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Ontogeny of long-range vocalizations in a Neotropical fossorial rodent: the Anillaco Tuco-Tuco (*Ctenomys* sp.)

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Adult vocalizations can develop following three alternative modes; by retention of juvenile vocalizations, through the modification of juvenile precursors or by a *de novo* appearance in adults. Vocalizations that develop from juvenile precursors may develop following two pathways; vocal learning (implying the ability of juveniles to modify their vocalizations based on an external auditory input) and/or vocal tract maturation (involving the improvement of the capacity of juveniles to generate progressively more adult-like vocalizations by the tuning of an innate motor program). The emission of adult vocalizations requires the synchronization of neuromuscular and anatomical structures, and the lack of maturation and/or precise coupling between them would lead to the production of abnormal vocalizations. Tuco-tucos (*Ctenomys* spp.) are subterranean rodents that produce territorial, high intensity long-range vocalizations (LRVs) of, broadband and low frequency that are essential for long-distance communication between individuals in different tunnel systems. Despite their importance, the developmental modes, pathways and developmental sequences of LRVs remain poorly understood. In adult Anillaco Tuco-Tucos (*Ctenomys* sp.) the LRV is composed by two types of syllables (series and individual notes) that are repeated a variable number of times. We studied the development of the LRV in 8 juveniles of the Anillaco Tuco-Tuco ranging from 14 to 108 days after birth. The LRV exhibited a mixed developmental mode: while series developed from juvenile precursors whose acoustic features gradually approached those of adults, individual notes appeared later in the ontogeny and *de novo*, with acoustic features undistinguishable from those of adults. Vocalizations became progressively longer, but the series types shown by adults were evident in juveniles at an early age. All three acoustic features of series (triad) notes studied correlated with age in both sexes (duration 90% increased through development, while bandwidth 90% and peak frequency decreased).

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tract maturation hypothesis and possibly rejecting the vocal learning hypothesis. Juveniles emitted a higher proportion (7.4%) of abnormal vocalizations than adults (0.3%), as expected in the development of any complex behavior that requires practice to be mastered. The maturation of the LRV occurred well before the sexual maturation, presumably due to the protracted time needed to acquire or build a burrow system long before mating is possible. We propose that protracted vocal development is another component in the slow developmental strategy of Ctenomys and subterranean rodents in general.
Ontogeny of long-range vocalizations in a Neotropical fossorial rodent: the Anillaco Tuco-Tuco (Ctenomys sp.)

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Abstract
Adult vocalizations can develop following three alternative modes; by retention of juvenile vocalizations, through the modification of juvenile precursors or by a de novo appearance in adults. Vocalizations that develop from juvenile precursors may develop following two pathways; vocal learning (implying the ability of juveniles to modify their vocalizations based on an external auditory input) and/or vocal tract maturation (involving the improvement of the capacity of juveniles to generate progressively more adult-like vocalizations by the tuning of an innate motor program). The emission of adult vocalizations requires the synchronization of neuromuscular and anatomical structures, and the lack of maturation and/or precise coupling between them would lead to the production of abnormal vocalizations. Tuco-tucos (Ctenomys spp.) are subterranean rodents that produce territorial, high intensity long-range vocalizations (LRVs) of, broadband and low frequency that are essential for long-distance communication between individuals in different tunnel systems. Despite their importance, the developmental modes, pathways and developmental sequences of LRVs remain poorly understood. In adult Anillaco Tuco-Tucos (Ctenomys sp.) the LRV is composed by two types of syllables (series and individual notes) that are repeated a variable number of times. We studied the development of the LRV in 8 juveniles of the Anillaco Tuco-Tuco ranging from 14 to 108 days after birth. The LRV exhibited a mixed developmental mode: while series developed from juvenile precursors whose acoustic features gradually approached those of adults, individual notes appeared later in the ontogeny and de novo, with acoustic features undistinguishable from those of adults. Vocalizations became progressively longer, but the series types shown by adults were evident.
in juveniles at an early age. All three acoustic features of series (triad) notes studied correlated with age in both sexes (duration 90% increased through development, while bandwidth 90% and peak frequency decreased). LRV developed normally in juveniles acoustically isolated from adults, supporting the vocal tract maturation hypothesis and possibly rejecting the vocal learning hypothesis. Juveniles emitted a higher proportion (7.4%) of abnormal vocalizations than adults (0.3%), as expected in the development of any complex behavior that requires practice to be mastered. The maturation of the LRV occurred well before the sexual maturation, presumably due to the protracted time needed to acquire or build a burrow system long before mating is possible. We propose that protracted vocal development is another component in the slow developmental strategy of *Ctenomys* and subterranean rodents in general.

