

## RESEARCH ARTICLE

### Factors shaping the ontogeny of vocal signals in a wild parrot

Karl S. Berg<sup>1,2,3,\*</sup>, Steven R. Beissinger<sup>3</sup> and Jack W. Bradbury<sup>2</sup>

<sup>1</sup>Laboratory of Ornithology and <sup>2</sup>Neurobiology and Behavior, Cornell University, Ithaca, NY 14850, USA and <sup>3</sup>Environmental Science Policy and Management, University of California, Berkeley, Berkeley, CA 94720-3114, USA

\*Author for correspondence (kberg@berkeley.edu)

#### SUMMARY

Parrots rely heavily on vocal signals to maintain their social and mobile lifestyles. We studied vocal ontogeny in nests of wild green-rumped parrotlets (*Forpus passerinus*) in Venezuela. We identified three successive phases of vocal signaling that corresponded closely to three independently derived phases of physiological development. For each ontogenetic phase, we characterized the relative importance of anatomical constraints, motor skills necessary for responding to specific contexts of the immediate environment, and the learning of signals that are necessary for adult forms of communication. We observed shifts in the relative importance of these three factors as individuals progressed from one stage to the next; there was no single fixed ratio of factors that applied across the entire ontogenetic sequence. The earliest vocalizations were short in duration, as predicted from physical constraints and under-developed motor control. Calls became longer and frequency modulated during intermediate nestling ages in line with motor skills required for competitive begging. In the week before fledging, calls drastically shortened in accordance with the flight-constrained short durations of adult contact calls. The latter constraints were made evident by the demonstrated links between wing-assisted incline running, a widespread prelude to avian flight, just before the shift from long-duration begging calls to short-duration contact calls. At least in this species, the shifting emphases of factors at different ontogenetic stages precluded the morphing of intermediate-stage begging calls into adult contact calls; as shown previously, the latter are influenced by sample templates provided by parents.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/216/2/338/DC1>

Key words: ontogeny, contact calls, vocal learning, *k*-means cluster, flight, wing-assisted incline running, Psittacidae, Venezuela.

Received 11 April 2012; Accepted 19 September 2012

#### INTRODUCTION

The ontogeny of animals between hatching/birth and maturity is complicated by interactions between growth, offspring environment, and learning (Baldwin, 1896; Gottlieb, 1991). Growth involves the serial process by which a small and uncoordinated neonate changes into a larger and fully functional adult. Species differ in the duration and scheduling of this process depending on the neonate's degree of precocity (e.g. extent of nervous system development) and the amount of physical transformation required to become an adult [e.g. gradual *versus* metamorphic changes (Finlay and Darlington, 1995; Starck and Ricklefs, 1998)]. Offspring environment exerts selection pressures on young that can be radically different from those on adults (e.g. flightless nestling birds are bound to a nest, whereas adults are highly mobile) and can induce changes during ontogeny. For example, as siblings mature, the levels of competition for parental resources can change as competitors acquire basic motor skills and as the number of siblings shifts due to mortality or to asynchronous dates of nutritional independence. Finally, it may be advantageous for juveniles to learn behaviors that are essential to adult survival and reproductive success before maturation is complete. Examples include recognition of kin, acquisition of cue and signal templates and refinement of other motor and cognitive skills that will be mainly used in subsequent life history stages.

There are likely to be developmental tradeoffs between growth, environment and learning. Some adjustments to the juvenile's environment may be impossible until a minimal level of skeletal,

muscle and nerve development has been achieved. Acquiring templates for use as adults may divert time and energy from maximizing growth in their current environment. The duration of the immature period may or may not be sufficient to lay all of the groundwork for adult traits. At present, there seem to be no general models predicting the optimal tradeoffs at any stage, much less all of ontogeny. In part, this is due to a focus on laboratory studies where only some of the selective forces that have shaped a particular ontogeny are present (Kroodsma, 1996). There is clearly a need to compare the relative importance of growth, offspring environment and learning during ontogeny in natural populations. Ideally, one would examine whether the relative importance of these three factors is constant throughout ontogeny or instead varies with the physiological stage of development. Finally, one would like to know the degree to which successive stages in ontogeny are constrained by having to serve as precursors for later stages (Alberts, 1985).

