song: HVC and RA. The midbrain and respiratory areas project back to HVC through Uva (Striedter & Vu 1998, Ashmore & Renk 2008), raising the possibility that syllables, which are tightly linked to respiratory patterning (Goller & Copper 2004), may be timed by respiratory oscillator circuits (Ashmore et al. 2005; Cooper & Goller 2006). It was suggested that HVC may contain multiple independent chains (or modules) (Glaze & Troyer 2006), which may be associated with syllables or long subsyllabic elements (Schmidt 2003). Thus each HVC can be seen as implementing a short-term clock or "music box" (Glaze & Troyer 2006) whose output is read by downstream nuclei such as RA and its brainstem targets which serves as a feedback connection through thalamus and back to HVC. The recurrent activity ascending from these target nuclei (such as DM and PAm) may then perform the role of a second, longer-term clock that governs overall tempo and synchronization (Ashmore et al. 2005). In this model, the HVCs would receive periodic signals from the brainstem clock (we do not know yet where in the brain stem it happens, but it should include connections to the respiratory nuclei if not to occur there) , and generate a small portion of its own temporal code in response to each global “tick”. This would ensure that the two halves of the song system (right hemisphere and left hemisphere) remained synchronized and coordinated sufficiently in their output to produce the temporal sequence of song with adequate precision (Ashmore et al. 2005). Respiratory feedback could provide crucial information about limitations at the periphery, such as the ability to maintain the high respiratory demands imposed by song (Wild et al. 1998). Integration of this feedback with descending motor commands may provide important signals for regulating both the tempo and duration of song production.

It has been shown that the respiration cycle has a mechanical impact on some motor cycles. For example, magpies have a constant wingbeat to respiratory cycle ratio per bird. That includes the ratio of 3:1 most commonly observed among birds (Berger et al. 1970) as well as ratios of 5:2 and 2:1. (Boggs 1997, Boggs et al. 1997). In galloping mammals the gait and respiratory cycles are phase locked on a 1:1 basis (Bramble 1989). If indeed song rhythm is as tightly linked to respiratory patterning as the model above suggests and the global clock is in respiratory related nuclei then we expect the rhythm generator to be related to the respiration pattern of the bird. The general respiration patterns do not change their steady state abruptly during development, i.e. there will be a temporary respiratory change in response to a stimuli such as a female bird (Cooper and Goller, 2006; Glaze and Troyer, 2006) but once the stimuli is over the bird will go back to its steady state respiratory cycle. Yet, song rhythm does change during development (Chapter 3). Hence we would expect those abrupt rhythm transitions to be limited to a ratio that relates to the respiratory cycle, as we see here. We observe one or two abrupt transitions in rhythms during development. In the birds we examined, the abrupt rhythm transitions had a small integer ratio such as 1:2 or 2:3. The relationship between the old rhythm and the new rhythm could originate from the relationship of the song rhythm to the respiratory cycle and the constraints it imposes on the system (for example the bird can not start a syllable because the respiration cycle indicates it should expire at that moment or it doesn't have enough air to sing that syllable). Note, as observed in the ratio of wing beat and respiratory cycle, the relationship between the old rhythm and the new rhythm might be different for different birds.

In chapter 3, we examined rhythm development in birds trained at early stages of the sensitive period. In the following chapter we will examine song development of birds that are socially and acoustically isolated until nearly the end of their sensitive period and compare it to rhythm development of juvenile birds.

Chapter 4. Rhythm development in late trained birds

Introduction

In zebra finches, a young male initially produces highly variable, unstructured vocalizations. The juvenile bird acquires his song during a sensitive period. By 3 month of age, the acoustic structure stabilizes into a stereotyped sequence of syllables (Immelmann 1969). Song development is to some extent hardwired. For example, socially and acoustically isolated birds (Price 1979) and birds deafened early in life (Nottebohm 1972) go through subsong and plastic song stages, comparable to those of intact birds. Nevertheless, the timing and the outcome of song development are strongly affected by the presence of a tutor, other social factors (Price 1979, Feher 2008), hormonal state (Nottebohm 1969) and nutrition (Nowicki 1998). Even the end of the sensitive period is not determined solely by age, but also by hormones and experience, e.g., castration can delay the end of the sensitive period for song learning. (Nottebohm 1993). The closure of the sensitive period could be delayed by withholding exposure to the song model (Eales 1985, Morrison and Nottebohm 1993, Jones et al. 1996).

Even though the sensory phase occurs from day 25-65 post hatching and the sensorimotor stage ends before day 90 (Immelmann 1969) birds that are isolated from conspecific songs can still learn to some extent even after day 90. However, while early trained birds achieve good imitation of the song model, most late trained birds imitate poorly (Figure 4.1, 4.2). In this chapter we explore why late learners cannot imitate well and try to predict which late trained birds will be able to imitate well. Does the rhythm of isolated birds 90 days old can indicate how well they will learn their song?

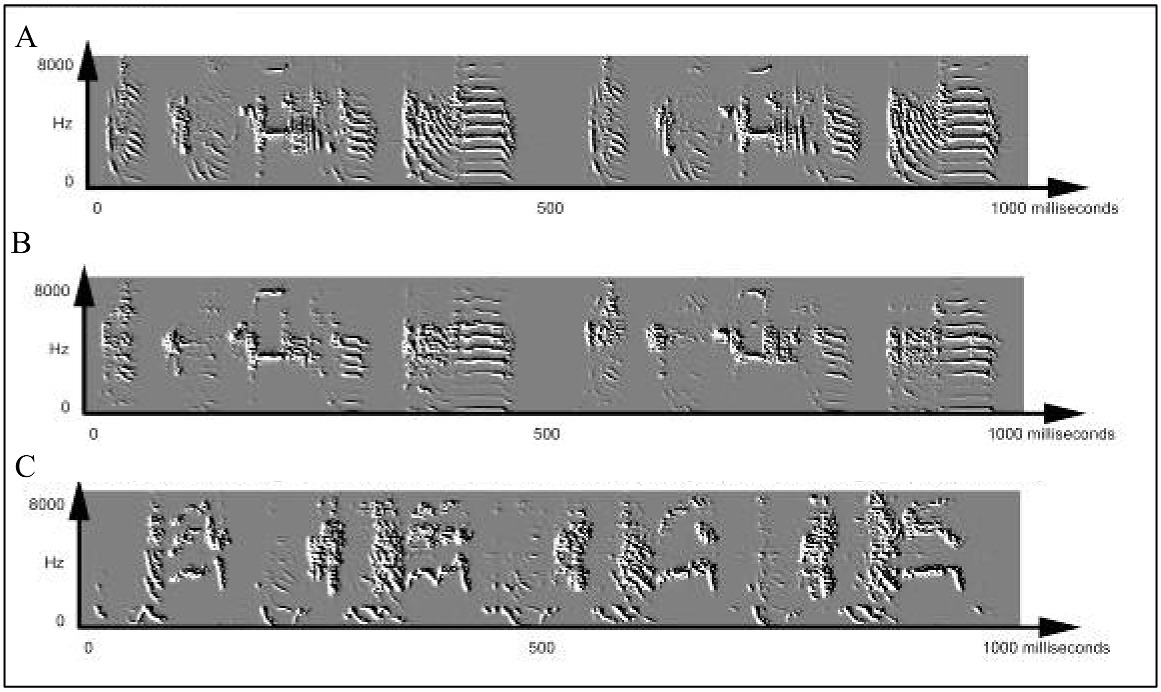


Figure 4.1 Examples of imitation. A. Socially and acoustically isolated birds were exposed to the song model shown here. B. This song is an example of an adult bird's song. This bird was socially and acoustically isolated until day 43. On day 43 it was exposed to the song model. The bird's adult song is very similar to the song model (84% similarity score). C. This song is an example of another adult bird. This bird was socially and acoustically isolated until day 90 and then exposed to the song model. It did not imitate the song model well (50% similarity score).

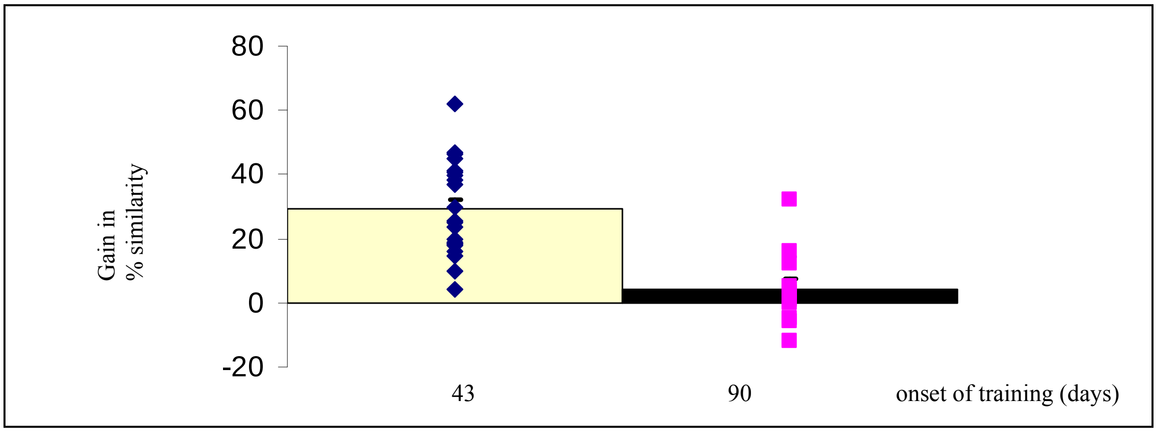


Figure 4.2 The gain in % similarity after 30 days of training. 23 birds trained from day 43, 12 birds trained from day 90. The gain in % similarity was averaged over three song models.

Methods

Subjects & training

We used 46 zebra finches (Taeniopygia guttata) from the City College of New York breeding colony. Sébastien Derégnaucourt and Ofer Tchernichovski trained and recorded the birds. All birds were kept in socially and acoustically isolation from day 30 to day 120 after hatching. 23 birds were trained with song playbacks starting from day 43 after hatching (Tchernichovski et al. 2001) 12 birds were trained with song playbacks starting from day 90 after hatching and 11 birds were kept in isolation until day 120. We used three different songs. Birds were raised from hatching under an artificial photoperiod of 12 h : 12 h LD (Deregnaucourt et al. 2005). Further information about the training procedure, song recording and analysis and song models can be found in the method section of Chapter 3.

Results

The age of the bird at the onset of training has a strong effect on the similarity of the adult's song to the song model. While early trained birds achieve good imitation of the song mode late trained birds imitate poorly (Figure 4.1, 4.2). The similarity to the song model of late-trained birds is not significant. Does that indicate that late-trained birds can not change their song? We will explore the reason for late-trained bird's inability to imitate across three time scales:

- Short time scale: milliseconds, where we look at the song's feature distribution
- Intermediate time scales: hundred of milliseconds, where we look at the song's syllables
- Long Time scale: hours, where we look at the song's rhythms

Short time scale

We compared the developmental changes of the distribution of acoustic features on a millisecond timescale. Acoustic features (amplitude, AM, FM, pitch, entropy, mean frequency, mean frequency amplitude and pitch goodness) were calculated using a sliding window of 10ms, for every 1ms (Figure 1.6). The magnitude of vocal changes was estimated between 30 days after the onset of training to the day before the onset of training, by using Kolmogorov Smirnov statistics, (which measures the difference between the cumulative distribution function of both days). Before applying the KS data we removed occurrences of long calls from the song. Long calls are not part of the bout, and could add noise to our analysis. Our analysis shows that, as expected, in the first 30 days of training birds trained from day 43 are able to change their millisecond distributions more than birds trained from day 90 (on average -- Figure 4.3 A). Between ages 90-120, birds trained from day 90 make more millisecond change between days 90-120 than isolated birds (on average), Figure 4.3 A. Most of the birds trained from day 43 changed their KS distribution significantly less than isolates and late trained birds between days 90-120, Figure 4.3 A and B. Note an interesting finding, young isolated birds (ages 43-73 PHD) make significantly more changes in their millisecond distribution than birds trained from age 43 at that age, Figure 4.3 A.

What is interesting about those results is that even though, on average, late trained birds do worse then early trained birds they still do better than the bottom 25% of early trained birds and some birds do better than 50% of the early trained birds, Figure 4.3 B.

In early trained birds there is a correlation between KS values and gain in % similarity ($r=- 0.47$, $n=23$, $p<0.03$). However, in late trained birds we don't see a significant correlation between the KS values and the gain (day 90 to day 120) in % similarity ($r=-0.016$, $n=6$, $p<0.97$). This result explains the phenomena that we visually see (Figure 4.4), some late trained birds might produce vocal changes on the millisecond level, but that does not necessarily improve their imitation. If the late trained birds who are bad learners can change their song at least to some extent, why can't they change it in a way that improves the imitation? Could it be that they can not add or remove syllables, or could it be that they can not modify their rhythm?

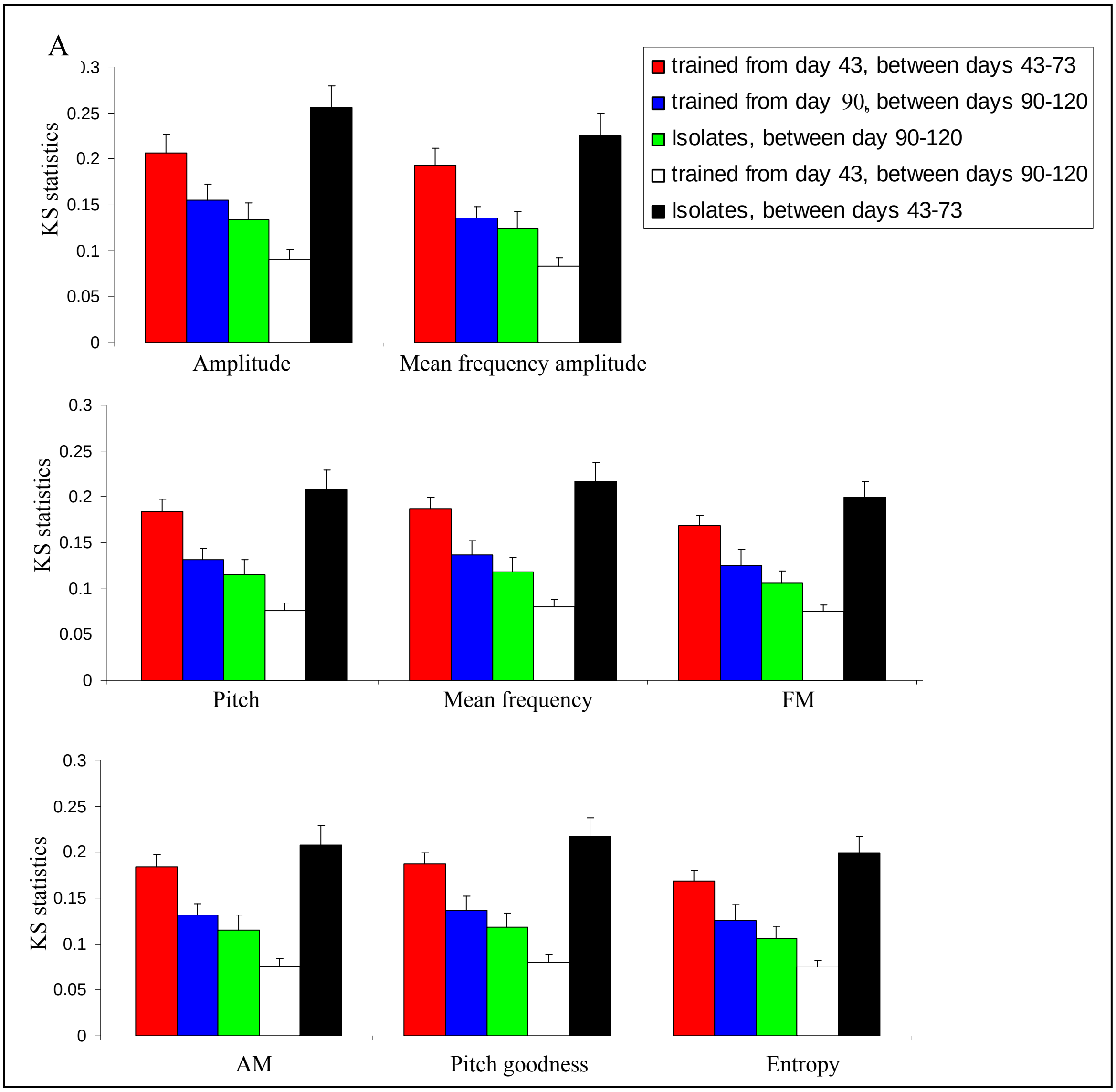
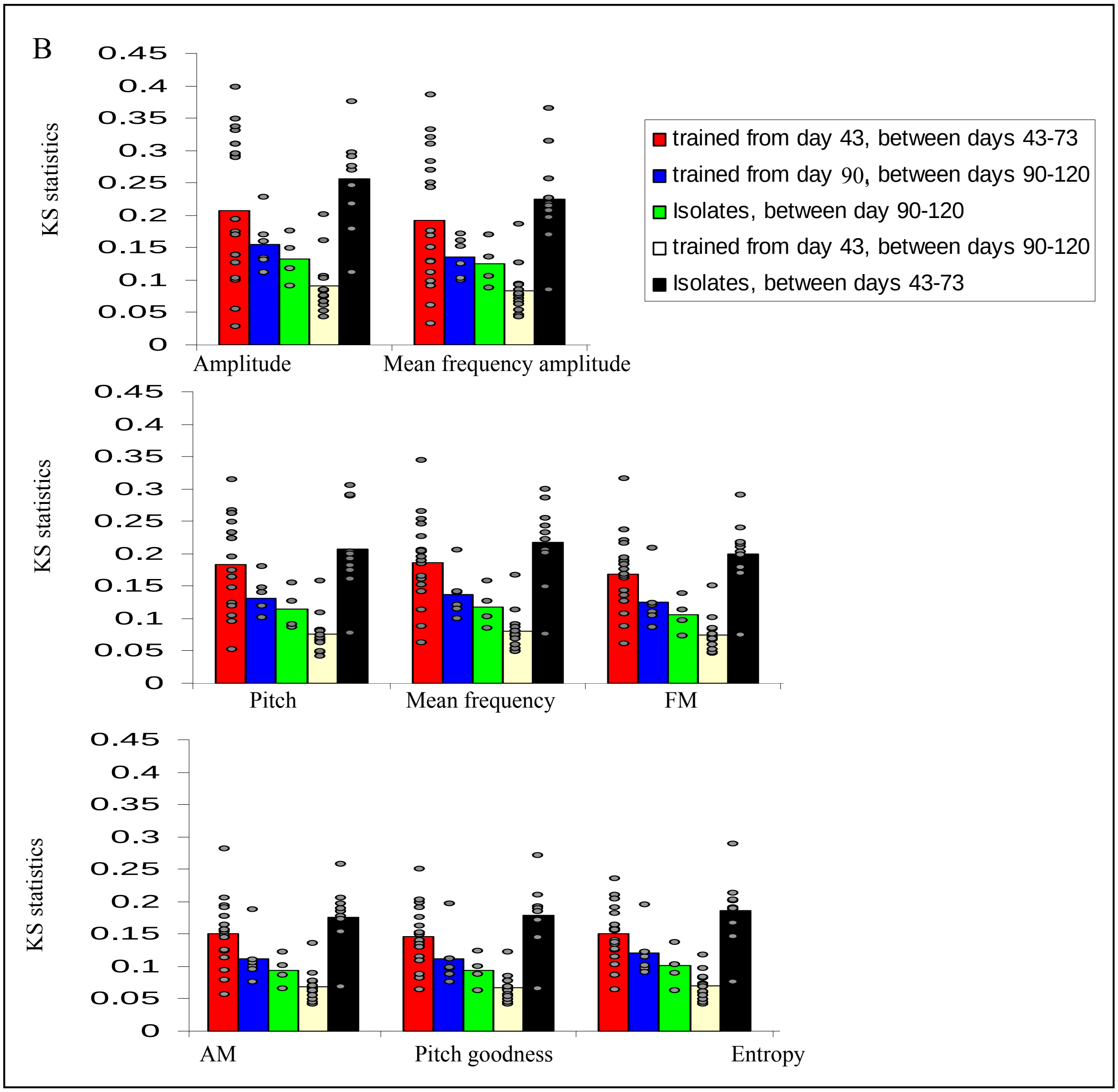