**Introduction**

Like many other aspects of biological systems, vocalizations experiment an ontogeny. In principle, adult vocalizations could develop following three alternative modes. Adults may just retain the vocalizations of juveniles (Campbell et al. 2014), vocalizations may develop through the modification of juvenile precursors that eventually reach adult features (Grimsley et al. 2011), or they may appear *de novo* in adults without the need of homologous juvenile precursors (Campbell et al. 2014). Additionally, vocalizations in the early life generally differ from those in adulthood, either because they perform specific functions that are lost with aging (e.g., begging vocalizations) (Hammerschmidt et al. 2000, 2001; Hauser 1989; Seyfarth & Cheney 1986) or because the rate of emission of the same vocalization changes from being common in juveniles to rare in adults or viceversa (e.g., the trill of *Cebus capucinus*) (Gros-Louis 2002).

Vocalizations that develop from juvenile precursors into adult ones, may follow two non-mutually exclusive developmental pathways: vocal learning and vocal tract maturation (Janik & Slater 1997). Development of vocalizations by vocal learning implies the ability of juveniles to modify their vocalizations based on the copying of sounds from an external auditory input, typically from conspecifics (Tyack 2016). Juveniles of vocal learners can modify the acoustic features of their vocalizations and also incorporate new vocalizations to their repertoire as a
result of the influence of social interactions (Janik & Slater 1997). On the other hand, development of vocalizations by vocal tract maturation involves the improvement of the capacity of juveniles to generate progressively more adult-like vocalizations by the tuning of an innate motor program to anatomical structures without the need of an auditory input (Winter et al. 1973; Tyack 2016).

The acquisition of adult vocal patterns requires practice, since it is necessary to synchronize the neuromuscular system and anatomical structures, either to match the vocal output to those signals acquired through external input or to achieve the successful deployment of instructions contained in the internal innate motor program (Boughman & Moss 2003). The lack of maturation and/or precise coupling between the morphology and the neuromuscular system of the vocal apparatus leads to the production of abnormal vocalizations. Consequently, regardless of the developmental pathway followed, juveniles are expected to produce a greater proportion of abnormal vocalizations during their practice period than adults during the emission of their mature vocalization (Konishi 1965).

Tuco-tucos (Ctenomys spp.) are subterranean rodents that produce long-range vocalizations (LRVs) of high intensity, broadband and low frequency. These vocalizations are essential for long-distance communication between individuals in different tunnel systems (Schleich & Busch 2002, Amaya et al. 2016). Multiple lines of evidence suggest that LRVs are territorial signals that facilitate the maintenance of individual territories and minimize aggressive encounters, especially between males (Francescoli 1999; Schleich & Busch 2002; Amaya et al. 2016). Despite their behavioral importance, the LRVs of adults of only three of the more than 60 recognized species of Ctenomys (Bidau 2015) have been characterized in some detail (Francescoli 1999, Schleich & Busch 2002, Amaya et al. 2016). Juvenile LRVs were simply mentioned in C. talarum (Zenuto et al. 2002) and C. mendocinus (Camin 2010), but there is no detailed information on the developmental modes, pathways or developmental times of LRVs. Learning how and when the LRVs reach maturity can help understand how life-history traits interact and influence each other in fossorial rodents. For example, since burrows are energetically expensive resources (Vleck 1979, 1981) it is conceivable that solitary species of Ctenomys secure or build their own burrow system before mating. This would imply the need of
having mature LRVs to help defend the burrows during the process of acquiring a mate, even
before reaching sexual maturity.

The LRV of adult Anillaco Tuco-Tucos (Ctenomys sp.) is composed by two types of
syllables (series and individual notes) that are repeated a variable number of times (Figure 1). In
normal vocalizations, each series begins with a note that is followed immediately by a soft note,
but adults occasionally produce structurally abnormal series by adding a soft note before the
initial note (Amaya et al. 2016). Depending on the number of notes sandwiching soft notes,
series can be classified as dyads (two notes, a soft note), triads (three notes, two soft notes), or
tetrads (four notes, three soft notes) (Amaya et al. 2016). In contrast, individual notes are single
notes emitted at a faster rate in succession and are always given after the series (Figure 1).

This paper aims to describe the ontogenetic changes of the LRVs in the Anillaco Tuco-
Tuco based on recordings of captive-reared pups, in order to 1) discuss which developmental
mode (juvenile retention, juvenile precursor, or de novo appearance) explains the ontogeny of
the two types of syllables of the long-range vocalization of the Anillaco Tuco-Tuco; 2) evaluate
whether the ontogeny of the long-range vocalizations of the Anillaco Tuco-Tuco fits the vocal
learning and/or the vocal tract maturation developmental pathways; 3) test the prediction that
juveniles should produce a greater proportion of anomalies in precursors of the LRV, than
adults when giving the fully mature rendering of this vocalization, and 4) discuss the
relationship between the development of LRVs and other life-history traits.