While physical development patterns have been assembled for a host of bird species (Starck and Ricklefs, 1998), behavioral ontogenies are less common and have proven more difficult to quantify (Düttmann et al., 1998; Leonard and Horn, 2006). One well-studied exception is song learning in oscine passerine birds (reviews in Kroodsma, 1996; Nelson, 1997; Janik and Slater, 2000; Tchernichovski et al., 2001; Nowicki and Searcy, 2005). However, song, which functions in mate attraction and territory defense, is usually employed after the dramatic transformations that lead to physical maturation and offspring independence. There is increasing

evidence that many birds also learn contact calls, which tend to emerge earlier in development and function to mediate other types of social interactions (Marler, 2004; Kondo and Watanabe, 2009; Sewall, 2009). However, vocal ontogeny in species known for contact-call learning has received less attention.

Parrots present a classic case of contact-call learning (reviews in Farabaugh and Dooling, 1996; Bradbury, 2003). In most parrot species studied to date, contact calls have been shown to be individually specific (Saunders, 1983; Farabaugh et al., 1994; Cortopassi and Bradbury, 2006; Moravec et al., 2006; Buhrman-Deever et al., 2008; Berg et al., 2011). Whereas singing is usually a more sedentary occupation, contact calls are commonly used to coordinate movements of social companions between roosting, foraging and nest sites (Bradbury et al., 2001; Kondo and Watanabe, 2009) and can be given before, during or after wing-powered flights. Forward flapping flight puts tremendous demands on the same respiratory systems that have been co-opted for vocal communication (Suthers et al., 1972; Cooper and Goller, 2004; Marler, 2004). Interestingly, nestling green-rumped parrotlets (*Forpus passerinus* L.) do not produce contact calls until shortly before wing-powered flight occurs [i.e. fledging (Berg et al., 2012)]. In contrast, nestlings of this and another parrot species, the budgerigar (*Melopsittacus undulatus*), produce 'begging calls' throughout most of their ontogeny to encourage nutritional provisioning from care-givers (Brittan-Powell et al., 1997) (present study), but their delivery is largely independent of locomotor activity. Although there may be some overlap, contact calls largely replace begging calls as wing-powered flight emerges. To determine any systematic differences in context and possible physiological constraints on the two call types (e.g. association with locomotor activity or not), careful studies of individual vocal ontogeny in natural populations are needed.

Here, we quantify vocal development in free-ranging green-rumped parrotlets from hatching to fledging. We track a wide set of acoustic measures throughout nestling ontogeny and use the results to estimate the relative roles of growth, environmental context and learning at each stage. We found no fixed weighting of these factors throughout parrotlet vocal ontogeny but, instead, their emphasis clearly changes with each stage of development. In particular, begging calls used at intermediate stages depend on the immediate context and do not serve as structural precursors to contact calls. Taken together, our results suggest that the two call types likely evolved under a different suite of selective pressures.

## MATERIALS AND METHODS

We studied a color-banded population of green-rumped parrotlets at Hato Masaguaral (8°34'N, 67°35'W), State of Guarico, Venezuela. The habitat consists of seasonally flooded tropical savanna and gallery forest. Parrotlets are year-round residents but breed monogamously during the wet season between June and December (Waltman and Beissinger, 1992). Parrotlet breeding, social system and population ecology have been monitored at this site since 1988, facilitated by 106 artificial nest cavities fashioned from 1×0.08 m PVC pipes (Beissinger, 2008).

### Nesting

Nest boxes were checked at three-day intervals to determine laying and hatching dates. Spotting scopes were used to identify breeding pairs by individuals' unique color band combinations. All eggs and nestlings were marked with non-toxic felt-tip markers. A week before fledging, nestlings were banded, weighed and wing-chord measured. On average, nestlings fledge at 30 days after hatching (Stoleson and Beissinger, 1997).