Figure 4.3 KS statistics of late trained birds, isolates and early trained birds.. 23 birds trained from day 43, 6 birds trained from day 90 and 4 isolates. Three song models. It is hard to interpret the standard errors from a scatter display of the individual birds, for that reason we plotted the same graph twice once with standard errors (A.) and once with scatter plots of individual bird data (B).


B
KS statistics
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Amplitude
Mean frequency amplitude
Pitch
Mean frequency
FM
AM
Pitch goodness
Entropy
🟥 trained from day 43, between days 43-73
🟦 trained from day 90, between days 90-120
🟩 Isolates, between day 90-120
☐ trained from day 43, between days 90-120
■ Isolates, between days 43-73

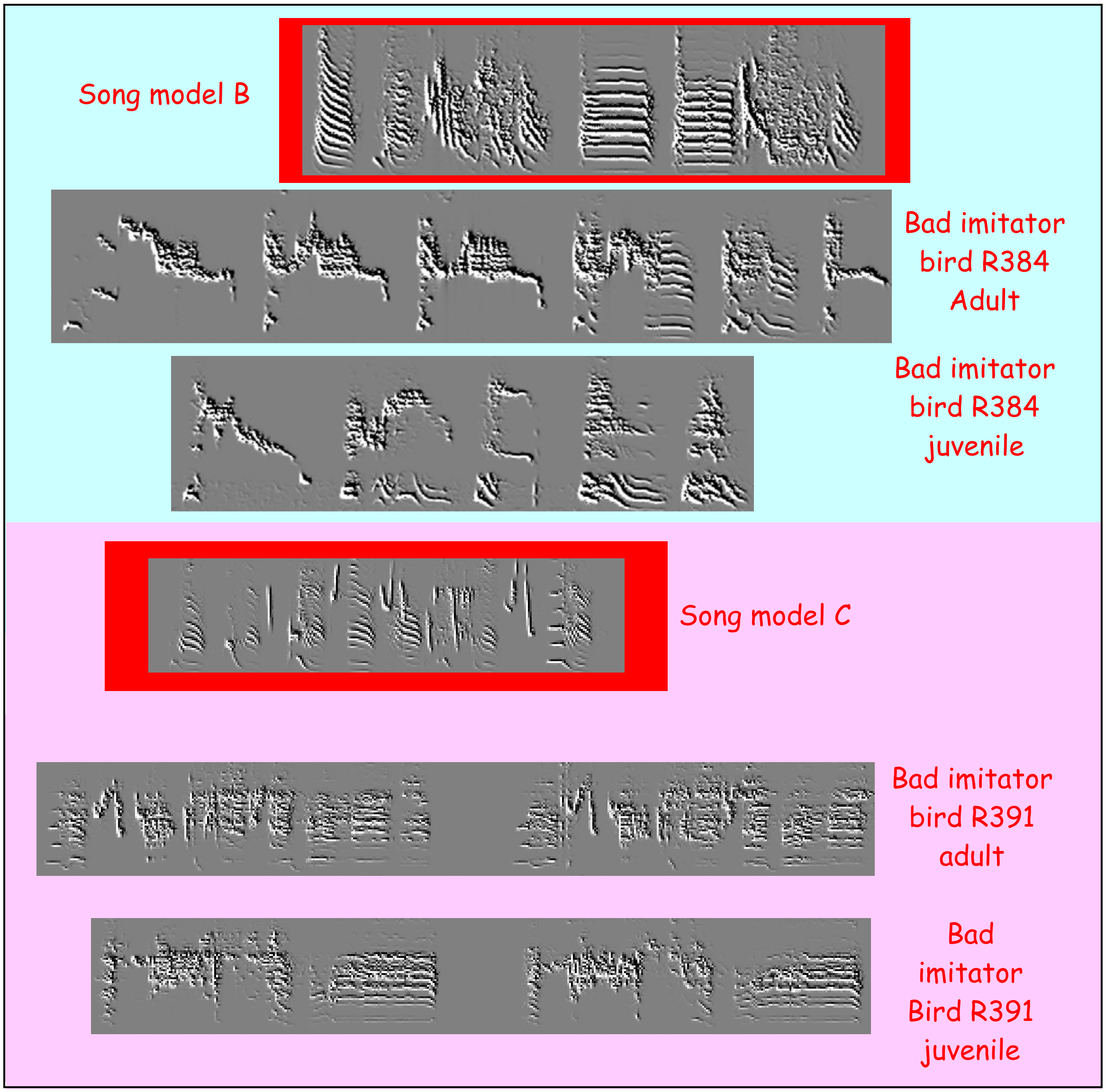


Figure 4.4 Examples of late trained birds who imitated badly despite changing their song on a millisecond level. A. Bird R384 achieved a gain in % similarity of 0. B. Bird R391 achieved a gain in % similarity of 5.

Intermediate time scales- syllable level

In order to study developmental effects on the syllable level, we first segment the syllables using an amplitude threshold and then summarize the structure of each syllable into an array of acoustic features (Figure 1.6). We compute the mean and variance of song features across the syllable. Together, the mean and variance of the feature values summarize the structure of each syllable in a simple and biologically intuitive manner (e.g., duration, mean pitch, variance of pitch, etc.). Syllables that belong to the same syllable type have similar features. Hence, when we plot scatter plots (Duration vs. mean entropy) , syllables that belong to the same syllable type group together and form a cluster. (Tchernichovski et al. 2004).

We studied distributions of syllable features and song ontogeny of three birds that did not achieve good imitation of the song model (Figure 4.5-4.7). All the birds modify their clusters (syllable features) and two of the three birds eliminate other clusters (syllables). Yet, after 30 days of training, their % similarity increased by less than 14%. Therefore, we see that late-trained birds can modify their syllables, insert stops, and eliminate syllables. Since that is the case, why are they not able to imitate well?

DVD (Dynamic Vocal Development) maps of early trained birds (Figure 1.3, 4.8 G-I) display new peaks that suddenly appear. Those peaks signify a new syllable which is introduced to the song. DVD maps of late trained birds (Figure 4.8 A-E) do not show that new syllables are added to the song. The DVD map of bird r404 (Figure 4.8 F) does show a new peak, which might signify the formation of a new syllable type. But closer inspection (Figure 4.5) reveals that the “new syllable” is actually caused by the merging of two old syllables (S1’ and S1 in Figure 4.5).

Inspection of the DVD maps for all the late trained birds does not reveal new peak formation, i.e. new syllables are not inserted to the song. In chapter 3 we showed that insertion of syllables causes an abrupt transition in the rhythm maps. Inspection of late trained bird's rhythm map (Figure 4.9, 4.10) did not reveal any abrupt transition in rhythms maps.

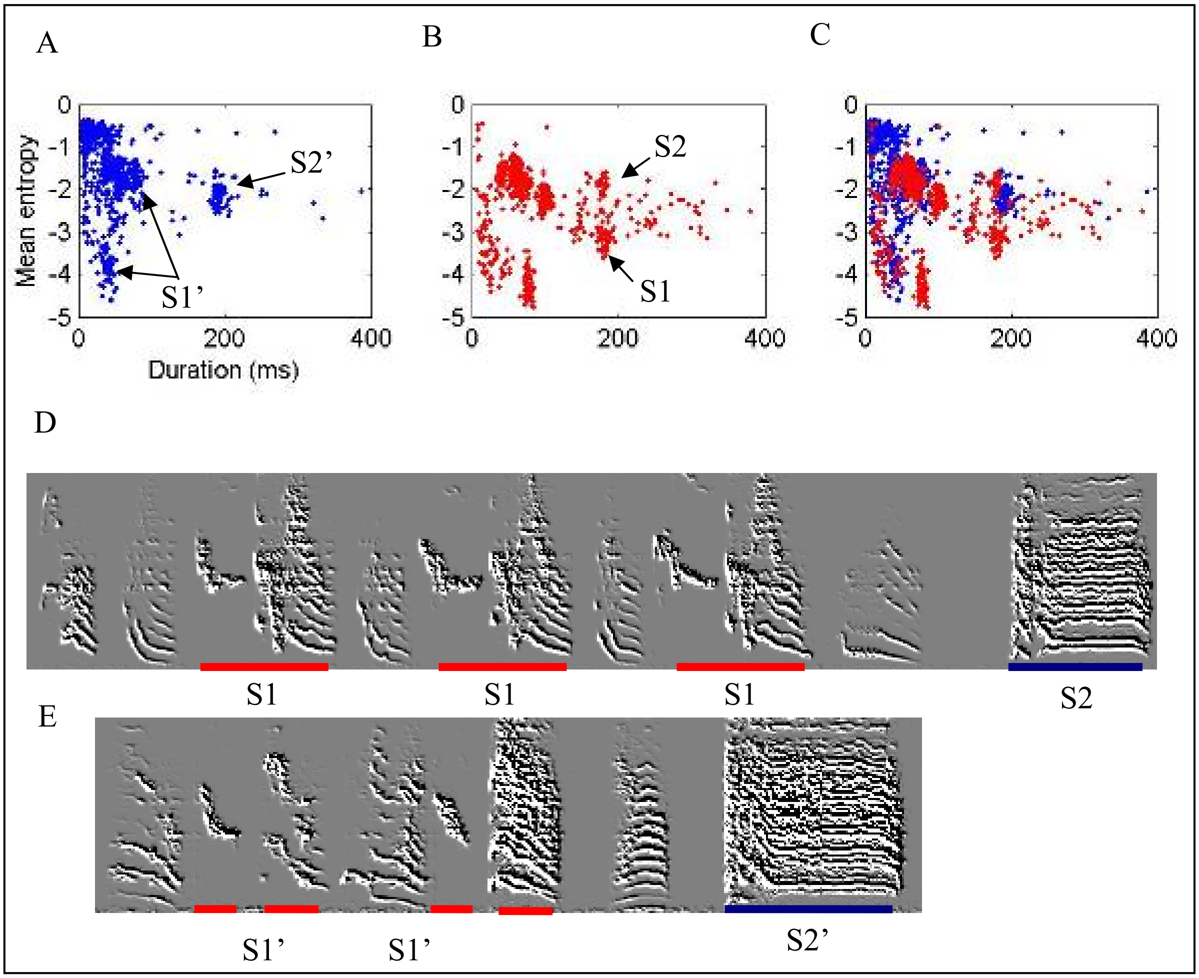


Figure 4.5 Distributions of syllables features and song ontogeny of bird r404. A. Distribution of syllable's features (Duration vs. mean entropy) before the onset of training (day 90). Note, syllable S2' and the two clusters that are marked by S1'. B. Distribution of syllable's features (Duration vs. mean entropy) after 30 days of training (day 120). Syllable S2 is a shorter version of syllable S2'. Its occurrence is less frequent than at age 90. The two syllables which composed S1' merged into one syllable (S1) C. An overlay of the two distributions. We can see that clusters shift, new clusters emerged, and the density of clusters changed. D. The song of Bird r404 on day 120. The gain in % similarity after 30 days of singing is -12%. Syllable S1 is marked in red while syllable S2 is marked in blue. E. Bird r404 song on day 90. The two syllables composing S1' are marked in red while syllable S2' is marked in blue.

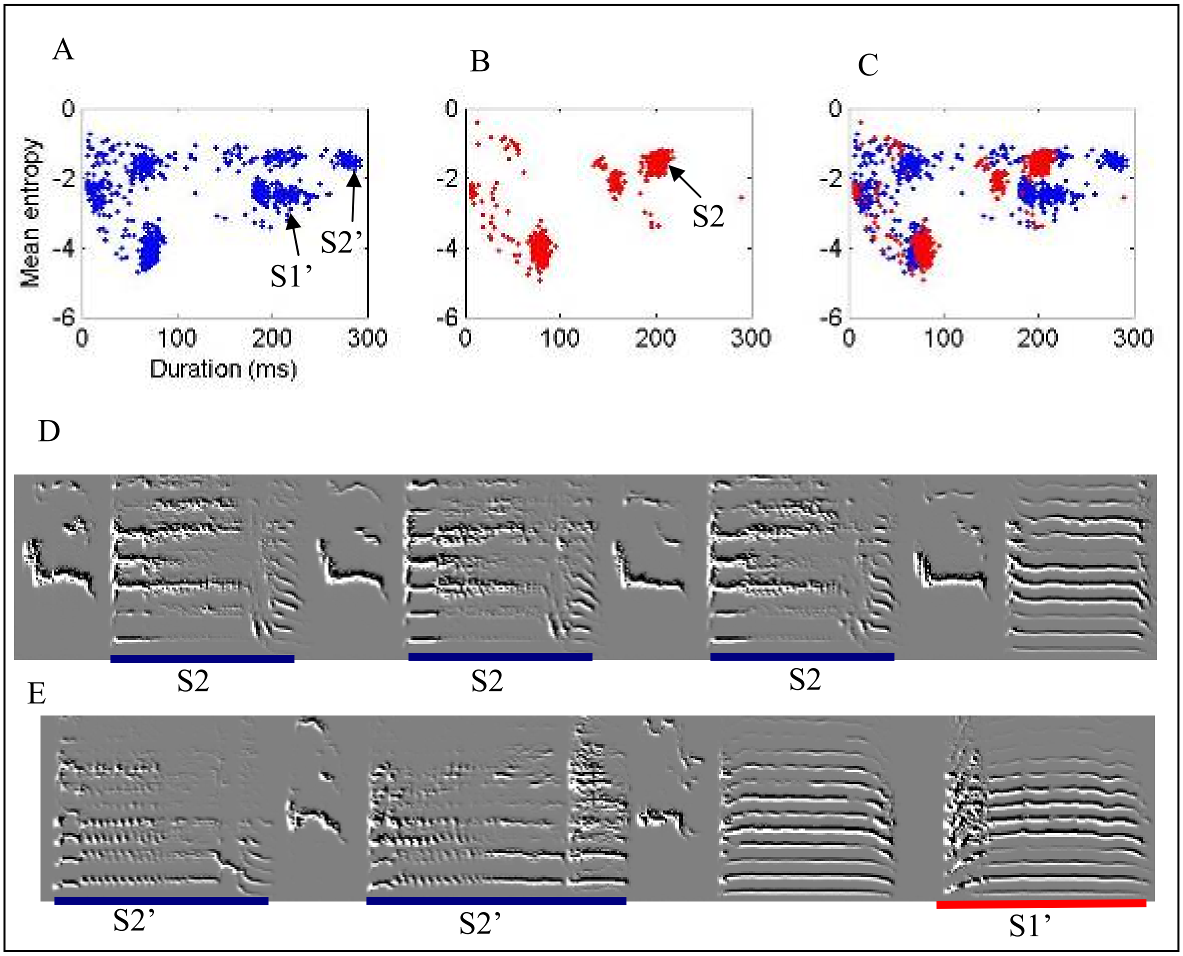


Figure 4.6 Distributions of syllables features and song ontogeny of bird r393. A. Distribution of syllable's features (Duration vs. mean entropy) before the onset of training (day 90). B. Distribution of syllable's features (Duration vs. mean entropy) after 30 days of training (day 120). Syllable S1' from panel (A) is eliminated (i.e. the bird does not produce it anymore). Syllable S2 is a shorter version of syllable S2'. C. An overlay of the two distributions. We can see that clusters shift, new clusters emerged, and some clusters are eliminated. D. The song of Bird r393 on day 120. The gain in % similarity after 30 days of singing is 14%. Syllable S2 is marked in blue. E. Bird r393 song on day 90. The syllable S1' is marked in red while syllable S2' is marked in blue.