Materials and methods
Experimental animals

We studied the development of the LRV in 8 juveniles (5 males and 3 females) of the
Anillaco Tuco-Tuco (see Amaya et al. 2016 for taxonomic background). Juveniles were trapped
at Anillaco, La Rioja, Argentina (28°48'50"S, 66°55'54"O; 1365 m asl) in November and
December yearly between 2013 and 2016. Immediately after trapping, juveniles were
acoustically isolated from adults and placed in individual enclosures in the Chronology and
Ethology lab in CRILAR (see Amaya et al. 2016 for enclosure features and animal care data).
Juveniles were weighed four times (when trapped and after 30-35 days in captivity; Table 1) and sexed \textit{a posteriori} because the genitalia were not mature at the time of capture.

Since we captured weaned juveniles of unknown biological age, we estimated their absolute ages at trapping by comparing their weights to those of juvenile \textit{C. mendocinus} of known age (Camin 2010). Adults of the Anillaco Tuco-Tuco are 10% heavier than adults of \textit{C. mendocinus} (Camin 2010, Amaya et al. 2016). Thus, we assumed a linear age-weight relationship such that 10% heavier juveniles of the Anillaco Tuco-Tuco where aged as equivalent to 10% lighter juveniles of \textit{C. mendocinus} (e.g., at day 14 juveniles of \textit{C. mendocinus} weigh on average 33 g, while at this age the weight of juvenile Anillaco Tuco-Tucos would be around 36.3 g). The minimum estimated age at trapping was 14 days (female 1; Table 1) and the maximum 28 days (male 1; Table 1).

\textbf{Recording of vocalizations}

We studied the development of LRVs of 8 juveniles of Anillaco Tuco-Tuco. Each juvenile was recorded in four 6-8hr sessions in alternate days within a week every four weeks (i.e., a week of recording with four sessions was separated by three weeks of not recording). Sessions began during the first week that individuals were trapped and finished after twelve weeks. Thus, each individual was recorded in 16 sessions spread along 12 weeks, except for male 3 that died in the ninth week and was recorded in only 12 sessions.

Recordings were made from outside the enclosure using a Zoom H4n digital hand recorder system with built-in microphones (sample rate of 44.1 kHz and 24 bit depth) mounted on a tripod with the microphones facing the enclosure. The gain setting of the recorder was the same for all recordings. Our recording protocol allowed us to detect and record the emission of the LRVs, which occur at a low rate and unpredictably. All the recording sessions were made during the night (between 22:00 and 6:00hs Argentina time, GMT -5), due the low level of ambient noise and to the high nocturnal activity of the species in laboratory conditions (Tachinardi et al. 2014). In total, we recorded 5 males for 448 hours in 76 sessions and 3 females for 276 hours in 48 sessions.
We searched for LRVs in recordings by manually examining on-screen spectrograms built with Raven pro 1.4 (http://www.birds.cornell.edu/Raven). We used the following spectrogram parameters to prioritize resolution in time to be able to easily detect vocalizations:

- Window: Hann; size: 256 samples (= 5.8 ms);
- 3 dB bandwidth-filter: 248 Hz;
- Time grid-overlap: 50%;
- Hop size: 128 samples (= 2.9 ms);
- Frequency grid–DFT size: 256 samples;
- Grid spacing: 172 Hz.

**Acoustic characterization of the long-range vocalization**

**Acoustic characterization of series.** We acoustically characterized triads of juveniles because they are the most common and characteristic series type of the LRV in adults of these species (Amaya et al. 2016). To compare triads of juveniles with those of adults we characterized triads in three adult males and three adult females (three triads per adult individual), using adult recordings from Amaya et al. (2016). We measured duration 90% (ms), bandwidth 90% (Hz) and peak frequency (Hz) of each note in one triad per vocalization in juveniles and adults. We calculated duration 90%, bandwidth 90% and peak frequency for each triad note as follows.

- First, we delimited triads with a selection border A (400 ms and 0-22.05 kHz) (Figure 2 A);
- second, we divided the selection border A in 20 equal selections borders B (20 ms and 0-22.05 kHz) (Figure 2 B);
- third, we calculated the energy contained within each B selection border (Figure 2 B); and fourth, we defined a selection border C (40 ms and 0-22.05 kHz) for each triad note choosing the two adjacent selection borders B with the highest energy values (Figure 2 B).

We calculated single duration 90%, bandwidth 90% and peak frequency values for each individual in each session by averaging the values of the three triad notes.