### Audio-video recording

In order to identify calls of individual nestlings, we audio-video (AV) recorded inside active nests at weekly intervals throughout nestling development during two different years (2007 and 2008). Recording sessions typically occurred in the morning and lasted 4 h. Parents feed nestlings on average once an hour (Stoleson and Beissinger, 1997), so our sessions usually captured two to three visits by parents. Details of AV recording methods are provided in Berg et al. (Berg et al., 2012). Briefly, multiple microphones [SM57 (Shure, Niles, IL, USA) and MKH816 (Sennheiser, Wedemark, Germany)] and multiple video cameras provided recordings of individuals inside and outside the cavity and were synchronized onto a Hi-8 video cassette recorder (GVA500, Sony, Japan). Tape-recordings were digitized with a Macbook (Apple, Cupertino, CA, USA) and saved as .mov files. Sound Track Pro (v. 1.3; Apple) was used to identify relevant video sequences, and calls of individuals were extracted and saved as .wav files for spectrographic analysis. Because not all nestlings vocalized during each recording session, and in other cases nestlings vocalized simultaneously, we focused the analysis on 20 nestlings from 12 nests for which we had at least three assignable calls from each individual for each week of the month-long nestling period (week 1=0–7 days; 2=8–14 days; 3=15–22 days; 4=23–30 days). Pairs fledged, on average, five individuals from each nest (range=2–7 fledglings), and all nestlings included in this analysis fledged successfully. Age did not differ significantly at time of sampling between sexes in any of the four weeks of nestling development (ANOVA, all  $P>0.35$ ), and age of sexes differed by one day or less, on average, during any of the weeks.

### Spectrographic measurements

We analyzed calls given by individuals in each of the nestling stages with a series of energy distribution measurements batch-extracted with code developed by Kathryn Cortopassi ([www.birds.cornell.edu/brp/research/algorithm](http://www.birds.cornell.edu/brp/research/algorithm)) in Matlab (v. 2009) using the platform XBAT (see also Berg et al., 2011). Spectrograms were created with a Hann window, 512-point fast Fourier transform (FFT) sample size and 50% overlap. To avoid extraneous sounds, only energy between 750 Hz and 12,500 Hz was analyzed. We chose eight spectrographic attributes that consistently explained separation of nestling stages: inter-percentile range time (hereafter duration), center frequency, first percentile frequency (hereafter lower frequency), second percentile frequency (hereafter upper frequency), inter-percentile range bandwidth (hereafter bandwidth) and average entropy. We then constructed center frequency contours (CFC) along each continuous signal and, from these, calculated the average derivative (an estimate of the general trend of the carrier frequency). We batch quantified the number of derivative sign changes in the CFC (hereafter inflections), which estimated the number of frequency modulations independent of the general trend of the carrier frequency.

### Locomotor ontogeny

Incidences of locomotor activity of nestling parrotlets were sampled from 22 nests, including the 12 nests mentioned previously. We scanned approximately 400 h of video and scored all unambiguous instances of vertical locomotor activity in offspring of known ages in each nest. Horizontal locomotor activity in the tight confines of the nest beyond the first week of development was minimal (<8 cm) and was not analyzed further. Vertical locomotor activity was larger in scale and defined as the ability to climb up any portion of the sides of the meter-long nest cavity. This likely requires considerably