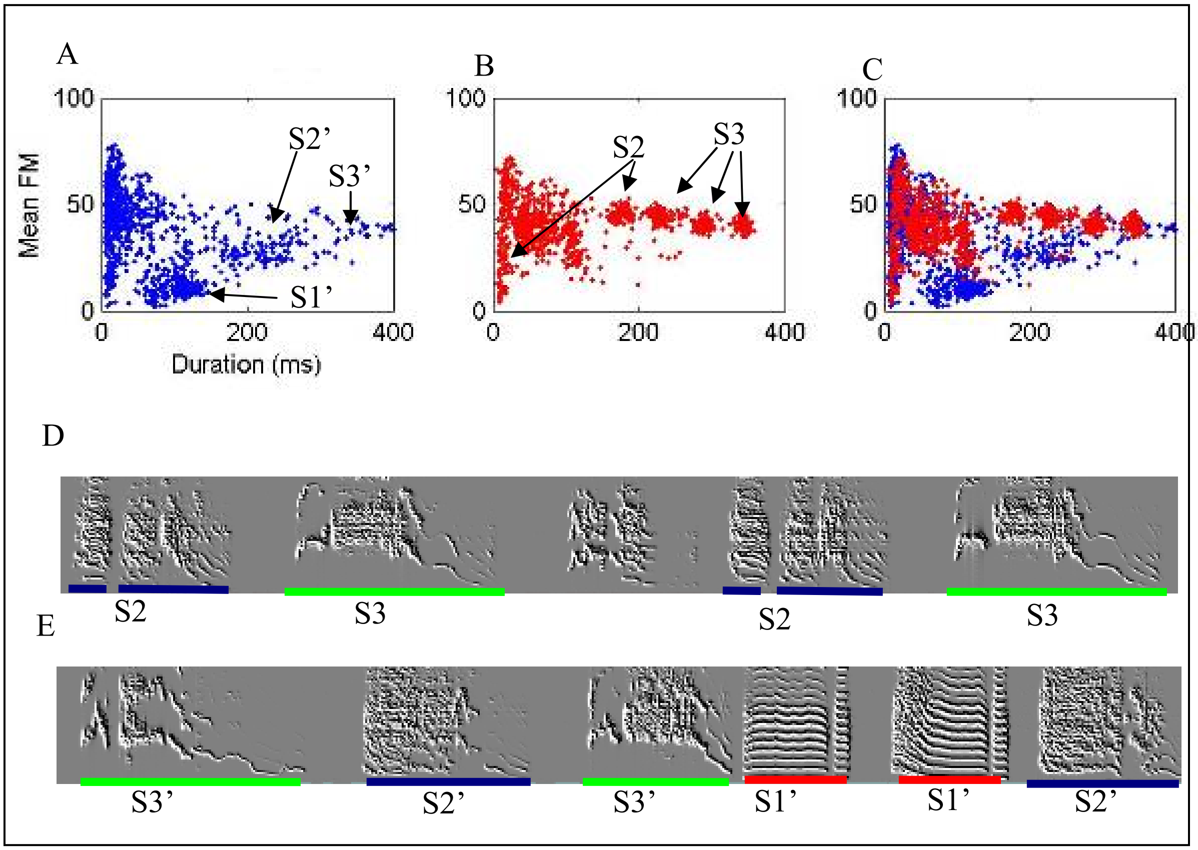


Figure 4.7 Distributions of syllables features and song ontogeny of bird r396. A. Distribution of syllable's features (Duration vs. mean FM) before the onset of training (day 90). B. Distribution of syllable's features (Duration vs. mean FM) after 30 days of training (day 120). Syllable S1' from panel (A) is eliminated (i.e. the bird does not produce it anymore). The bird inserted a stop in syllable S2' and split the adult version of the syllable, S2, into two syllables. Syllables S2' and S3' are variable in their duration and FM, while the adult versions, S2 and S3, are more stereotyped and sung more often. There are three clusters representing syllable S3 because the edges of the syllable have variable and low amplitude which are cut of at times by the segmentation algorithm. C. An overlay of the two distributions. We can see that clusters shifted, new clusters emerged, and some clusters were eliminated. D. The song of Bird r396 on day 120. The gain in % similarity after 30 days of singing is 12%. The two syllables that make S2 are marked in blue, syllable S3 is marked in green. E. Bird r396 song on day 90. Syllable S1' is marked in red while syllable S2' is marked in blue and syllable S3' is marked in green.

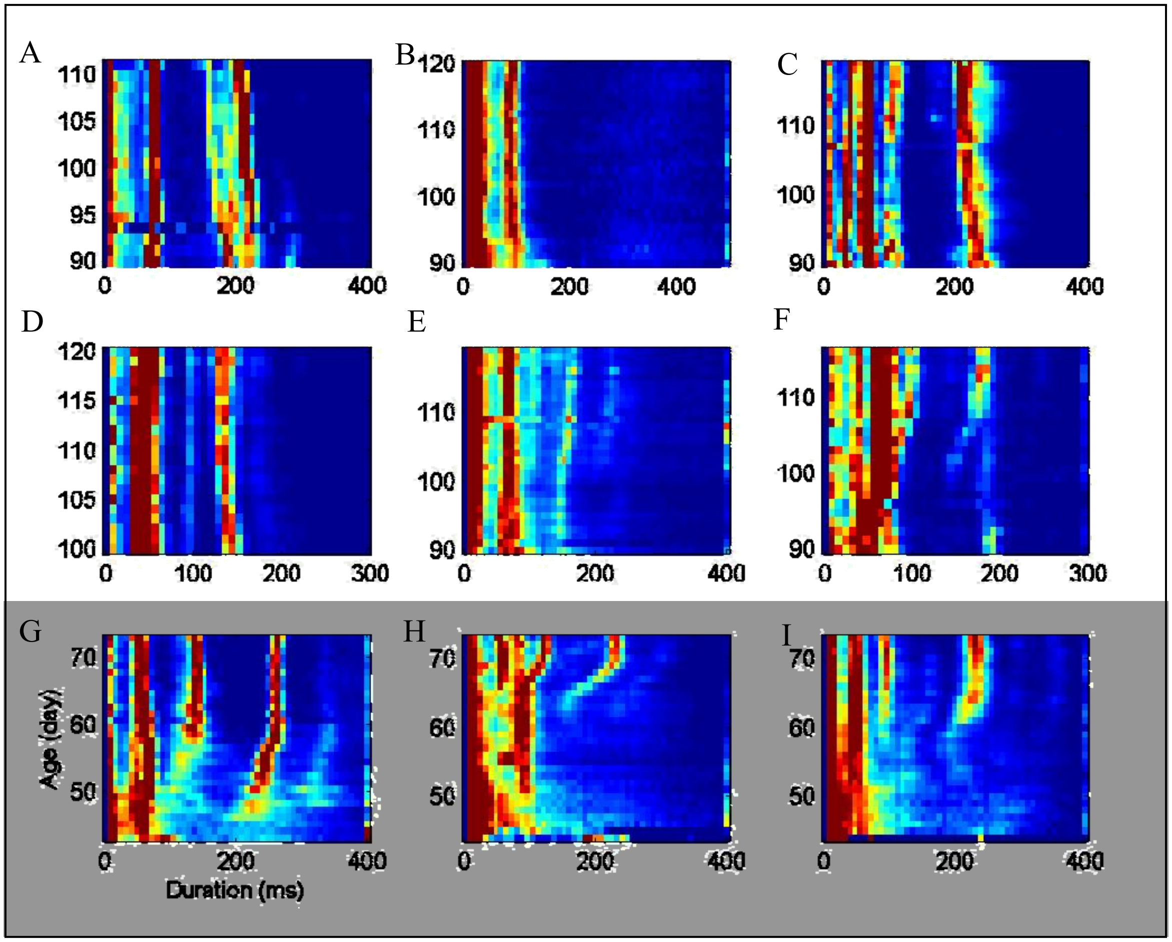


Figure 4.8 DVD maps of syllable duration. A-F. Late trained birds. G-I. Early trained birds.

Long time scales- rhythm level

Visual inspection of rhythm maps of birds trained from different ages (43, 90 and isolated birds) show remarkable visual differences between birds trained from day 43 (Figure 3.2 3.4), birds trained from day 90 (Figure 4.9-4.10) and isolates (Figure 4.11). Since some of the data is missing (due to technical difficulties in recording), the missing data is interpolated in Matlab for display purposes only.

We summarize the visual differences by comparing the rhythm entropy, the fundamental frequency of rhythm harmonics and the number of rhythm peaks of late trained birds with birds trained from day 43 and isolates.

Fundamental frequency:

The fundamental frequency of the rhythm harmonics indicate the duration of the repeating unit. Late trained birds decrease their fundamental frequency after training. Birds trained from day 43 have a lower fundamental frequency than birds trained from day 90 and isolates (Figure 4.12 A,B). In chapter 3 we saw that even birds trained from a juvenile age have higher fundamental frequency (i.e. indicating their motif duration) than expected from the song model's duration. Late trained birds have a higher fundamental frequency than early-trained birds, which means that their repeating unit is shorter. Figure 4.12 (b) shows that late trained birds are able to decrease their fundamental frequency, but their decrease is equivalent to the decrease in fundamental frequency of isolated birds which were not exposed to a tutor. Despite this decrease, they still have a higher fundamental than early trained birds, Figure 4.12 A. As in early trained birds, late-trained birds can have additional song elements that are not incorporated in their song.

Rhythm entropy:

Rhythm entropy is a measurement for the rhythmicity of the song. Arrhythmic birds such as juvenile birds have high rhythm entropy. Adult birds, trained from age 43, that imitate well have low rhythm entropy (Figure 3.2- 3.4). How does the entropy of late trained birds compare to early-trained birds? The difference between the rhythm entropy of late trained birds before training and after 30 days of training is not significant, but we do see a certain increase in rhythm entropy. We found that birds trained from day 43 start with higher rhythm entropy than birds trained from day 90. However, 30 days after training the entropy of early trained birds is lower than late trained birds at age 120 PHD (Figure 4.13). This means that in 30 days of training the early-trained birds improve their rhythm entropy much more than late-trained birds. The songs of birds trained from day 43 are more rhythmic (steadier period) and stereotyped than songs of late trained birds.

The number of rhythm peaks:

Another descriptive feature is the number of rhythm peaks. In Chapter 3 we saw that juvenile birds have fewer rhythm peaks than adult birds (Figure 3.28), and that the number of rhythm peaks correlates with imitation goodness for adult birds trained from age 43 (Figure 3.29). How does the number of rhythm peaks of late trained birds compare to early-trained birds? We found that birds trained from day 43 start with fewer peaks in their rhythm spectrum than birds trained from day 90, but 30 days after training early trained birds have more peaks in their rhythm than late trained birds at age 120 PHD (Figure 4.14).

Late trained birds are capable of vocal changes, to some extent, on the millisecond level and on the syllable level, but those changes do not necessarily improve their imitation. The cause for the limited effect of the millisecond changes and the syllabic changes could be the constraints we observed on the rhythm level. Even though late trained birds decrease their rhythm's fundamental frequency, this decrease would have happened regardless of training. Furthermore, late-trained fundamental frequency is not as low as the fundamental frequency of early-trained birds (Figure 4.12 b). Late trained birds increase the number of peaks in their rhythm maps but the number of peaks is not as large as the number of peaks in early trained bird's rhythm maps (Figure 4.14). Late trained birds do not change their rhythm entropy significantly, and it remains higher than early-trained birds (Figure 4.13). Therefore, late-trained birds have rhythmic developmental constraints.

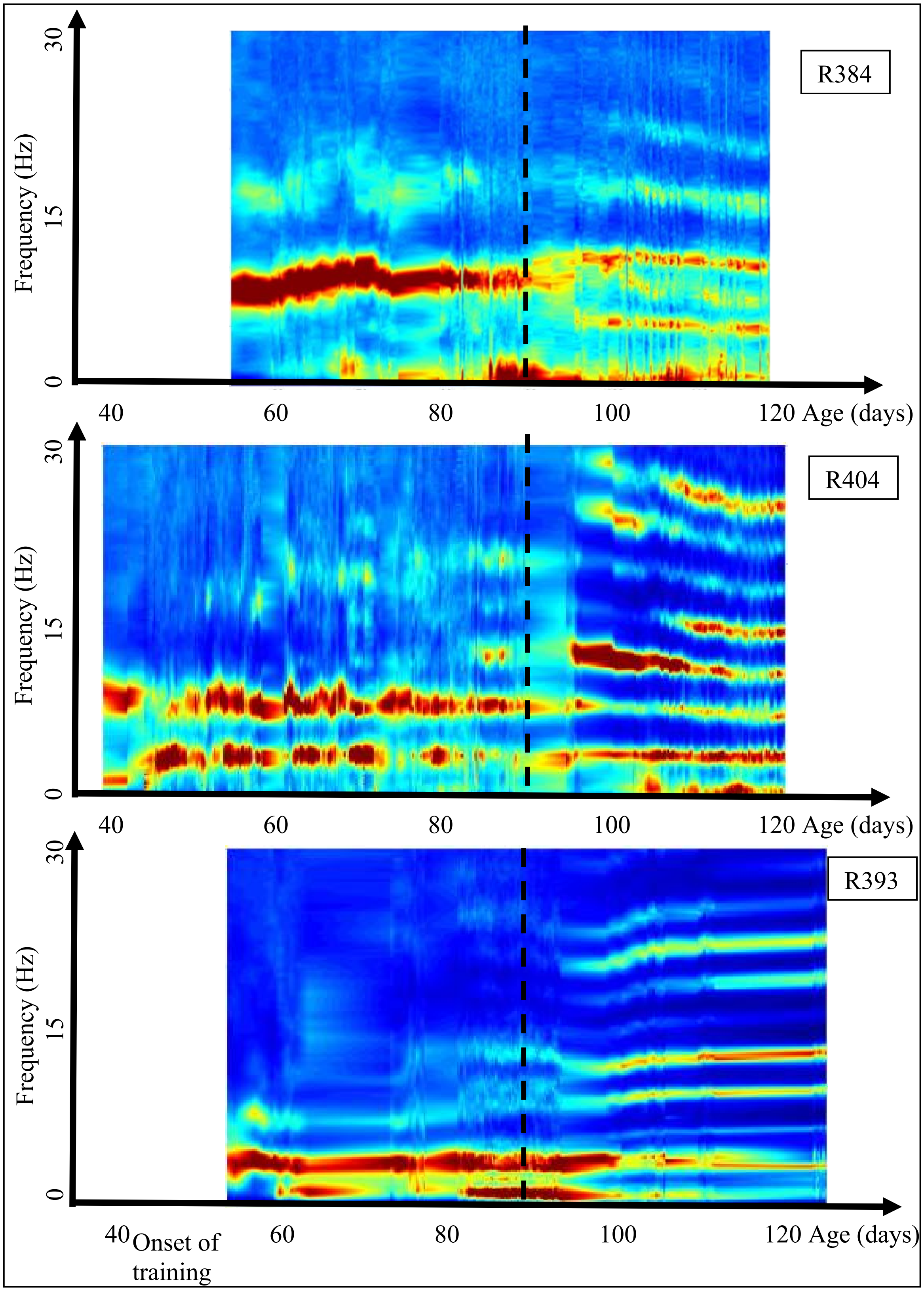


Figure 4.9 Examples of rhythm maps of birds trained from day 90 with song model Simple