**Acoustic characterization of individual notes.** We analyzed all juvenile vocalizations to quantify the number of individual notes emitted per vocalization and acoustically characterized them. To compare individual notes of juveniles with those of adults we characterized individual notes in three adult males and three adult females, using adult recordings from Amaya et al. (2016). We
measured duration 90% (ms), bandwidth 90% (Hz) and peak frequency (Hz) in three individual notes per vocalization in juveniles and adults. We calculated duration 90%, bandwidth 90% and peak frequency for each individual note as follows. First, we delimited individual notes with a selection border D (80 ms and 0-22.05 kHz) (Figure 2 B); second, we divided the selection border D in 4 equal selections borders E (20 ms and 0-22.05 kHz) (Figure 2 C); third, we calculated the energy contained within each E selection border (Figure 2 C); and fourth, we defined a selection border F (40 ms and 0-22.05 kHz) for each individual note choosing the two adjacent selection borders E with the highest energy values (Figure 2 C). We calculated single duration 90%, bandwidth 90% and peak frequency values for each individual in each session by averaging the values of the three individual notes.

**Spectrogram parameters.** All acoustic measurements were made with Raven Pro 1.4. We used different spectrograms parameters that allowed us to obtain accurate time and frequency domain measurements. For duration 90% we used the following spectrogram parameters: Window: Hann; size: 256 samples (= 5.8 ms); 3 dB bandwith-filter: 248 Hz; Time grid-overlap: 50%; hop size: 128 samples (= 2.9 ms); Frequency grid–DFT size: 256 samples; grid spacing: 172 Hz. For bandwidth 90% and peak frequency the spectrogram parameters were: Window: Hann, size: 1024 samples (= 52.4 ms), 3 dB bandwith-filter: 27.4 Hz; Time grid–overlap: 50%, hop size: 512 samples (= 26.2 ms); Frequency grid–DFT size: 4,096 samples, grid spacing: 4.7 Hz. All recordings were band-pass filtered between 80–5,000 Hz in Raven Pro 1.4 to eliminate sources of disturbance and distortion in acoustic measurements as in Amaya et al. (2016).

**Series types and abnormal series**
We analyzed all juvenile vocalizations to quantify the occurrence of each of the three normal series-patterns (dyad, triad and tetrad). We first identified the series-patterns in the corresponding waveform and spectrogram by counting the number of notes and soft-notes per series, and then counted the number of series-patterns per vocalization. Following the same procedure, we quantified the occurrence of abnormal series in juvenile vocalizations. We considered a series to be abnormal if it had a soft-note before the note 1 in dyads, triads and
tetrads or if it was composed by a single note followed by a soft-note (i.e., it was a monad) (Figure 3).

In order to test the prediction that juveniles produce proportionally more abnormal series than adults, we compared the proportion of abnormal series in adults (Amaya et al. 2016) to the proportion of abnormal series in juveniles. To calculate these proportions we counted the number of abnormal series and divided these counts by the total number of series in juveniles and in adults (325 juvenile series recorded for the present study and 703 adult series from Amaya et al. [2016]).

**Statistical analyses**

To evaluate if the acoustic features of juvenile triads notes changed during their development, we performed Spearman correlations between duration 90%, bandwidth 90% and peak frequency (one average value per variable was calculated using the three triad notes for each individual at each sampling week) against absolute age for each individual. To evaluate if the number of series per vocalization changed along the development we performed a Spearman correlation between the number of series per vocalization and absolute age for each individual.

To examine if juvenile triads changed more quickly at some point in time or whether developmental changes were even through the sampled period, we averaged acoustic features from all recording sessions within a week for all individuals of the same sex and of similar age (i.e., within an 11 day range in males and a 9 day range in females) and compared these values between all weeks and against adult values of the corresponding sex using a Kruskall-Wallis test. The same procedure was applied to compare the acoustic characteristics of the individual notes of each juvenile male and of the average value of all males of similar age (i.e., within a 108-118 day range) against adult values.

All procedures followed the guidelines of the American Society of Mammologists for the use of wild mammals in research (Sikes & Gannon, 2011). All experiments were performed at the CRILAR in Anillaco and were authorized by the Environmental Department of La Rioja (permits 028–10 and 062–08) and approved by the Ethics Committee of the Faculty of
Veterinary Sciences of La Plata National University, Argentina (permit 29-2-12). All individuals were returned to their natural environment after the study was complete. For statistical analyses we used InfoStat (Di Rienzo et al. 2012).