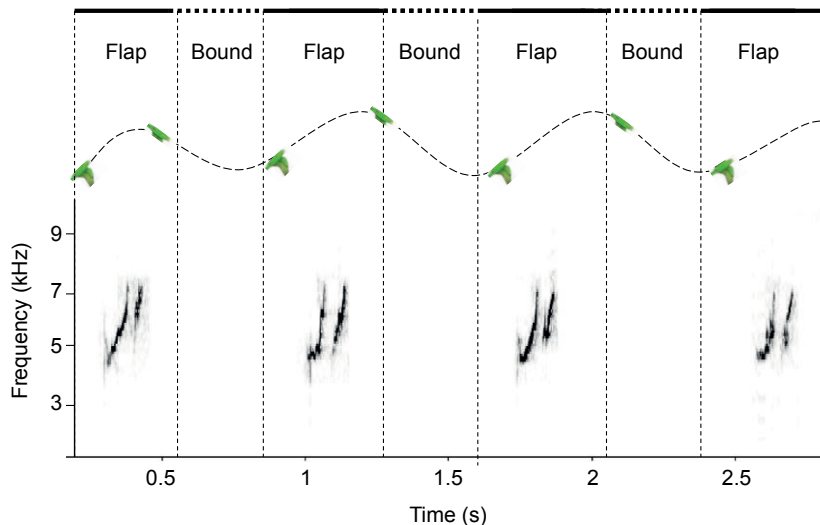


Fig. 1. An example of contact call production during intermittent flight in an adult female green-rumped parrotlet recorded at Hato Masaguaral, Venezuela in 2008. Contact calls only occurred during flapping ascents. Video was made at a distance of ~25 m. Timing of contact call production on video was adjusted for 0.073 s audio delay (i.e. 25 m/345 ms<sup>-1</sup>).

more energy and dexterity of leg and foot muscles than the short horizontal displacements; ascension of approximately 80 cm to the cavity entrance is also required for survival to adulthood. Because nestlings commonly used their wings to aid upward movement along the cavity walls, wing-flap frequencies were estimated by counting the video frames in Sound Track Pro ( $\pm 0.033$  s). Data on adult phenotypes were collected from five free-flying adults recorded in the same population in 2011 with a camcorder (HD170, Drift Innovation, UK or a HDR-CX360V, Sony, Japan) set to record in high definition (H.264) at 60 frames s<sup>-1</sup> and AV converted to .mov files (Aimersoft v. 2.5.2, Aimersoft Studio, Houston, TX, USA). Adult parrotlets have bounding intermittent flight (Fig. 1) as described for the similar-sized budgerigar (Tucker, 1968; Tobalske and Dial, 1994), so mean wingbeat frequencies were calculated based on the flapping portions of flight paths.

#### Statistical analyses

To distinguish individual differences in call attributes from ontogenetic changes that can be generalized to the population or species, we compared spectrographic variation within and between age groups, while controlling for individual differences, sex and sibling affiliation with generalized linear mixed models (GLMM, SAS, v. 9.2; SAS Institute, Cary, NC, USA). The eight spectrographic measurements were included separately as Gaussian-distributed, untransformed response variables, with each linked to an identity function. Week of development and sex were included as fixed, main effects. Individual and sibling groups were included as random effects because our main objective was to compare developmental stages and whether these might vary depending on the sex of the individual. *Post hoc* comparisons between successive weeks were made with Tukey–Kramer honestly significant difference (HSD) tests in JMP (v. 8; SAS Institute, Cary, NC, USA) and significance values adjusted for False Discovery Rates using the MULTTEST Procedure in SAS.

To estimate the separate stages in calls independent of age classes, we used *k*-means clustering algorithms with spectrographic measurements included as dependent variables (JMP, v. 8). The method first collapsed the dependent variables into two principal components (PC). Upper frequency was strongly correlated with bandwidth ( $r=0.85$ ,  $P<0.001$ ), and duration was strongly correlated with inflections ( $r=0.80$ ,  $P<0.001$ ). Because including these highly correlated variables added little residual explanatory power, and could introduce problems associated with co-linearity, we restricted

the dependent variables in the *k*-means clustering to duration, lower and upper frequencies, average derivative and average entropy. However, the strong correlation between inflections and duration was biologically significant, and we return to this issue later. The number of clusters was selected as a first approximation. Calls were then randomly assigned to one of the cluster seeds, and centroids of PC scores were calculated for each group. An iterative process then re-assigned observations to more similar centroids and updated the centroids. The process continued until variation was minimized within clusters. Despite the staggered ages and periodicity of sampling, the number of individuals sampled was evenly distributed across all age classes. We ran diagnostic checks of the method by repeating the analysis with two, three and four cluster seeds. We only report results of three clusters, which was the only model that produced non-overlapping 95% confidence circles, indicative of statistically significant differences between all clusters.