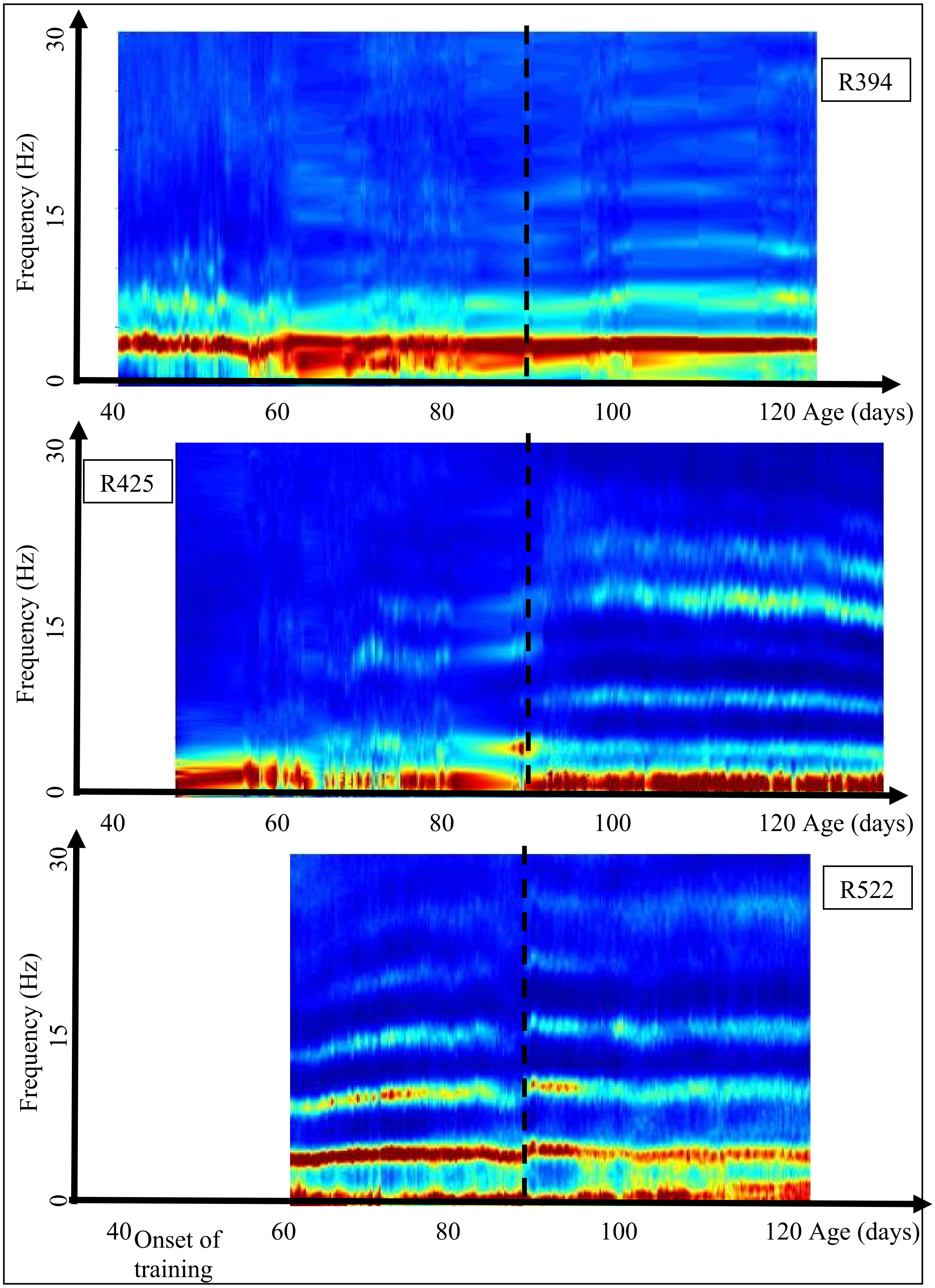


Figure 4.10 Examples of rhythm maps of birds trained from day 90 with song model Samba

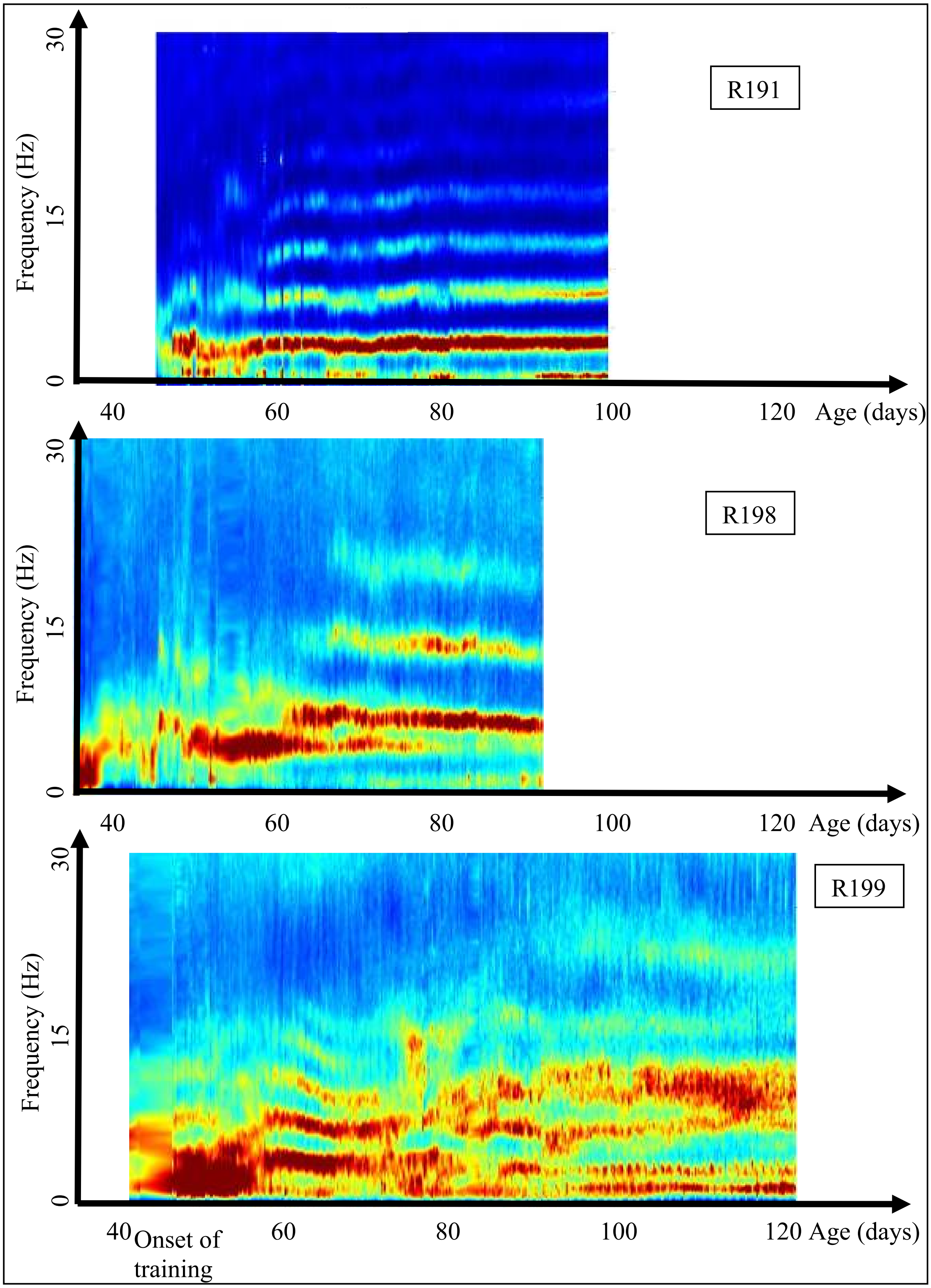


Figure 4.11 Examples of rhythm maps of isolated birds

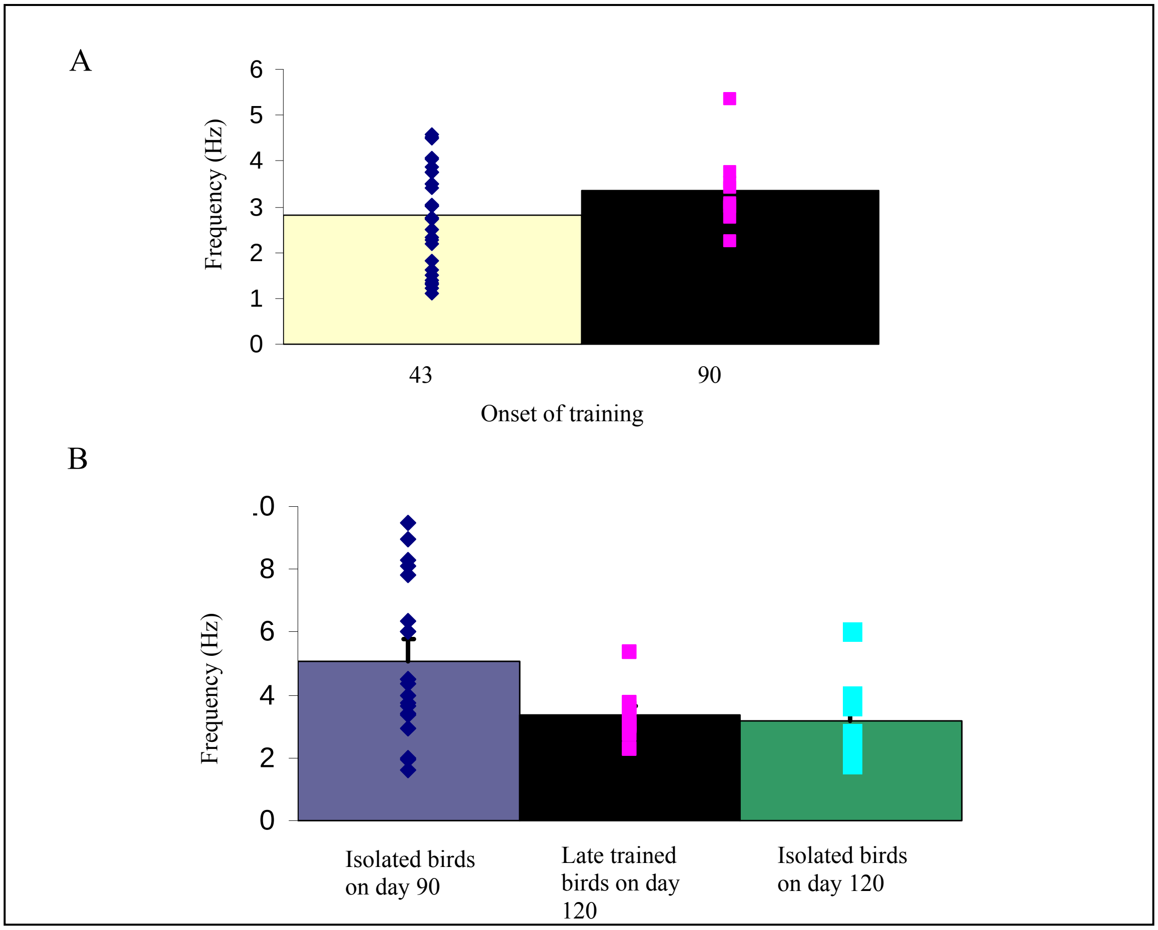


Figure 4.12 Average fundamental rhythm frequency of late trained birds. A. The average fundamental frequency after 30 days of training. Birds trained from day 43 have lower fundamental frequency than birds trained from day 90. B. The average fundamental frequency of isolated and late trained birds at age 90 and age 120 PHD. Late trained birds are able to decrease their fundamental frequency, but their decrease is equivalent to the decrease in fundamental frequency of isolated birds. Despite this decrease, they have a higher fundamental than early-trained birds (panel A). 34 birds trained from day 43, 11 birds trained from day 90 and 11 isolated birds.

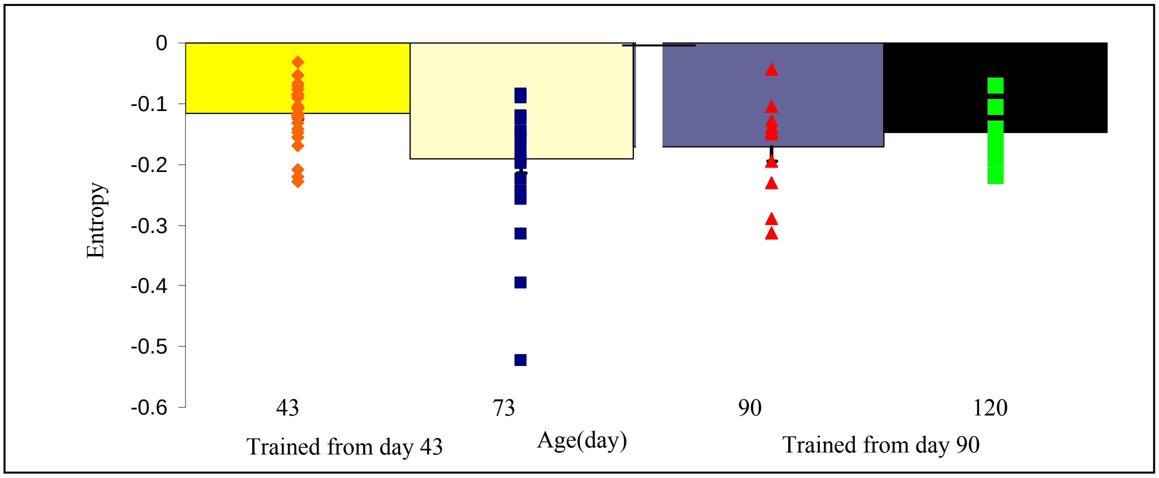


Figure 4.13 Average rhythm entropy at the onset of training and 30 days after training for birds trained from age 43 and 90. The difference between the rhythm entropy of late trained birds before training and after 30 days of training is not significant. Birds trained from day 43 start with higher rhythm entropy than birds trained from day 90, but after 30 days of training, their entropy is lower than late trained birds on day 120PHD. 19 birds trained from day 43, 9 birds trained from day 90.

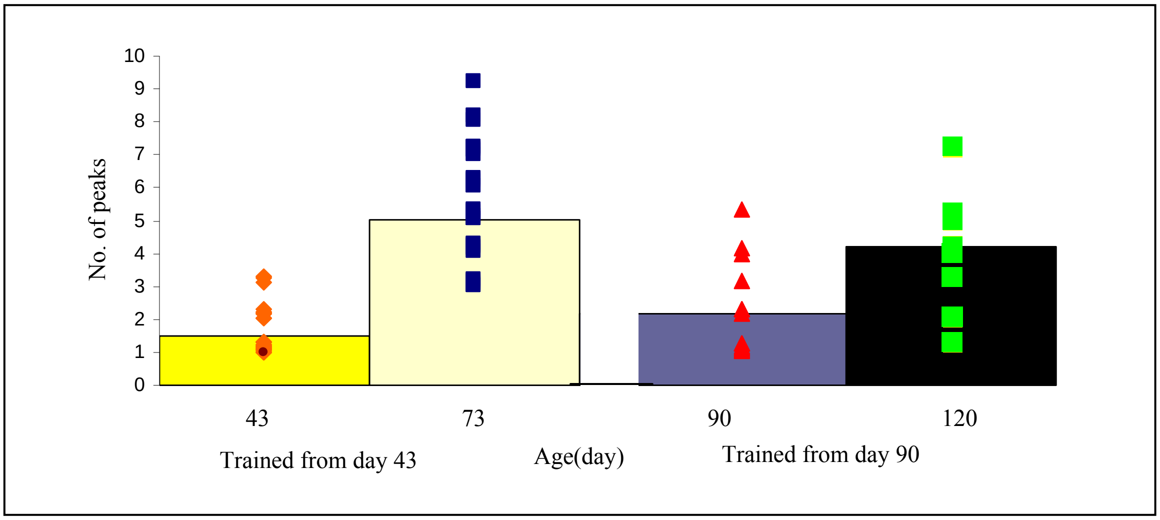


Figure 4.14 Average number of peaks in the rhythm maps at the onset of training and 30 days after training for birds trained from age 43 and 90. Birds trained from day 43 start with fewer peaks in the spectrum than birds trained from day 90, but 30 days after training have more peaks in their rhythm. 12 birds trained from day 90, 22 birds trained from day 43. The peaks have been jittered a bit for display purposes.