Results
Seven juveniles of the Anillaco Tuco-Tuco (five males and two females) produced 71 LRVs in the 12 study weeks; while an eighth juvenile (female 3) did not produce any vocalization (Table 1). The two syllable types of LRVs, series and individual notes, appeared sequentially in the vocal development (Figure 4). Series appeared first and were given by juveniles as young as 14 days old (39 g). Individual notes were given considerably later in the development, when juveniles were as young as 108 days old (156 g) (Figure 4, Table 1). Accordingly, most vocalizations were composed only by series (95.77%, N=68), and fewer vocalizations were composed by series and individual notes (4.22%, N=3) (Table 1).

Development of series
Developmental changes caused juvenile triads to progressively approach the acoustic parameters of adult triads (Figure 5). The seven juveniles that vocalized gave series (Table 1). The number of series per vocalization increased through development and varied from 1 to 25 in juvenile males (Table 1, Table S3) and from 1 to 6 in juvenile females (Table 1, Table S3). The first vocalizations of all juveniles contained a single series, and the highest numbers of series were recorded in juvenile males between 108 to 118 days old, and in juvenile females between 104 to 113 days old (Table 1).

Juveniles produced the three series-patterns present in the adults. Triads were by far the most common series-pattern (82.76%, N=269), followed by dyads (16.61%, N=54), and tetrads (0.61%, N=2) (Table 1). Triads were produced by all vocalizing juveniles, dyads were produced by all juveniles (except male 3 which died prematurely), and tetrads were produced by only two juveniles (female 1 and female 2) (Table 1).

Within individual developmental trajectories, duration 90% exhibited a directional increase through development and correlated with age in two out of four males males and in
both females (Table 1, Table S3), whilst bandwidth 90% of triad notes decreased in all but one individual (Table S3), and peak frequency of triad notes decreased during the development of all individuals (Table S3). When individuals were lumped by sex, all three acoustic features of triad notes correlated with age: duration 90% increased through time (males: \( r = 0.25,  P<0.0001 \); females: \( r = 0.55,  P <0.0001 \)), while bandwidth 90% (males: \( r = -0.49,  P<0.0001 \); females: \( r= -0.75,  P<0.0001 \)) and peak frequency decreased (males: \( r = -0.58,  P<0.0001 \); females: \( r= -0.49,  P<0.0001 \)).

Acoustic parameters progressively approached features of adults in both sexes and achieved mature features at different developmental stages (Table 2). All acoustic parameters passed through one or two intermediate developmental stages in which parameters were statistically different from adults as they approached adult values (Table 2).

**Abnormal series in juveniles and adults**

Juveniles emitted two types of abnormal series during the development of the long-range vocalization. In the first type of abnormal series a soft note preceded the first series note, and included abnormal triads (Figure 3A), dyads (Figure 3B) and tetrads (Figure 3C). In the second type, the series was conformed by a single monad that consists of a single note followed by a soft-note (Figure 3D). Monads are unknown in adults (see Amaya et al. 2016).

The proportion of abnormal to normal series was obviously larger in juveniles than in adults. While in juveniles 7.38% of the series were abnormal (8 abnormal triads; 11 abnormal dyads; 3 abnormal tetrads; 4 monad vs 325 normal series), in adults only 0.28 % of the series were abnormal (one abnormal triad and one abnormal dyad vs. 701 normal series).

**Development of individual notes**

Only 3 of the 5 males and none of the two females that vocalized gave individual notes. These notes were given by males 1, 2 and 4 when they had between 156-162 g and were 108-118 days old (Table 1); while male 3 died at an early age, male 5 approached this weight and was within this age range but never gave individual notes (Table 1). Individual notes were recorded in only a single vocalization in each male, and ranged in number from 22-31 notes always
emitted after a sequence of series and never in isolation (Table 1). Acoustic parameters (duration 90%, bandwidth 90% and peak frequency) of individual notes of all juveniles were undistinguishable from adults both individually and when averaged (Kruskal Wallis test, p>0.05) (Table 1, Figure 5).

Discussion

In this paper we have shown that the LRV of the Anillaco Tuco-Tuco (*Ctenomys* sp.) exhibits a mixed developmental mode in which the two syllables types of the vocalization develop differently: while series developed from juvenile precursors whose features gradually approach those of adults, individual notes appeared later in the ontogeny and de novo, with acoustic features undistinguishable from those of adults. We also conclude that the LRV develops normally in juveniles acoustically isolated from adults, supporting the vocal tract maturation hypothesis and possibly rejecting the vocal learning hypothesis. Finally, we found that juveniles emit a higher proportion of abnormal vocalizations than adults, as expected in the development of any complex behavior that requires practice to be mastered.