To test for systematic differences in the ages at which individuals first produced contact calls compared with vertical locomotor displacements, we used a GLMM with age at first appearance of each behavior modeled as a Poisson-distributed response variable (link function) and a fixed effect of behavior (i.e. contact call or locomotor displacement) as a categorical variable. This divided the global age distribution into two smaller distributions, one for each behavior, and then tested for significant differences between the two distributions. Nest (i.e. sibling group) and individual were included as random effects (i.e. repeated measures, residual random effects model) (2008 SAS User's Manual), which combined the utility of a mixed model with that of a paired *t*-test. Call durations just prior to fledging and those given upon virgin flights were compared with a GLMM, using a Gaussian response distribution for call duration in flight (linked to an identity function) and call duration prior to fledging modeled as a fixed effect, controlling for random effects of nest and individual as in the previous model.

## RESULTS

### General ontogenetic patterns

Controlling for individuals and sibling groups, there were no significant sex differences in call attributes (Table 1). However, all spectrographic measurements of calls underwent significant changes during the nestling period (Tables 1, 2, Fig. 2, supplementary material Figs S1, S2). Among the most prominent of these patterns was a marked increase in call duration between the first and second weeks,

Table 1. Results of generalized linear mixed models for main effects of four age classes and two sex classes on eight spectral attributes (dependent variables) of vocalizations in 20 green-rumped parrotlet nestlings at 12 nests

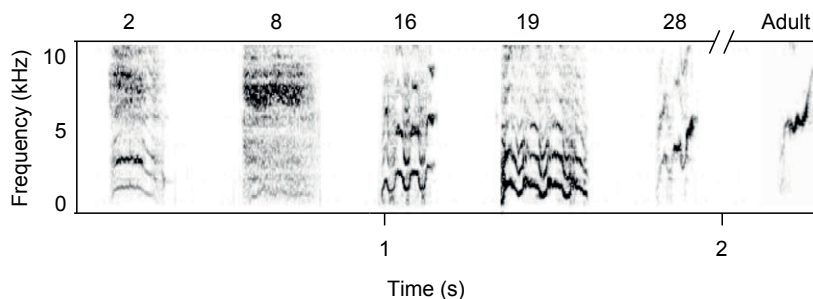
	Individual estimate ± s.e.m.	Nest estimate ± s.e.m.	Sex		Age	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Duration	0.0008±0.0006	0.0008±0.0004	3.75	0.0530	319.58	<b>0.001</b>
Center frequency	149±157	173±111	0.03	0.8640	92.85	<b>0.001</b>
Lower frequency	272±133	34±22	4.81	0.0284	72.63	<b>0.001</b>
Upper frequency	180±131	134±77	1.70	0.1927	284.41	<b>0.001</b>
Bandwidth	75±83	153±81	0.00	0.9577	137.52	<b>0.001</b>
Derivative	2.4×10 <sup>11</sup> *	7170*	0.09	0.7693	203.81	<b>0.001</b>
Inflections	17±12	19±9	3.02	0.0826	263.51	<b>0.001</b>
Entropy	0.006±0.01	0.041±0.02	1.62	0.2026	293.49	<b>0.001</b>

Covariance parameter estimates are provided for random effects of individual and nest. Degrees of freedom in numerator: sex = 2, age = 3, denominator = 2494. Bold-faced terms indicate statistical significance after controlling for multiple comparisons. \*s.e.m. was not uniquely estimable given the positive and negative values of the Average Derivative.

followed by a major reduction in duration between the third and final week. The decline in duration between the final week was accompanied by a rise in the fundamental frequency during the course of the call (i.e. average derivative; Table 2, Fig. 3, supplementary material Figs S1, S2), culminating in an adult-like contact call.