Can we predict if a late-trained bird will be able to learn well?

Can we look at a bird's rhythm map before the onset of training on day 90 and predict how well that bird will imitate, i.e. can we predict from the socially and acoustically isolated bird's song how well it will be able to imitate when trained? We will show here three features that summarize spectral properties and predict imitation: rhythm entropy, fundamental frequency of rhythm harmonics and number of rhythm peaks. In Chapter 3, we showed that those features correlate with goodness of imitation of adult birds trained from day 43. Here we will show that those features also predict the imitation abilities of late trained birds.

Rhythm entropy:

Rhythm entropy is a measurement for the rhythmicity of the song. Arrhythmic birds have high rhythm entropy than birds with a stereotyped song (Figure 3.22, 3.23). The correlation between the gain (day 90 to day 120) in entropy and the gain in % similarity after 30 days of late training ($r=0.858$. $n=9$, $p<0.004$) tells us that birds that increased their entropy (decreased their song stereotypy) imitated better, Figure 4.15 C. **This means that birds who imitated well destabilized their song in order to rebuild it again.**

The rhythm entropy of isolated birds on day 90 (just before the onset of training) correlates with the gain in % similarity they achieved in 30 days of training ($r=-0.91$, $n=10$, $p<0.001$), Figure 4.15 A. This means that birds with lower rhythm entropy at the onset of training (day 90) have a better chance to imitate even as late learners. This is counter intuitive. How come birds that are more stereotyped before the onset of training are able to change their song to produce a better imitation?

To further complicate the matter, we see indications (though they not statistically significant) that at age 120, late-trained birds that have low entropy achieved a better gain in % similarity during the 30 days of training (r=-0.51, n=8, p<0.2), Figure 4.15 B. This indicates that at age 120 birds with a more stereotyped song are more likely to improve their imitation than other birds.

Fundamental frequency of rhythm harmonics:

The solution to the enigma is revealed when we inspect another feature- the fundamental frequency of the rhythm harmonics. The fundamental frequency of the rhythm harmonics indicates the duration of the repeating unit. The fundamental frequency of isolated birds on day 90 (just before the onset of training) correlates with the gain (day 90 to day 120) in % similarity during 30 days of training (r=-0.64, n=10, p<0.05), Figure 4.16 A. This means that birds with a longer repeating unit have a better chance to imitate even as late learners. One possible explanation, considering our finding that late trained birds do not add syllables to their song, is that **a longer repeating unit means the bird has more prototypes (or raw material that is incorporated in the rhythm) to manipulate to match the model.**

The fundamental frequency of late trained birds 30 days after training (age 120 PHD) is not significantly correlated with the gain in imitation over those 30 days of training (r=-0.42637, n=8, p<0.3), Figure 4.16 B. The cause for the reduced correlation might be the general tendency of late trained birds to decrease their fundamental frequency regardless of imitation when exposed to a song model. Most of the adult late-trained bird's fundamental frequency is within a small interval of 2-4Hz. In birds that do not imitate well or do not change their song, the decrease in fundamental frequency happens because their song becomes more stereotypical despite the lack of imitation. In birds that imitate well, the decrease in fundamental frequency happens because they form their new song into stereotyped motifs, Figure 4.17. We don't see a significant correlation between the gain in fundamental frequency and the gain in % similarity during the first 30 days of training ($r=0.294$, $n=12$, $p<0.4$), Figure 4.16 C.

Correlation between fundamental frequency and rhythm entropy

We saw before (Figure 4.15 A) that isolated birds with a stereotyped song before the onset of training are able to change their song to produce a better imitation. We also saw that isolated birds with longer repeating units before the onset of training are able to change their song to produce a better imitation. Is there a relation between entropy and fundamental frequency? When we calculate the linear regression between the entropy and the fundamental frequency of isolated birds on day 90, we get $r=0.775$, $n=10$, $p<0.01$, Figure 4.18 A. Hence, birds with long repeating units also have higher entropy. Is it because the entropy measurement is skewed toward long repeating motifs? In Figure 4.19, we show an example of two sinusoids, one with short repeating unit (higher frequency), and another with long repeating unit. The entropy of the vector with the short repeating unit is higher than the entropy of the other vector- the opposite of what we would expect if there were an artifact.

Therefore, the reason that low rhythm entropy correlates with improvement in imitation is that isolated birds with low rhythm entropy also have long repeating units that have more prototypes (or raw material that is incorporated in the rhythm) which the bird can use to manipulate its song to match the model.

The Number of peaks in the song

The third descriptive feature of rhythm maps is the number of peaks in the song. A few factors influence the number of peaks.

- The duration of the motif determines how many possible harmonics can be in a frequency interval.
- The song becomes more rhythmic with development (Figure 3.23). As the repeating unit stabilizes, we see higher harmonics appearing.
- Finally, the specific shape of the repeated waveform may cause missing harmonics. In the presence of a steady period (rhythm), we usually see up to two missing harmonics in the interval of 0-30Hz.

The number of spectral peaks for isolated birds on day 90 (just before the onset of training) correlates with the gain (day 90 to day 120) in % similarity during 30 days of training ($r=0.625$, $n=12$, $p<0.05$), Figure 4.20 A. This means that birds with more spectral peaks on day 90 have a better chance to imitate even as late learners. The correlation of the number of peaks vs. gain in % similarity on day 120 is not significant, and the correlation of the gain in the no. of peaks vs. gain in % similarity is not significant either, Figure 4.20 B ($r=0.155$, $n=12$, $p<0.7$), C ($r=-0.25$, $n=12$, $p<0.5$).

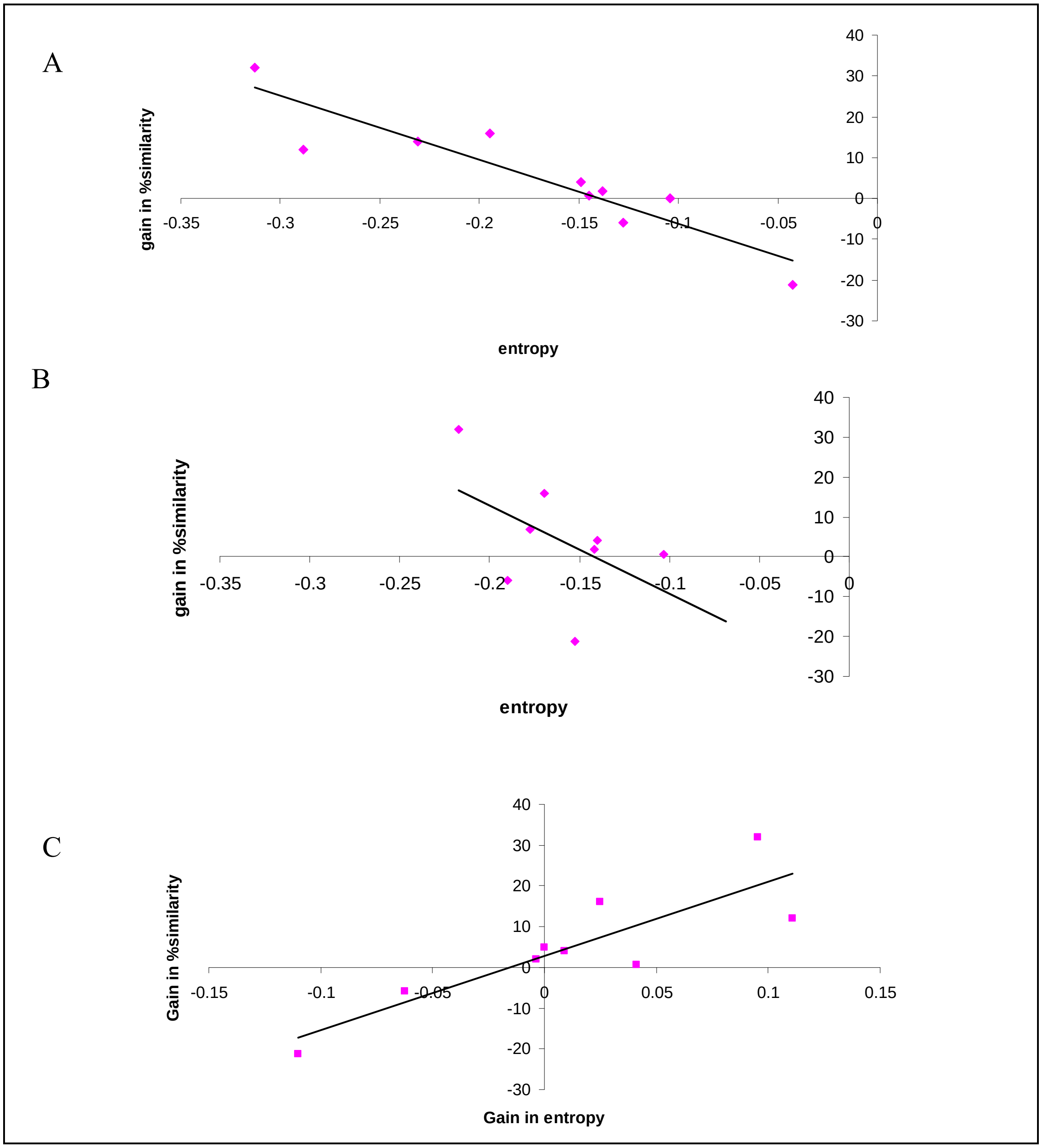


Figure 4.15 The rhythm entropy of isolates on day 90 (just before the onset of training) predicts how well those birds will imitate. A. Rhythm entropy of isolated birds on day 90 (just before the onset of training) correlates with the gain in % similarity after 30 days of training (r=-0.91 , n=10 , p<0.001). B. Rhythm entropy of isolated birds 30 days after training begins (age 120) correlates (though not significantly) with the gain in % similarity after 30 days of training (r=-0.51 , n=8 , p<0.2). C. The correlation between the gain in entropy and the gain in % similarity after 30 days of late training (r=0.858, n=9, p<0.004) tells us that birds that increased their entropy (decreased their song stereotypy) imitated better. This means that birds who imitated well destabilized their song in order to rebuild it again.

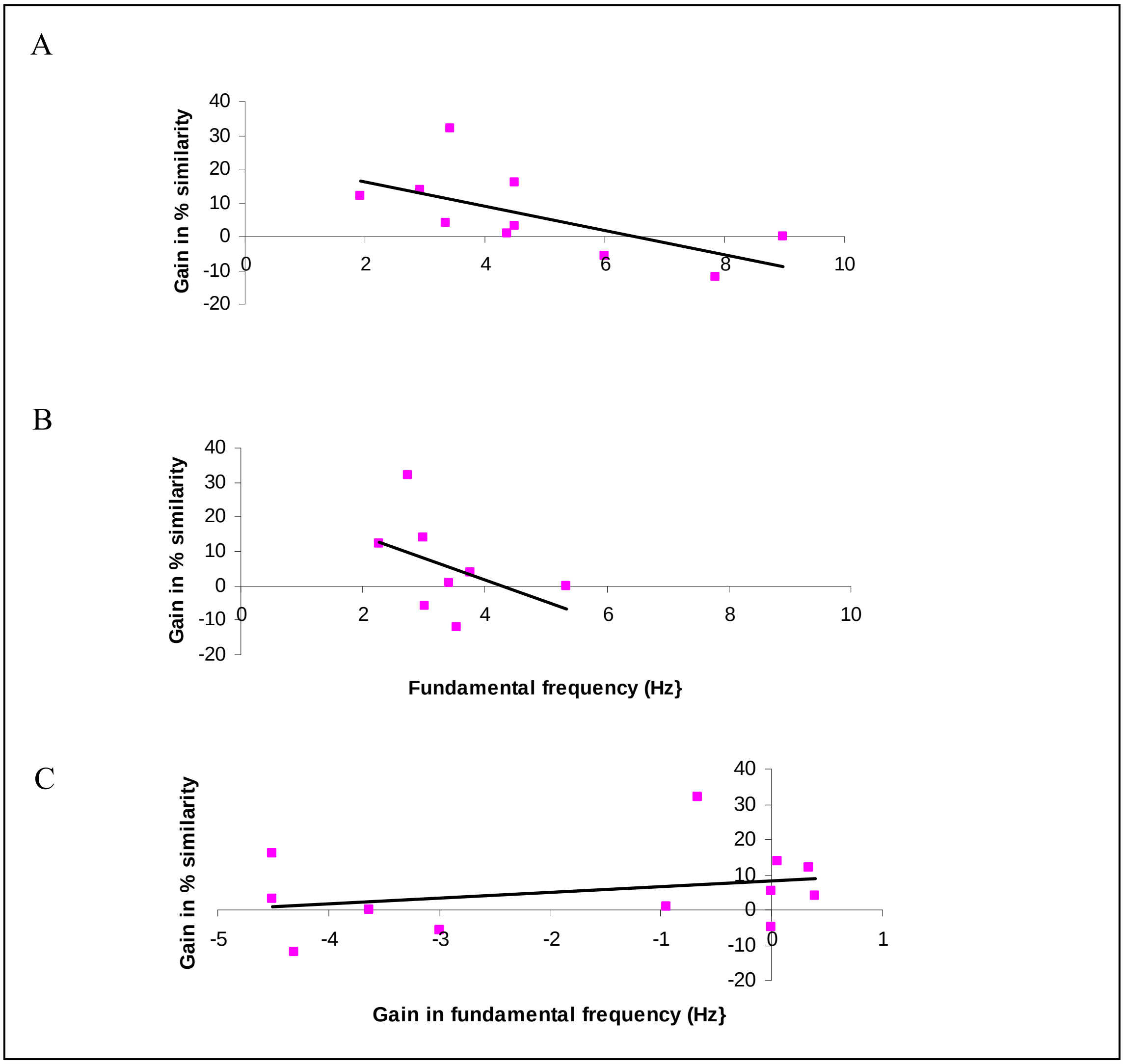


Figure 4.16 The fundamental frequency of isolates on day 90 (just before the onset of training) predicts how well those birds will imitate. A. The fundamental frequency of isolated birds on day 90 (just before the onset of training) correlates with the gain in % similarity during 30 days of training(r=-0.64, n=10 , p<0.05). This means that birds with a longer repeating unit have a better chance to imitate even as late learners. One possible explanation is that a longer repeating unit means more prototypes (or raw material) that the bird can use to manipulate its song to match the model. B. The fundamental frequency of late trained birds 30 days after training (age 120) is not significantly correlated with the gain in imitation over those 30 days of training (r=-0.42637 , n=8 , p< 0.3). C. The correlation between the gain in fundamental frequency and the gain in % similarity during the first 30 days of training is not significant either (r=0.294, n=12, p<0.4).