Development of the long-range vocalization

The LRV in the Anillaco Tuco-Tuco exhibits a mixed developmental mode, since it includes a syllable type (series) that develops gradually from structurally conserved ontogenetic precursors in the juveniles and another syllable type (individual notes) that appears de novo with adult features. This sort of mixed development is novel and has not been reported in the vocalizations of other rodents. For example, while the vocalization of *Mus musculus* develops from precursors of the syllables that increase in duration and decrease in fundamental frequency (Grimsley et al. 2011), the acoustic characteristics of the notes in *Scotinomys* spp. do not experience ontogenetic changes (Campbell et al. 2014) and are therefore the product of retention of juvenile vocalizations.

Triads are by far the most common series pattern in isolated captive juveniles and in free-ranging adults of the Anillaco Tuco-Tuco (Amaya et al. 2016). Early in the ontogeny,
juveniles of the Anillaco Tuco-Tuco produce the three series-patterns present in adults (dyads, triads and tetrads), but the early acoustic features of triads differ from those of adults. Juvenile precursor triad notes decrease bandwidth and peak frequency gradually until reaching the values of adult triads. Thereby, juvenile precursors develop into adult series by gradual changes in their acoustic parameters but without changing their basic structure. This lack of changes in the structure of series is interesting since a priori, other developmental mechanisms could have occurred. For instance, it would have been possible to begin with the emission of separate notes and soft-notes, passing to their joint emission in the shape of monads, subsequently leading through the repetition of this basic structure to the creation of the other types of series composed by more notes and soft-notes. We interpret the early appearance of triads and their ubiquitous presence as evidence of a substantial genetic makeup in the structure of series in Ctenomys. In fact, other series-patterns predominate in other species of tuco-tucos. For example, monads are characteristic of Ctenomys talarum (Schleich & Busch 2002) and dyads are characteristic of Ctenomys mendocinus (Amaya et al. 2016, Amaya and Areta, unpublished data).

The lower degree of morphological and neuromuscular maturation and tuning in the vocal tract of juvenile in comparison to adult tuco-tucos may account for the higher proportion of abnormal series in young individuals. This explanation may be more general and could also apply to juveniles of two species of singing mice (Scotinomys teguina and S. xerampelinus). Five types of notes were described in juvenile Scotinomys spp. (Campbell et al. 2014); one very frequent note (note A) exists at all ages and is almost the only note sang by adults, while four less frequent notes are more frequent in the first 12 days of life and become almost non-existent after 30 days (Campbell et al. 2014). We speculate that the four types of less frequent notes are not different notes per se, but that they could simply represent anomalies of the note A. Hence, their decrease in frequency could be explained by the improved performance of the vocal tract and associated neuromuscular apparatus through development.

Individual notes of LRVs appear late, de novo and with acoustic parameters indistinguishable from those of adults, showing a developmental mode that is strikingly different to that of series. The first individual notes are given by juveniles when the series have
already acquired adult acoustic characteristics through maturation from precursors. This suggests that the appearance of individual notes with their definitive characteristics without the existence of ontogenetic precursors is possible because the neuromuscular system and the vocal apparatus have acquired the necessary "fine tuning" through practice of the series during their development. The passive maturation of the individual notes might be, at least in part, a co-product of the active maturation of the series.

The LRVs in the Anillaco Tuco-Tuco exhibit a large variety of syntactic patterns (Amaya et al. 2016). These syntactic patterns undergo some general and predictable ontogenetic changes that include, first an increase in both the number of series and series-patterns per vocalization, and then an increase in syllable types given by the late appearance of individual notes. The increment in song length and the late appearance of individual notes might be widespread in *Ctenomys*. These patterns appear to occur also in the vocal development of *C. mendocinus*, where "short calls" presumably conformed only by series occur at 33 days and "long calls" presumably conformed by series and individual notes occur at 70 days (Camín 2010). In contrast, the highly stereotyped pattern of *Scotinomys* adult song appears *de novo* in the first vocalization of juveniles after a period of vocal inactivity (Campbell et al. 2014).

**Vocal learning vs vocal tract maturation in the LRV**

Vocalizations of rodents appear to develop by maturation of the vocal tract and not by vocal learning since adult vocalizations develop normally without the need of an external auditory model from which to copy (Scherrer & Wilkinson 1993, Grimsley et al. 2011). In this paper we demonstrated that juveniles of the Anillaco Tuco-Tuco develop normal LRVs without an external auditory input between 14 and 108 days. This would indicate that the development of vocalization is a product of vocal tract maturation and not of a vocal learning process (Efremova et al. 2011). However, juveniles were exposed to adult LRVs from birth until they were captured, and *Ctenomys* are born with an open auditory meatus (Zenuto et al. 2002, Camín 2010). Thus, it remains possible that adult vocalizations heard at an early age have served as an auditory model for the subsequent vocal development. To thoroughly test the importance of vocal learning in the development of LRVs in *Ctenomys*, future studies should focus on
discovering when juveniles acquire the ability to listen and on evaluating if deafened juveniles develop normal LRVs when devoid from acoustic self-stimulation (Kroodsma & Konishi 1991; Campbell et al. 2014).