*k*-means cluster analysis showed that vocal ontogeny is represented by three call categories (Fig. 4). One well-defined cluster included calls that came mainly from individuals during their first week and were characterized by short durations, high frequencies and broadband noise (i.e. high average entropy). The second cluster included calls that were evenly distributed between weeks 2 and 4 and had long durations, low frequencies, low entropies and a repetitious frequency modulation (FM) component. In fact, controlling for sibling group affiliation, the longer call durations were significantly correlated with the number of inflections in each call (GLMM,  $F_{1,9}=14.06$ ,  $P<0.001$ ). The third cluster was dominated by calls given during the fourth and final week of the nestling period and was characterized by calls with short durations and strong upwards modulation of the fundamental frequency (e.g. highest average derivative; Figs 3, 4), i.e. the contact call.

Although both contact calls and middle-stage begging calls showed frequency modulations, we found no significant correspondence between the number, shape, frequency ranges or other measures in a nestling's begging calls and contact calls (e.g. Fig. 3). Thus, the attributes of the begging call in this species do not appear to carry over into the signature attributes of the contact call. The context in which contact calls were given also suggested a functional discreteness from begging calls. The latter usually did not occur until parents were inside the cavity and feeding had begun whereas contact calls were routinely given once parents had arrived in the vicinity of the nest but before any visual contact between nestlings and parents had been established. This is similar to the context in which incubating-brooding females use contact calls to identify mates when visually separated inside nest cavities prior to feedings (Berg et al., 2011).



### Vocal and locomotor integration

All adults analyzed showed intermittent flight patterns. Flapping portions lasted  $0.70\pm 0.75$  s (mean ± s.d.), contained  $5\pm 2.3$  full flaps and a wingbeat frequency of  $19.6\pm 5.8$  Hz. Bounding (i.e. non-flapping) periods of flight lasted  $0.41\pm 0.16$  s but were not significantly shorter than flapping periods (GLMM,  $F_{1,18}=1.24$ ,  $P<0.2804$ ). Contact call production was invariably synchronized with the flapping phases of intermittent flight and was never observed during bounding phases ( $\chi^2_{1,24}=24$ ,  $P<0.001$ ; Fig. 1).

Nestlings began vertical locomotor displacements, on average, six days prior to fledging ( $24\pm 3.8$  days post-hatch,  $N=53$  nestlings), as they climbed up the vertical sides of the interior of the nest cavity just prior to the arrival of one or both of the parents. They usually began with short ascents and lacked coordination. By fledging age, many could run quickly up the meter-long cavity. Controlling for siblings and individuals, this behavior began  $3.2\pm 0.02$  days earlier than our estimate of the onset to contact call production (GLMM,  $F_{1,84}=35.01$ ,  $P<0.001$ ), and in 83% of cases ( $N=53$ ) nestlings showed evidence of climbing before we recorded their first contact call. In all instances, nestlings used wing-flaps to aid upward movement along the vertical walls of the cavity, highly suggestive of wing-assisted incline running (WAIR) (Dial, 2003). Wing-flaps during ascents were commonly synchronized with contact call production (Fig. 5; see <http://macaulaylibrary.org/video/470662> to <http://macaulaylibrary.org/video/470669>). Fledglings also predictably coupled forward flapping flight with the emission of contact calls on their virgin flights from the nest ( $N=9$  of 12 fledging events). Calls underwent further decreases in duration once nestlings began regular flight [mean duration (±s.e.m.) prior to fledging= $0.076\pm 0.004$  s versus mean duration (±s.e.m.) in flight= $0.061\pm 0.003$  s; GLMM  $F_{1,9}=14.69$ ,  $P<0.004$ ; Fig. 6].