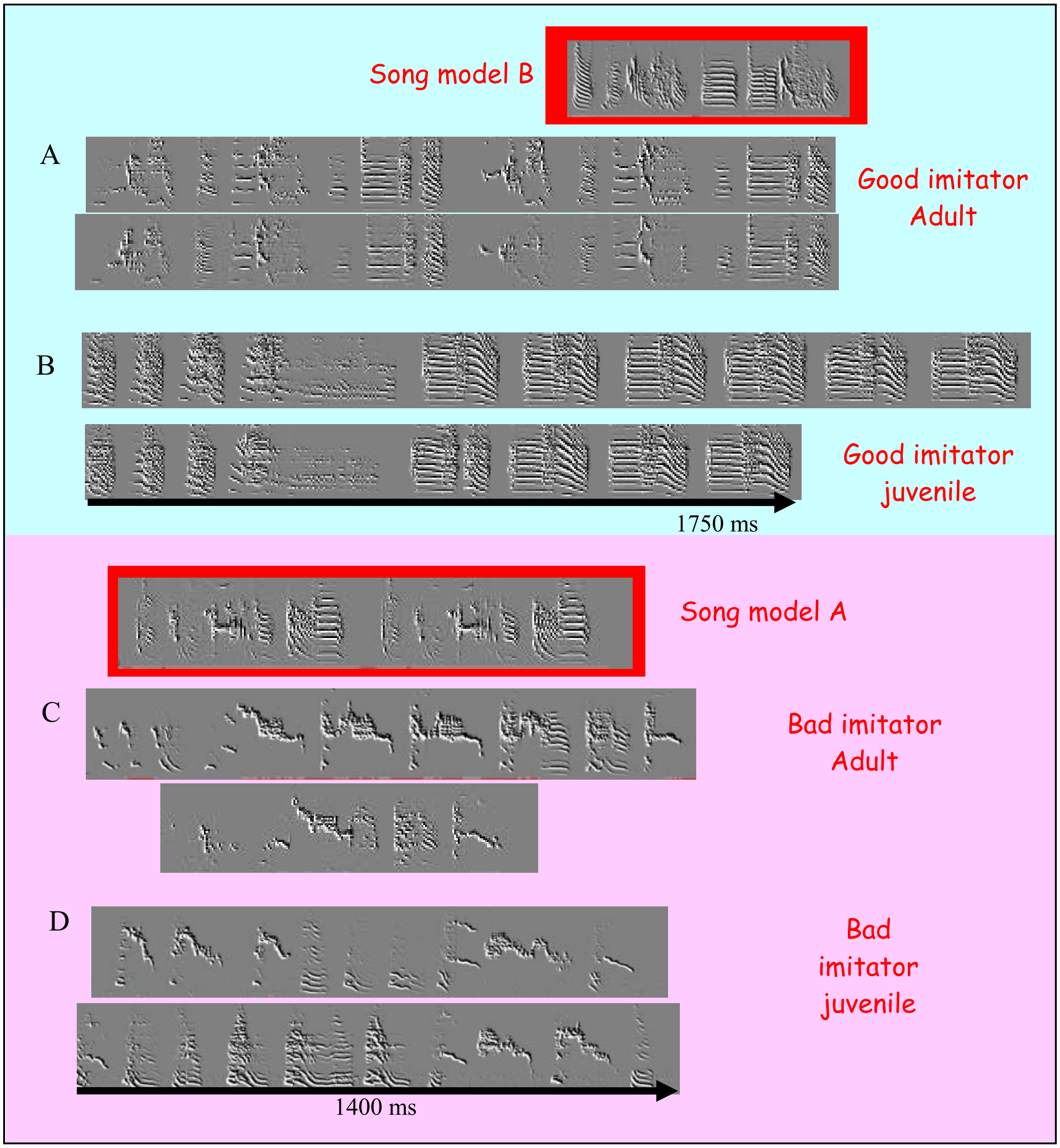
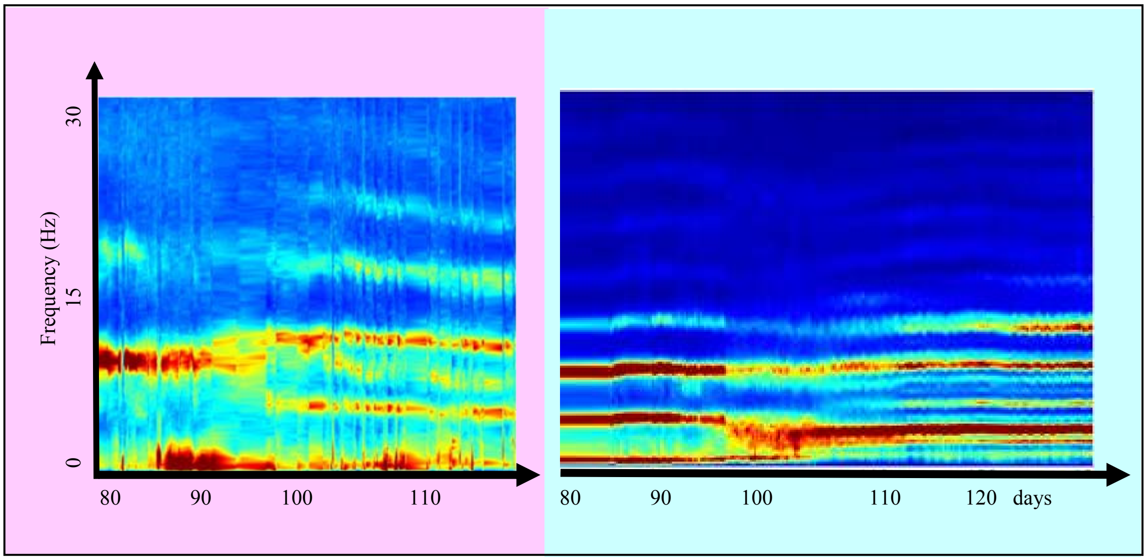


Figure 4.17 Late trained birds songs. A. An example of a late-trained bird that imitated well, 30 days after training. B. An example of a late-trained bird that imitated well, just before the onset of training. In birds that imitate well, the decrease in fundamental frequency happens because they form their new song into stereotyped motifs. C. An example of a late-trained bird that did not imitate well, 30 days after training. D. An example of a late-trained bird that did not imitate well, just before the onset of training. In birds that do not imitate well or do not change their song, the decrease in fundamental frequency happens because their song becomes more stereotypical despite the lack of imitation. E. Rhythm maps of the bad imitator (right) and the good imitator (left).



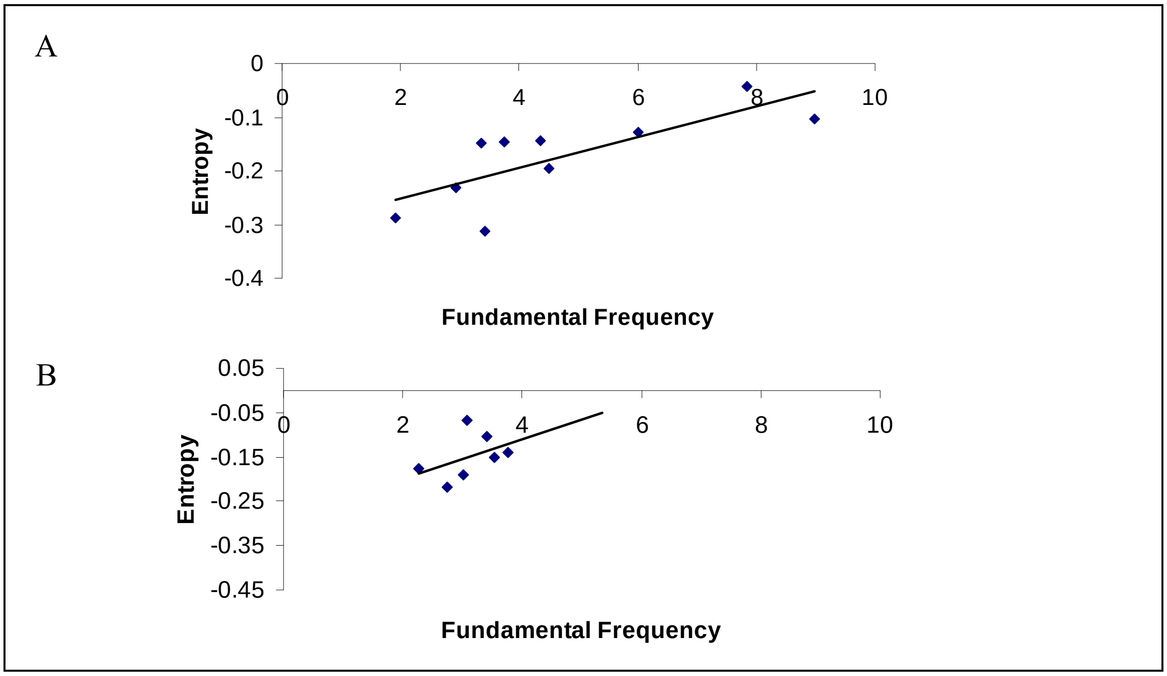


Figure 4.18 Correlations between entropy and fundamental frequency. A, There is a correlation between the entropy and fundamental frequency of isolated bird's rhythm at age 90 PHD (r=0.775, n=10, p<0.01). B. The correlation between rhythm entropy and fundamental frequency at the age of 120 (r=0.44, n=7 , p<0.4).

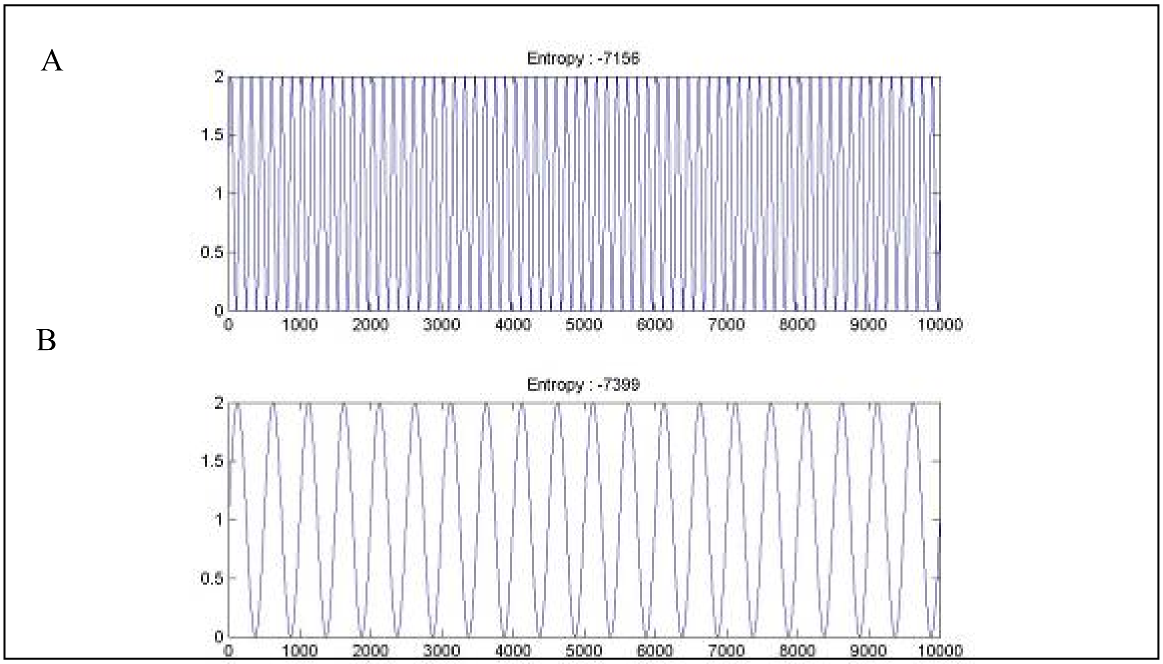


Figure 4.19 An example of two sinusoids and their entropy. An example of two sinusoids, one with short repeating unit (A), and another with long repeating unit (B). The entropy of the vector with the short repeating unit (-7156) is higher than the entropy of the other vector (-7399).

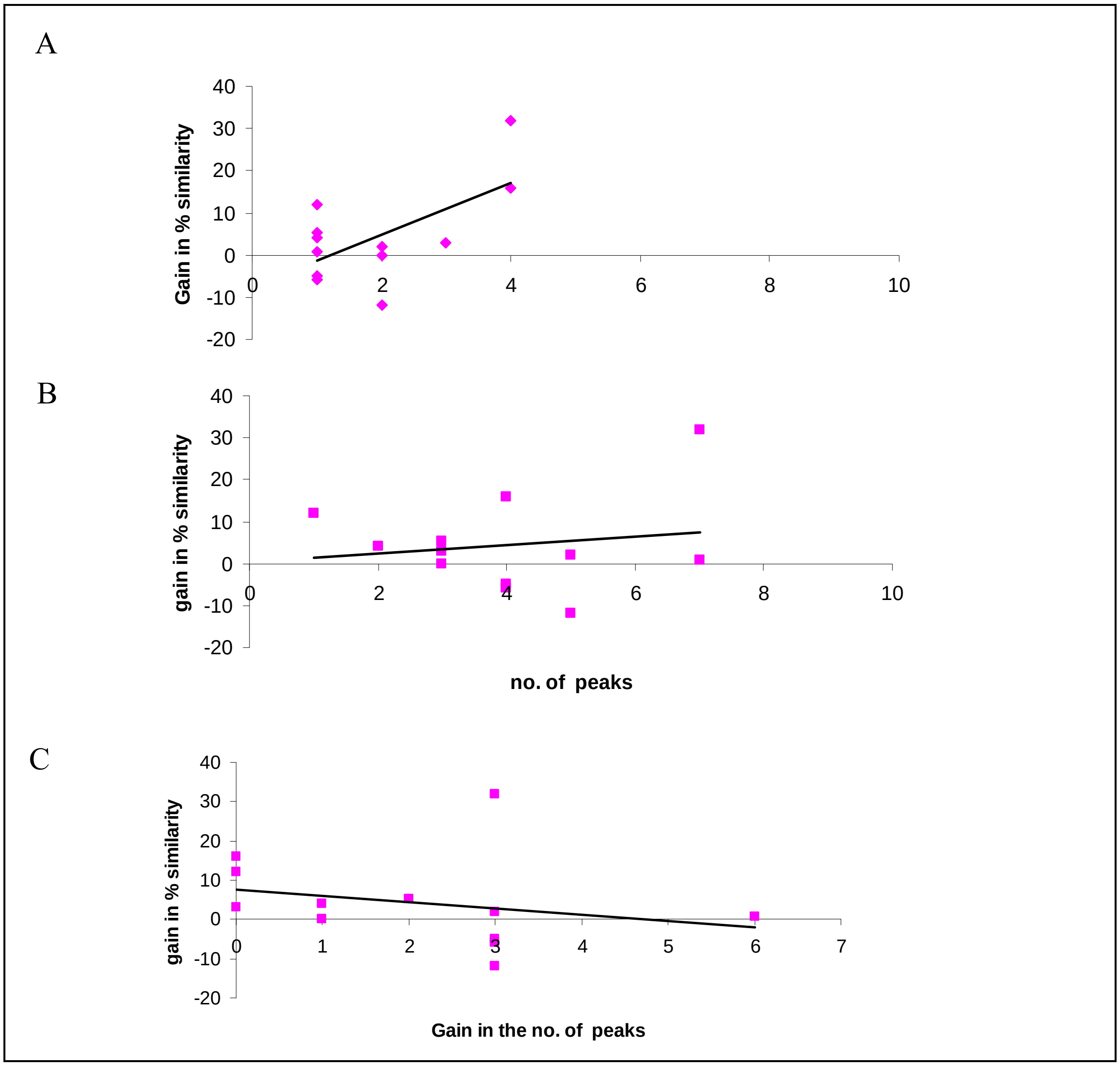


Figure 4.20 The number of peaks of isolates on day 90 (just before the onset of training) predicts how well those birds will imitate. A. The number of spectral peaks for isolated birds on day 90 (just before the onset of training) correlates with the gain in % similarity during 30 days of training (r=0.625, n=12, p<0.05). B. The correlation of the number of peaks vs. gain in % similarity is not significant (r=0.155, n=12, p<0.7), and in C. gain in the no. of peaks vs. gain in % similarity (r=-0.25, n=12, p<0.5).

Discussion

Birds that are socially and acoustically isolated until the end of their sensitive period do not imitate well (Figure 4.1-4.2). Yet, this work and previous work in adult birds proved that they are capable of modifying their song to some extent. It was shown (Tumer & Brainard 2007, Sober & Brainard 2009) that adult Bengalese finches can change their syllable notes either by associating small variations in their vocal behavior with differential outcomes, or due to artificially perturbations of the auditory feedback's pitch. These data suggest that adult song might not be as stereotyped as previously believed (Tumer & Brainard 2007, Sober & Brainard 2009). Nevertheless, late trained birds can not imitate well (Figure 4.1).

We saw in this chapter that late trained birds are capable of some vocal changes. Late trained birds are capable of vocal changes on the millisecond level and on the syllable level but those vocal changes do not necessarily improve their imitation. Birds that change their song features more than other birds might still sing a bad imitation of the song model. We found that late trained birds do not insert new syllables into their song (Figure 4.8). If birds are stuck with the syllables they possess at age 60d, those syllables might not be an easy starting line for imitation. This hints of a problem on the rhythm level. If rhythm level is constrain (or stereotyped), i.e. abrupt rhythm changes can not happen in the rhythm level than the bird wont be able to add a new syllable easily since that will change its rhythm abruptly. And indeed, even though exposure to song, even at the late age of 90 days, decreases the rhythm's fundamental frequency and increases the number of peaks in the spectrum (Figure 4.12-14) there are no abrupt rhythm transitions. Even though rhythm maps of adult late-trained birds have higher entropy, higher fundamental frequency and fewer peaks than adult early-trained birds (Figure 4.12-14) they achieve that by smooth changes of rhythm. Therefore, late-trained birds have developmental constraints at the rhythm level.

We can predict how well birds will imitate. Birds that have low fundamental frequency, which indicates long repeating units, are able to imitate better (Figure 4.16 A).

A longer repeating unit means more song prototypes (or raw material that is incorporated in the rhythm) that the bird can use to manipulate to match the model. DVD maps of syllable duration, rhythm maps and song ontogeny did not reveal new syllable that were added into the song (Figure 4.8). Therefore, late-trained birds have an arsenal of syllables they can use to imitate, but they cannot add new material to their arsenal like early trained birds (Figure 4.8). The result is that birds with longer repeating units tend to improve their imitation more than birds with short motifs. To do that, birds with long repeating units and stereotyped song destabilize their song in order to rebuild it again (Figure 4.15 C).

We saw that late trained birds are capable of changing their syllables, and their millisecond distribution – to some extent. But they are not able to insert new syllables into the song. This is consistent with the neuronal model we presented in the discussion of chapter 3. In that model, the timing of the millisecond sub-notes and the fine structure of the syllables is controlled by HVC. The global timing is controlled by the brainstem clock. There could be different critical periods for different nuclei in the feedback loop. In the discussed model, a possible explanation for the lack of syllable insertion keeping the ability to change millisecond song features (at least to some extent) is that HVC and RA forebrain nuclei possible close their critical periods later than the brainstem "clock" nuclei. Hence, HVC and RA allow modulation of song features, but there are no additional units in the song because the rhythm generator is crystallized.