Life-history traits and territorial vocalizations in subterranean rodents

Subterranean rodents exhibit slow life-histories, which are characterized by slow speed of tissue growth due to a low basal metabolic rate selected for by the high energetic demands of burrowing (Henneman, 1983, 1984; Hofman, 1983; Martin, 1984a, 1984b; Glazier 1985) leading to delayed sexual maturity (Busch et al. 2000). In *Ctenomys talarum* and *C. mendocinus* gestation lasts around 95 days (Zenuto et al. 2002; Camín 2010); sexual maturity takes 180-240 days in *C. talarum* (Busch et al. 2000) and presumably adult vocalizations have been reported long before sexual maturity at 65-70 days (Zenuto et al. 2002) and the same pattern is evident in *C. mendocinus* which reaches sexual maturity around 180-270 days (Camín 2010) and alleged vocal maturity at 70 days (Camín 2010). We lack precise information on the age of sexual maturity in the Anillaco Tuco-Tuco, but the patterns described for *C. talarum* and *C. mendocinus* suggest that vocalizations mature well in advance of sexual maturity in *Ctenomys*.

The solitary bathyergids, *Bathyergus janetta, B. siullius* and *Georinchus capensis* exhibit a moderate gestation period of around 50 days and presumably delayed sexual maturation (Jarvis 1969; Bennett & Jarvis 1988; Bennett et al. 1991, 2000). In *B. janetta* and *B. siullius* juveniles begin to produce seismic signals (foot-drumming) at 80 days, after dispersion at 60-65 days from the maternal burrow system, while in *Georinchus capensis* juveniles produce foot-drumming at 50 days of age before dispersion at 55-60 days of age (Bennett & Jarvis 1988). Dispersion in these bathyergids, occur when they have the capacity to dig and protect their own burrow systems (Bennett & Jarvis 1988). Thus, territorial acoustic signals of solitary ctenomyids and bathyergids seem to mature approximately at the time of dispersion but long before sexual maturity.

The protracted development of the LRV of the Anillaco Tuco-Tuco (ca. 110-120 days) herein reported contrasts markedly with the short developmental times of vocalizations of
superficial rodents such as *Scotinomys* (35 days; Campbell et al. 2014) and *Mus musculus* (at 13 days individuals had some adult vocal characteristics, Grimsley et al. 2011). The altricial *Ctenomys* contrast with the precocial *Scotinomys* in which gestation takes 30 days (Hopper & Carleton 1976) and which reach sexual maturity after 30-40 days concomitantly with the maturation of adult vocalizations (Hopper & Carleton 1976, Campbell et al. 2014).

We propose that protracted vocal development is another component in the slow developmental strategy of *Ctenomys* and subterranean rodents in general. The underground burrow systems have a central role in the ecology of subterranean rodents (Busch et al. 2000) and their defense is of key importance. Burrows are costly resources that serve for breeding and that are defended by the use of long-range vocalizations in *Ctenomys* (Amaya et al. 2016). The maturation of vocalizations in advance of sexual maturation might be explained by the protracted time needed to acquire or build a burrow system long before mating is possible.

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**Figure 1**

General structure and syllable types of the long-range vocalization of the Anillaco Tuco-Tuco (*Ctenomys* sp.).

General structure (above) and syllable types (series and individual notes) (below) of the long-range vocalization (LRV) of the Anillaco Tuco-Tuco (*Ctenomys* sp.). Waveforms are shown above and corresponding spectrogram below in both figures. Triad series are composed by three notes (N1, N2 and N3) and two soft-notes (Sa and Sb), and individual notes are simple sounds.
Figure 2

Acoustic characterization of the long-range vocalization during the vocal development of juveniles and in adults of the Anillaco Tuco-Tuco (*Ctenomys* sp.).

Delimitation of selection borders used for the quantitative acoustic characterization of triads (A and B) and individual notes (C and D) of the long-range vocalization (LRV) during the vocal development of juveniles and in adults of the Anillaco Tuco-Tuco (*Ctenomys* sp.). Waveforms are shown above and corresponding spectrogram below in all figures. A. Selection border A delimiting a triad. B. Selection borders B and selection border C delimited by the two adjacent selection borders B with the highest energy values. C. Selection border D delimiting an individual note. D. Selection borders E and selection border F delimited by the two adjacent selection borders B with the highest energy values. We measured duration 90%, bandwidth 90% and peak frequency in selection borders C (in triads) and F (in individual notes).
Figure 3

Abnormal series produced by juveniles of the Anillaco Tuco-Tuco (*Ctenomys* sp.)