A songbird has four distinct stages of song production:

1. Juvenile birds that are at their sensitive period for learning a new song. In chapter 3 we examined rhythm development in juvenile birds.
2. Young birds toward the end of their sensitive period. In chapter 3 we examined the imitation abilities and constraints of isolated birds trained at the end of their sensitive period.
3. Middle aged birds after their sensitive period for learning. Immelmann, Dietrich and Clayton discovered that later in life middle aged songbirds do not modify their song (Immelmann 1969, Dietrich 1980, Clayton, 1987, 1988, 1989).
4. Aged birds toward the end of their life.

So far, in this dissertation we explored rhythm development and rhythm constraints in juvenile and young birds as well as analytic techniques for analyzing song at a shorter timescale. We would like to use the rhythm method to examine song changes in the last unexplored stage of the bird's life - old age.

Chapter 5. Old Age Slows the Song in Bengalese finches

Collaborative work with Brenton G. Cooper, Jorge M. Mendez, Addison G. Whetstone, Ron A. Meyers, and Franz Goller

Introduction

We know that in humans there are some speech changes that occur with normal aging and with Parkinson's disease (Calhoun & Eibling 2006, Linville 2001). Songbirds provide an excellent animal model system for exploring the central and peripheral mechanisms of learned vocal behavior. Numerous parallels between song learning in young birds and the acquisition of speech in infants have been described (Doupe & Kuhl 1999). Carolyn Pytte (Pytte 2007) observed that zebra finches changes are not striking large. However, bengalese finches change their song at late age more than zebra finches. It is important to find an animal model for speech changes at old age so we used the animal model bengalese finch. Changes in song structure with old age have not been explored and compared to those that occur in speech. We would like to utilize the rhythm analysis method to better understand song changes between middle aged and aged songs.

Unlike most songbirds including zebra finches, bengalese finches (Lonchura striata var. domestica) sing non-deterministic songs that can be described by a finite-state syntax. The Bengalese finches' song depends upon auditory feedback (Okanoya & Yamaguchi 1997). When deafened, the temporal organizations of the song immediately deteriorate.

Each male Bengalese finch sings a unique song with a unique set of song elements and individual specific finite-state syntax. Two to five song elements are organized into a "chunk". Several chunks are further organized into phrases by passing through a finite-state syntax. Several phrases are in turn arranged based on the finite-state syntax. A bengalese finch can produce infinite varieties by taking different paths on the finite-state automaton. A finite-state automaton or simply a state machine is a model of behavior composed of a finite number of states, transitions between those states, and actions. A finite state machine is an abstract model of a machine with a primitive internal memory. For comparison, the zebra finch is a much simpler song where the syllable order is the same every time. A bengalese finch would repeat certain syllables a few times before moving on to the next syllable. That next syllable could be one of a number of options.

Using a cross-sectional and longitudinal analysis, we show the first evidence that aged Bengalese finches (*Lonchura striata*) undergo substantive changes in rhythm and acoustic structure of song.

Method

We recorded song from males over 6 years old (n = 4), which is near the average life-span of this species. For comparison, we analyzed the songs of randomly selected middle-aged adult finches (n = 18, approx. age 1 – 3 years old) from the same captive colony. To characterize changes in song associated with aging, we measured the duration of song syllables, inter-syllable intervals, pitch and range of frequency modulation

(Figure 1.6). In two of the birds, we recorded song at both ages with 4 and 6 years between the recordings, allowing a direct comparison within individuals. The results of the cross-sectional and longitudinal analyses were similar.

Results

We used spectral rhythm analysis (Saar & Mitra 2008) to explore the periodic song structure- i.e. song rhythm. Rhythm analysis quantifies the global repeating unit of the song which in Bengalese finches is the syllable and silence gap interval (Saar & Mitra 2008). The repeating unit increased significantly in old age (Two-sample T-test p < .00075), (Figure 5.1). Therefore, similarly to humans, in old age the song slows down. Why is the song slowing down in old age?

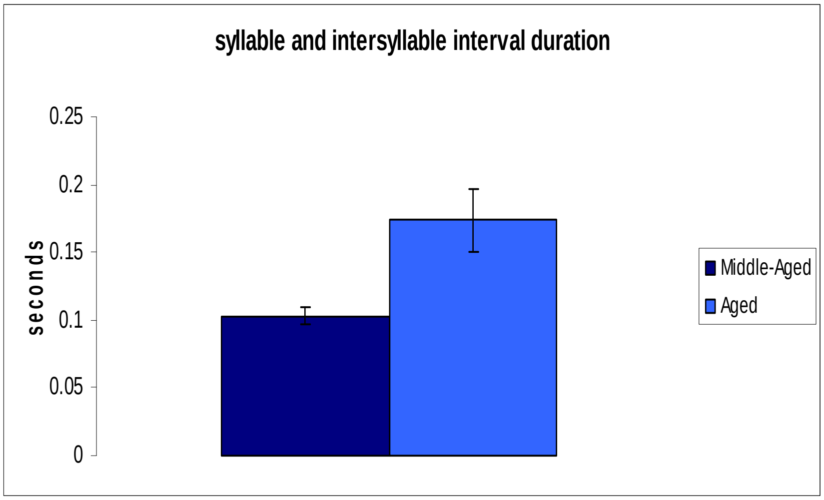


Figure 5.1 Rhythm analysis shows that the repeating unit (syllable and intersyllable interval) increased significantly in old age.

In order to study developmental effects on the syllable level, we first segment the syllables using an amplitude threshold and then summarize the structure of each syllable by an array of acoustic features (Figure 1.6). We compute the mean and variance of song features across the syllable. Syllables that belong to the same syllable type have similar features (Tchernichovski et al. 2004).

Syllable and gap analysis revealed the cause for the slowing down of the song. There was a large and consistent increase in the intersyllable interval in the aged finches (Figure 5.2). This increase in silent intervals, which correspond to short inhalations (minibreaths) slowed the song rhythm.

Are there additional differences between aged birds and middle aged birds?

According to the 2 birds recorded in middle age and aged years, visual inspection and similarity analyses show that all of the syllables composing the song were retained to the older age (Figure 5.3). There were subtle changes in syllable duration (Figure 5.2 A,B, 5.4), but they did not reach significance. However, the distribution of syllable occurrences changed. The number of syllable repetitions decreased. For example, one male sang a repeated syllable on average 11.3 times per motif during the middle-age years, but only 5.5 times at older age (Figure 5.3). A scatter plot of the distribution of syllable features shows syllable features were similar but not identical across the lifespan of the bird (Figure 5.4).

Why is the song slowing down?

To investigate the possibility that respiratory dynamics are slowed at old age causing minibreaths to last longer and making high syllable repetition rates more difficult, we measured percentage and fiber diameters of the respective muscle fiber types in an inspiratory muscle, *m. scalenus*, and in the muscles of the vocal organ, the syrinx. There were no significant changes in fiber type composition or diameter between aged and middle-aged birds (SOM), suggesting that the behavioral changes result from changes in innervation patterns or the neural motor program of song.

Another possibility for the slowing down of the rhythm is hearing loss in aged birds. Deafening results in rapid and profound deterioration of Bengalese finch song syllables (Woolley & Rubel 2002). It is unlikely that hearing loss is the cause of the observed effects because song syllable similarity remained high across the lifespan of the birds in this study (over 60%).

So why is the song slowing down? The avian basal ganglia modulate song tempo in middle-aged birds (Kao 2005). This suggests that the observed tempo change is, in part, caused by age-related deterioration in these sensorimotor control areas. Indeed, loss of dopaminergic function in the human basal ganglia is thought to underlie some of the speech changes that occur with normal aging and in Parkinson's disease (Linville 2001).

Analysis of song amplitude and call amplitude reveals that in old birds the amplitude of the song is lower compared to their calls than in young birds (Figure 5.5). Unlike the learned song that is produced by the song system (RA, HVC, LMAN), calls are not learned and produced in the sub-cortical area dorsomedial intercollicular (DM). The decrease in old age of song amplitude compared to the call amplitude suggests there is a loss of cortical inputs which are driving the song, i.e. a deterioration of components of the song system at old age.

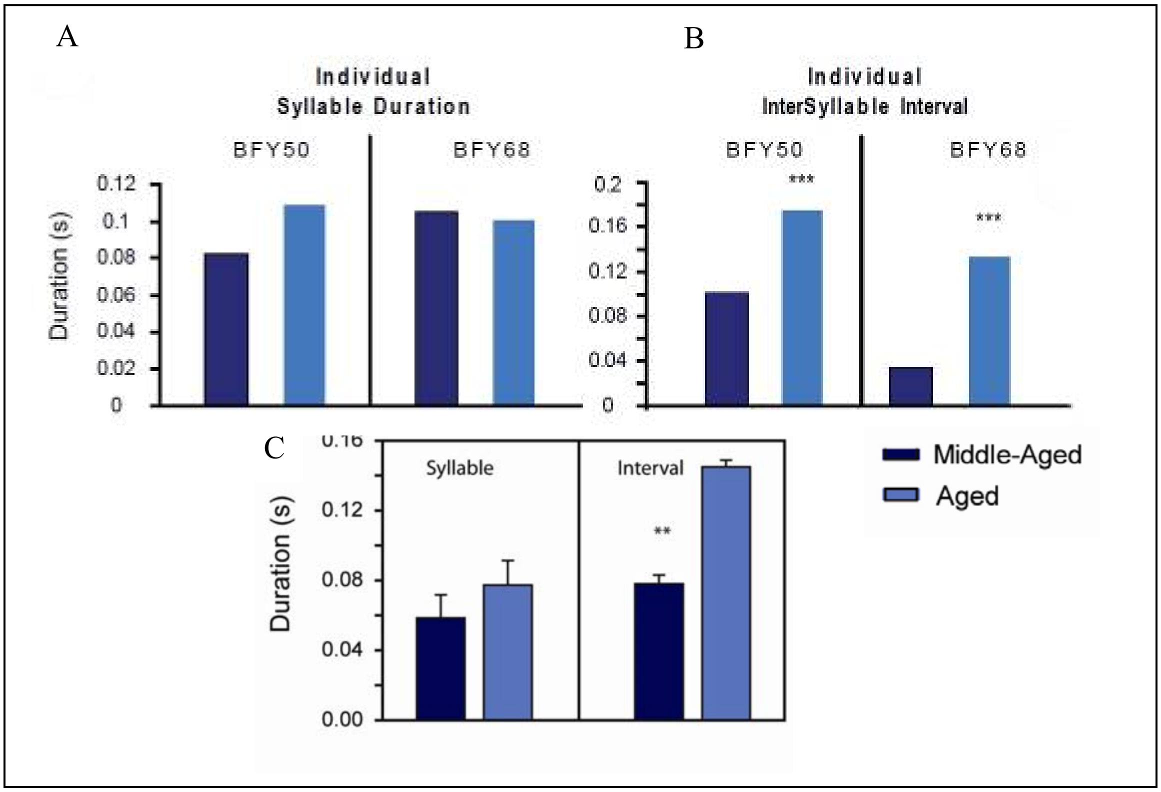


Figure 5.2 Syllable features reveal that there was a large and consistent increase in the intersyllable interval in the aged finches. This increase in silent intervals, which correspond to short inhalations (minibreaths) slowed the song. A Examples of two individual bird's syllable duration. B Examples of two individual bird's intersyllable interval. C. overall analysis for 4 birds.

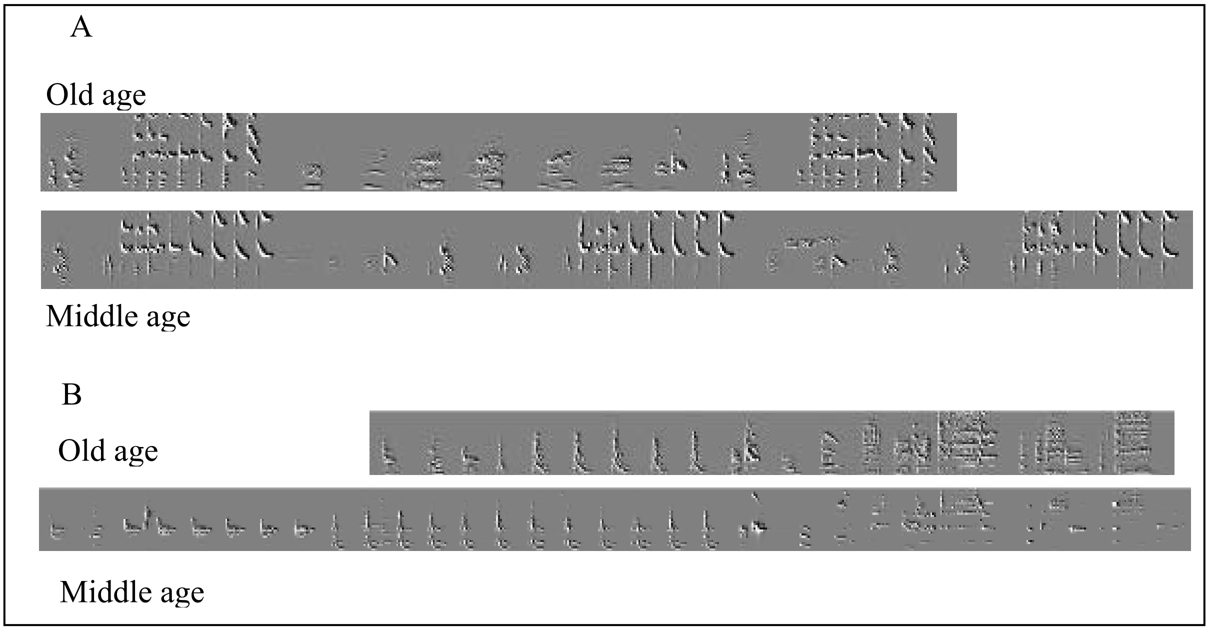


Figure 5.3 Visual inspection shows that all of the syllables composing the song were retained to the older age but the number of syllable repetitions decreased. (A) Bird BFY50 (B) Bird BFY68

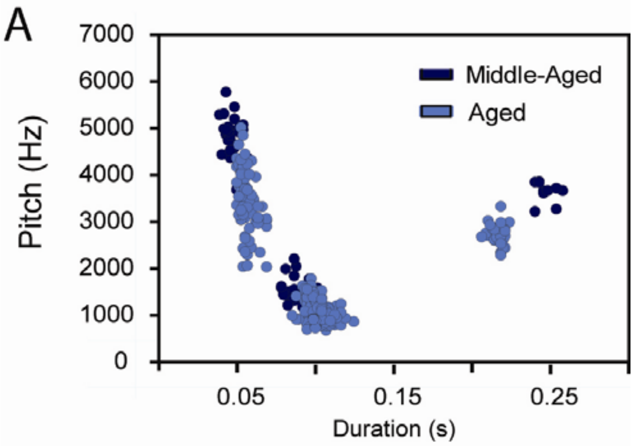


Figure 5.4 An example of a scatter plot of the distribution of syllable features for BFY50 which shows that syllable features were similar but not identical across the lifespan of the bird.

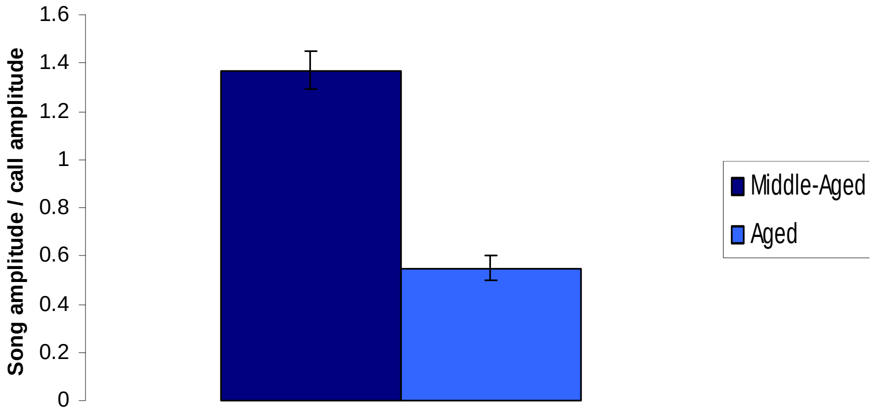


Figure 5.5 Analysis of song amplitude and call amplitude reveals that in old birds the amplitude of the song is lower compared to their calls than in young birds.

In women the fundamental frequency of speech decreases with age, whereas in men it increases (Linville 2001). Compared to middle-aged birds, both pitch (roughly corresponding to f_0) and degree of frequency modulation decreased in the songs of aged Bengalese finches (Figure 5.6 A,B). These acoustic changes may be caused by physical modifications in the syrinx, such as changing mass or tensile properties of the vibrating labia or surrounding connective tissues, or reflect changes in neural control.

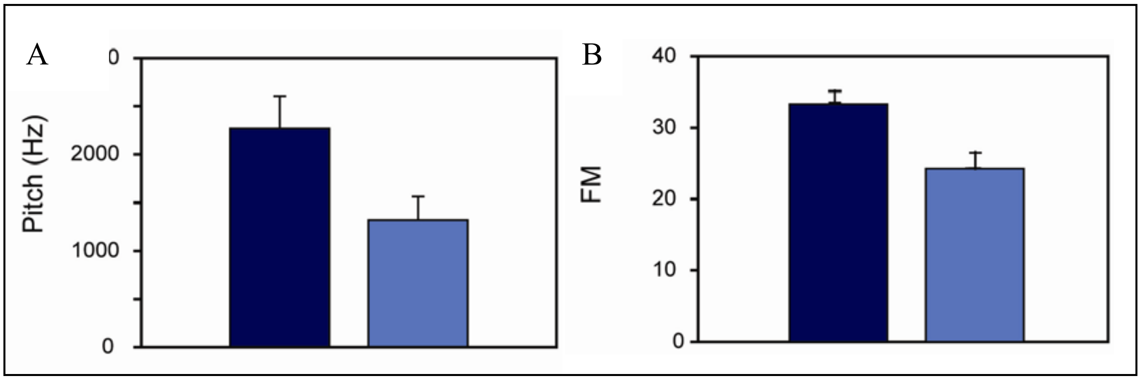


Figure 5.6 Compared to middle-aged birds, both pitch (A) and degree of frequency modulation (B) decreased in the songs of aged Bengalese finches.

Discussion

These changes in temporal and acoustic features of song may provide female birds with clues regarding the age of the individual, potentially indicating the fitness of the singing bird. It is likely that such drastic changes in song affect mate selection, because female canaries prefer songs with a faster tempo (Drăgănoiu et al. 2002). The selective changes to the rhythm and acoustic structure of the song with advanced age reveal another striking parallel between human speech and birdsong. Furthermore, they show that the motor program of song is modified throughout the lifespan of the bird. In human speech, age-related slowing of speech and changes in fundamental frequency result from the complex interaction of multiple peripheral and neural changes (Linville 2001). Aged songbirds could provide an animal model system for exploring how peripheral and neural changes interact to induce age-related deterioration in learned vocal patterns in elderly populations.

Chapter 6: Conclusions

During the course of a bird's lifespan, zebra finch has four distinct stages of song production:

1. Young juvenile birds (23-90 PHD) are acquiring their song (memorization phase).
2. Young acoustical and socially isolated birds (60-120PHD) at the end of their song's learning period (Postponement of full crystallization).
3. Middle aged birds (120 PHD-4 years) are well beyond their sensitive period for learning, and are sexually mature.
4. Aged birds (5 years +) are nearing the end of their life

Each of these life stages is characterized by different singing abilities, which constrain song development and song production. For example, we saw that juvenile birds are able to improve their imitation significantly more than young 90 day old isolates (Figure 4.2). Juvenile birds that are exposed to a song model are able to make abrupt rhythm transitions and insert syllables into their song while young 90 day old isolate can not. (Chapter 2). We know that middle aged birds have a crystallized song which is highly stereotyped from one rendition to the next (Immelmann 1969, Dietrich 1980, Clayton, 1987, 1988, 1989, Doupe & Kuhl 1999), but saw (Chapter 5) that at old age bengalese finches had modified their song analysis suggests that . Evidences suggests that due to a loss of cortical inputs which are driving the song, i.e. a deterioration of components of the song system, old Bengalese finches sing slower (slower rhythms), Chapter 5.

The rhythm generator

When we listen to the zebra finch song it sounds very rhythmic and periodic because of its repeating nature. However, it is not known yet what mechanisms generate the rhythm of the zebra finch song. There are additional uncertainties concerning which brain regions are responsible for the timing of motifs, syllables, and sub-syllabic structure. Furthermore, throughout the bird's development its rhythm changes, we do not know how is the new rhythm acquired, nor if the transition from one rhythm to another is random or constrained in some way.

Two forebrain nuclei in particular have been implicated in the control of the temporal structure of song: HVC and RA. The midbrain and respiratory areas project back to HVC through Uva (Striedter & Vu 1998, Ashmore & Renk 2008), raising the possibility that syllables, which are tightly linked to respiratory patterning (Goller & Cooper 2004), may be timed by respiratory oscillator circuits (Ashmore et al. 2005; Cooper & Goller 2006). It was suggested that HVC may contain multiple independent chains (or modules) (Glaze & Troyer 2006), which may be associated with syllables or long subsyllabic elements (Schmidt 2003). Thus each HVC can be seen as implementing a short-term clock or "music box" (Glaze & Troyer 2006) whose output is read by downstream nuclei such as RA and its brainstem targets which serves as a feedback connection through thalamus and back to HVC. The recurrent activity ascending from these target nuclei (such as DM and PAm) may then perform the role of a second, longer-term clock that governs overall tempo and synchronization. In this model, the HVCs would receive periodic signals from the brainstem clock, and generate a small portion of its own temporal code in response to each global “tick”. This would ensure that the two halves of the song system (in the right hemisphere and the left hemisphere) remained synchronized, and coordinated sufficiently in their output to produce the temporal sequence of song with adequate precision. Respiratory feedback could provide crucial information about limitations at the periphery, such as the ability to maintain the high respiratory demands imposed by song (Wild et al. 1998). Integration of this feedback with descending motor commands may provide important signals for regulating both the tempo and duration of song production.

It has been shown that the respiration cycle has a mechanical impact on some motor cycles. For example, magpies have a constant wing beat and respiratory cycle ratio per bird. That includes the ratio of 3:1 most commonly observed among birds (Berger et al. 1970) as well as ratios of 5:2 and 2:1. (Boggs 1997, Boggs et al. 1997). In galloping mammals the gait and respiratory cycles are phase locked on a 1:1 basis (Bramble 1989).

If indeed song rhythm is as tightly linked to respiratory patterning as the model above suggests and the global clock is in respiratory related nuclei than we expect the rhythm generator to be related to the respiration pattern of the bird. The general respiration patterns do not change their steady state abruptly during development, i.e. there will be a temporary respiratory change in response to a stimuli such as a female bird (Cooper and Goller, 2006; Glaze and Troyer, 2006) but once the stimulus is over the bird will go back to its steady state respiratory cycle. Yet, song rhythm does change during development (Chapter 3). Hence we would expect those abrupt rhythm transitions to be limited to a ratio that relates to the respiratory cycle, as we see in Chapter 3. In chapter 3 we observe abrupt transitions in rhythms during development. In the birds we examined, the abrupt rhythm transitions had a small integer ratio such as 1:2 or 2:3. The relationship between the old rhythm and the new rhythm could originate from the relationship of the song rhythm to the respiratory cycle and the constraints it imposes on the system. Note, as observed in the ratio of wing beat and respiratory cycle, the relationship between the old rhythm and the new rhythm might be different for different individual birds.

In chapter 4 we studied late trained birds. It is known that the closure of the sensitive period could be delayed by withholding exposure to the song model. Nevertheless, late trained birds do not imitate well (Figure 4.1). In Chapter 4 we saw that late trained birds are capable of changing their syllables, and their millisecond distribution – to some extent. But they are not able to insert new syllables into the song. This is in par with the model we presented above. In that model, the timing of the millisecond sub-notes and the fine structure of the syllables is controlled by HVC. The global timing is controlled by the brainstem clock. There could be different critical periods for different nuclei in the feedback loop. In the discussed model, a possible explanation for the lack of syllable insertion is that HVC-RA forebrain nuclei close their critical periods later than the brainstem “clock” nuclei.

Rhythm analysis

In order to study rhythm development in songbirds we had to introduce a new concept to bird song research, the quantification of rhythm and periodicity in song structure. By developing a new technique that gives us insights into the song rhythm we have studied how song structure changes at each stage of life. This provided a level of analysis that was previously unexplored because rhythm analysis provides four key advantages over previous song analysis techniques:

1. Rhythm analysis is a systematic method for quantifying periodic or quasi-periodic behavior. We saw that many bad imitators are stuck with a compound song. (Figure 3.28) Therefore, they are stuck with a fast rhythm, and once in a while (usually at the end or the beginning - see Chapter 3) they will be able to incorporate the additional syllable into their song. Rhythm analysis distinguishes between compound songs and periodic songs (see Chapter 3).
2. There is no need for segmentation or identification of syllable prototypes. This is an important advantage because segmentation and syllable prototype cannot be clearly and objectively identified in early song. For example, even in early ages rhythm analysis can at times identify repeating elements that are hardly visible by the naked eye (Figure 2.27).
3. Developmental data is abundant. Rhythm analysis reduces the dimensionality of the data. It summarizes an entire bird's song development (120 days of development) into two easy-to-interpret vectors. First, the duration of the repeating unit in the song (the bird's motif) is identified and the stereotypy is quantified. Furthermore, it helps us identify the rare but important days when the bird modified its song by presenting a rhythm map with high entropy or a different new spectrum. Therefore, in order to understand song ontogeny one

can focus its efforts on those specific significant days when changes in the song occur. For example, analysis of bird R109 has been ongoing for years using ontogeny visualization and interval analysis. Rhythm analysis could have shortened this analysis time by focusing the researchers on the interesting phenomena on day 60. On day 60, bird 109 rhythm map shows an abrupt transition in rhythm that corresponds to the integration of a new syllable in the motif (Figure 3.16).

4. Rhythm analysis is not sensitive to phase shifts in the data. Let's say there is a periodic structure (syllable or note) that is "jittered" with respect to syllable onset, syllable interval analysis will be affected. Spectra analysis is more robust to such jitter. Such "onset-unlocked" periodic structure is likely to be present in the song during development while song rhythm has not crystallized.

Rhythm analysis was used to study rhythm development in juvenile birds (Chapter 3). Song learning in zebra finch and Bengalese finches, similar to language learning in humans, is based on a process of memorization and imitation (Konishi 1965, Kuhl 1994, Doupe & Kuhl 1999). Learning begins when a young bird is exposed to the song of an adult 'tutor'. Subsequently, during a sensitive period, the young bird refines his initially disordered vocalizations into a mature, 'crystallized' song that is very similar to that of the tutor (Arnold 1975, Immelmann 1969, Dietrich 1980, Clayton, 1987, 1988, 1989).

We were interested in the question whether rhythm is an epiphenomena of the repetitive song structure of the zebra finch song. We discovered that rhythm transitions occur in two forms: smooth downward modulation and abrupt transition in rhythms, where the new frequency and the old frequency seem to be related to the old rhythm. We see the smooth down-modulations when the bird is time-warping (usually stretching) specific syllables that are already included in the motif. Abrupt transitions occur when the bird inserts a new syllable into its song, or differentiates a prototype into two distinct syllables.

We also found that rhythm entropy, which indicates the periodicity of the bird song, is correlated with imitation abilities (Figure 3.27). This indicates that birds that imitate well sing a stereotyped song. Bad imitators have a more complex song, with a shorter repeating unit (Figure 3.29, 3.30) and may have additional elements that are not incorporated in to the rhythm (Figure 3.31).

The constraint of incorporating additional elements into the motif is exhibited by the relationship between the old rhythm and the new rhythm. The smooth rhythm transitions that are observed in some birds before an abrupt rhythm transition could be a preparation for an abrupt rhythm transition. This indicates that that rhythm is not an epiphenomena but has an underlying neuronal constraints. The neuronal constraints might stem from the brainstem clock, which is related to respiratory pattenrs (for a more in-depth explanation see the model of the rhythm generator at the beginning of this chapter).

In Chapter 4 we use the dynamic spectrum to study the development of rhythm in acoustical and socially isolated zebra finches that we exposed to a training model at the end of their sensitive period. Zebra finches that are isolated until the end of their sensitive period and then trained do not imitate well (Figure 4.1). Those finches can change the millisecond distribution of their song but those changes do not necessarily improve their imitation. They can modify their syllables and eliminate the production of other syllables. We found that their inability to imitate well is caused by their inability to add new syllables to the songs. Zebra finches that sing a long motif before the onset of training, that is, they have more raw materials to work with, were able to imitate better than finches with a short motif. This indicates that at the end of the sensitive period there are developmental constraints on the level of song rhythm. Those constraints could stem from a neuronal segregation between the coarse rhythmic control in the brainstem clock and the fine temporal control in HVC and RA. The two neuronal areas might have different critical periods, i.e. the HVC-RA forebrain nuclei close their critical periods later than the brainstem clock.

In chapter 6 we used rhythm analysis to examine the song of old birds. We know that in humans there are some speech changes that occur with normal aging and with Parkinson's disease. However, changes in song structure with old age have not been explored and compared to those that occur in speech. We wanted to utilize the rhythm analysis method to better understand song changes between middle aged and aged songs. We found that old Bengalese finches have slower rhythms compared to middle aged birds, and the amplitude of their song compared to call amplitude decreases. This hints of a possible loss of the cortical inputs which are driving the song, i.e. a deterioration of components of the song system at old age. Our study of old Bengalese finches shows that the motor program of the song is modified throughout the lifespan of the bird. In human speech, age-related slowing of speech and changes in fundamental frequency result from the complex interaction of multiple peripheral and neural changes (Linville 2001). Aged songbirds could provide an animal model system for exploring how peripheral and neural changes interact to induce age-related deterioration in learned vocal patterns in elderly populations.

Future directions

The work presented here leaves a number of intriguing questions unanswered. Perhaps of foremost interest is the exact nature of the rhythmic constraints on the juvenile birds. Is there a basic unit in the rhythm generator? Is it related to the respiratory cycle? It would be interesting to statistically compare the developmental motif durations extracted from pressure data before and after abrupt transitions, the ratio of the abrupt transition with respiratory cycles in rest and during singing. In chapter 3 we saw an example where a bird inserts a syllable into a time slot, and than increases the silence gap between the syllables (Figure 3.23, 3.24). Since birds inhale during gaps of silence, from a respiratory point of view the bird started with a hard task, and than as it became more proficient with producing the motif it “made its life easier”. One would think that the opposite would occur, the first times the bird produces a new syllable it won’t rush (shorter silence gap) to produce the new syllable. This hints that the temporal location when the syllable is sung is necessarily due to temporal alignment constraints not due to physical constraints such as lack of air. It would be interesting to record from HVC->RA neurons at the time of insertion and during compound bouts. How does the reorganization of the firing cycle happen? If we could find the neuron that fires in the time point of the insertion, how will it modify its firing pattern? Will it be temporally associated with the previous syllable, the new syllable, or both syllables?

Another interesting topic is if we can teach late trained birds to imitate well. We preformed a few experiments where we trained late trained birds with a model song that has a similar temporal alignment compared with the bird's song. We did not reach conclusive results. Our best learners imitated very well compared to other late trained birds (Figure 6.1) despite having short motifs which, as we saw in chapter 4, correlates with bad imitation (Figure 4.16), but other birds did not increase their similarity to the song model. The bad imitation results could be explained by our lack of understanding which temporal markers we should aligned the song to. It would be interesting to redo those experiments, and try to improve our understanding of late trained imitation from the birds' successes and failures.

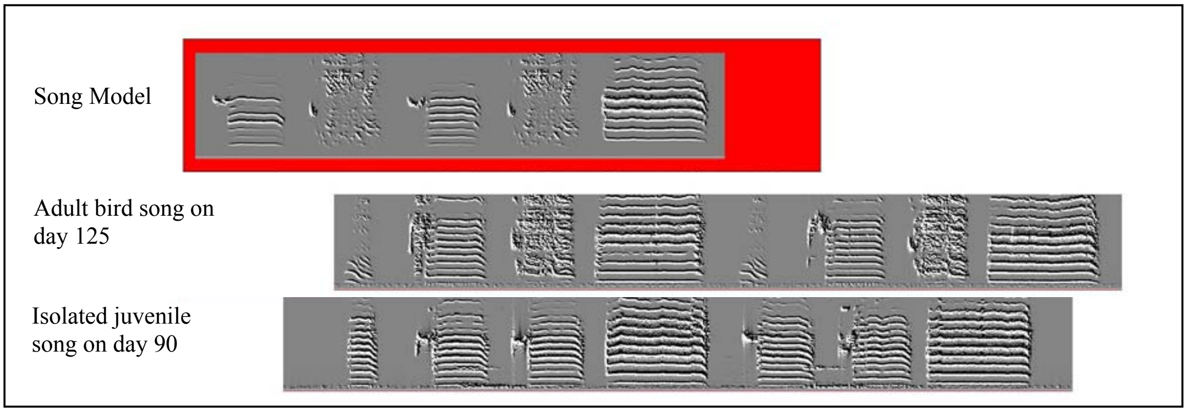


Figure 6.1 Can we design a song model specific for a late trained bird that will enable the bird to imitate well? Here is an example of a late trained bird that was able to imitate a song model that was tailored for him.

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