Abnormal series produced by juveniles of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during the development of the long-range vocalization (LRV). Waveforms are shown above and corresponding spectrogram below in all figures. A. Abnormal triads. B. Abnormal dyads. C. Abnormal tetrad. D. Monad. Previous soft-note (P S); note (N); soft-note (S).
Figure 4

Development of the long-range vocalization

Development of the long-range vocalization (LRV) in a male 2 of the Anillaco Tuco-Tuco (Ctenomys sp.) during 4 sampling weeks over 12 study weeks. During the sampling period this individual (male 2) gave 11 vocalizations. Note the increase in the number of series per vocalization and the late appearance of individual notes. These two features are representative of the development of the LRV in this species. For each sampling week we indicate weight (g) and estimated age (days after birth).
Figure 5

Developmental changes in acoustic features in the long-range vocalization of the Anillaco Tuco-Tucos (*Ctenomys* sp.)

Developmental changes in acoustic features of triad notes and individual notes in the long-range vocalization (LRV) of the Anillaco Tuco-Tucos (*Ctenomys* sp.) and comparison to adult values. All data from juveniles and adults in Table S1 and S2. A. Duration 90%. B. Bandwidth 90%. C. Peak frequency.
Table 1 (on next page)

Long-range vocalizations emitted by eight juveniles of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during vocal development

Long-range vocalizations emitted by eight juveniles (five males and three females) of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during 4 sampling weeks over 12 study weeks. For each juvenile individual we indicate weight (*W*) (g), estimated age in days (*D*), and recording time in hours (*R*). For each vocalization recorded (Roman numerals) we indicate number of each normal series-pattern (triads in bold font; dyads in normal font; and tetrads in italics), and number of individual notes per vocalization (underlined). Deceased (†), no vocalization (-).
| Juveniles individual | Week 1 | Week 4 | Week 8 | Week 12 |
|---------------------|--------|--------|--------|---------|
|                     | W D R  | W D R  | W D R  | W D R   |
|                     | Vocalizations I II III IV | Vocalizations I II III IV | Vocalizations I II III IV | Vocalizations I II III IV |
| Male 1              | 73 28 22 1 1 1 - | 101 58 24 1 1 1 - | 125 88 18 2 2 1 - | 162 118 24 2 10 2 - |
| Male 2              | 47 18 22 1 1 - | 85 48 20 1 1 1 - | 119 78 24 1 2 6 | 156 108 24 2 2 8 - |
| Male 3              | 45 17 20 1 1 - | 80 47 22 1 2 - | 117 77 20 4 7 - | + + + + |
| Male 4              | 71 27 24 1 1 3 - | 97 57 24 1 3 5 - | 123 87 28 2 13 2 - | 158 117 28 13 4 - |
| Male 5              | 49 19 24 1 1 - | 81 49 24 2 4 - | 111 79 28 1 2 4 - | 150 109 28 5 1 15 - |
| Female 1            | 39 14 22 1 1 1 - | 68 44 24 1 1 1 - | 95 74 22 1 2 3 - | 125 104 20 5 6 - |
| Female 2            | 59 23 24 1 2 1 - | 85 53 24 1 1 - | 110 83 28 1 3 - | 142 113 28 2 3 6 - |
| Female 3            | 55 21 20 - - - | 70 51 18 - - - | 92 81 22 - - - | 115 101 24 - - - |
Table 2 (on next page)

Pairwise comparisons of acoustics parameters in series notes of the long-range vocalization of the Anillaco Tuco-Tuco (*Ctenomys* sp.).

Pairwise comparisons (Kruskal-Wallis test) of duration 90%, bandwidth 90% and peak frequency of series notes at different developmental segments of the long-range vocalization (LRV) of the Anillaco Tuco-Tuco (*Ctenomys* sp.). Shared letters indicate no statistical differences, different letters indicate significant statistical differences between developmental segments.
### Males

| Acoustic parameter | Juveniles (days of age) | Adults |
|--------------------|-------------------------|--------|
|                    | 17-28 | 47-58 | 77-88 | 108-118 |
| Duration 90%       | A     | B     | B     | B       | B       |
| Bandwidth 90%      | A     | A     | B     | B       | B       |
| Peak frequency     | A     | B     | B     | C       | C       |

### Females

| Acoustic parameter | Juveniles (days of age) | Adults |
|--------------------|-------------------------|--------|
|                    | 14-23 | 44-53 | 74-83 | 104-113 |
| Duration 90%       | A     | B     | B     | B C     | C       |
| Bandwidth 90%      | A     | A     | A     | B       | B       |
| Peak frequency     | A     | B     | BC    | BC      | C       |