### DISCUSSION

Our *k*-means clustering analysis of spectrographic measures indicates that green-rumped parrotlet nestlings pass through three stages during vocal ontogeny: (1) an early, short-duration, noisy stage; (2) a middle

Fig. 2. Representative spectrograms of the chronological sequence of call development recorded from a nestling green-rumped parrotlet at Hato Masaguaral in 2007, with an adult contact call example provided as a reference. Age of nestling (±1 day) is provided above each call example.



Table 2. Mean number of calls analyzed per individual (*N*) and mean  $\pm$  s.d. of eight spectrographic parameters measured in a sample of calls from 20 green-rumped parrotlets during each of four weeks of nestling development

	Weeks			
	1	2	3	4
<i>N</i>	35	22	20	65
Duration (ms)	165 $\pm$ 33	213 $\pm$ 53	185 $\pm$ 63	107 $\pm$ 53
Center frequency (kHz)	5.47 $\pm$ 0.9	4.59 $\pm$ 0.7	4.26 $\pm$ 0.8	4.85 $\pm$ 0.6
Lower frequency (kHz)	2.32 $\pm$ 0.5	1.89 $\pm$ 0.5	1.58 $\pm$ 0.6	2.07 $\pm$ 0.7
Upper frequency (kHz)	9.85 $\pm$ 0.9	8.18 $\pm$ 1.0	7.66 $\pm$ 0.7	8.29 $\pm$ 0.7
Bandwidth (kHz)	7.53 $\pm$ 1.0	6.30 $\pm$ 0.9	6.08 $\pm$ 0.6	6.21 $\pm$ 0.7
Derivative (000s)	-10 $\pm$ 10	-2 $\pm$ 4	0 $\pm$ 9	19 $\pm$ 17
Inflections (kHz)	19.6 $\pm$ 10	25.9 $\pm$ 11	20.7 $\pm$ 7	11.2 $\pm$ 7
Entropy (kHz)	3.8 $\pm$ 0	3.6 $\pm$ 0	3.4 $\pm$ 0	3.3 $\pm$ 0

Derivative = average derivative of the center frequency contour (CFC); inflections = the number of derivative sign changes in the CFC; entropy = average entropy.

stage with longer durations and repetitive FM; and (3) a final stage with short-duration, highly inflected contact calls. The timing of each stage fits surprisingly well with the timing of three stages recently reported for physiological development of parrotlets (Pacheco et al., 2010). Below, we review each stage individually and examine the relative roles of growth, environment and learning.

#### Early noisy stage

Between 0 and 13 days, parrotlet nestlings show spikes in their uptake of sodium, lipids and energy (Pacheco et al., 2010). This is consistent with high growth rates and establishment of basic skeletal, muscular and neural structures. Our study showed that calls during this stage had short durations, high frequencies and large amounts of broadband noise (i.e. high entropy). Short-duration calls are consistent with the fact that air cavities and respiratory organs were likely still small and undergoing substantial growth during this period (Vleck and Bucher, 1998). High frequencies are synonymous with short wavelengths of sound and are predicted from the resonant properties of small vocal tracts (Podos, 1996; Brittan-Powell et al., 1997; Hoese et al., 2000; Suthers and Zollinger, 2004; Beckers et al., 2004; Bradbury and Vehrencamp, 2011; Ohms et al., 2012). Broadband noise has been

found in calls of several nestling bird species (Redondo, 1991; Brittan-Powell et al., 1997; Baker et al., 2003; Sharp and Hatchwell, 2006) and perhaps reflects a basic lack of syringeal and respiratory control (Heaton and Brauth, 2000) or that organs were growing too fast to coordinate the production of more precise tonal or FM sounds. In short, this stage in vocal ontogeny emphasizes growth of the physical structures and neuro-muscular connections necessary for more complex vocal communication at later stages.

#### Repetitive FM stage

The second stage occurred between 14 and 22 days, when growth slowed, nutrient assimilation shifted to emphasize protein, calcium and phosphorus uptake, and nestlings became increasingly feathered (Pacheco et al., 2010). Calls during this period showed large, precise FMs that could be repeated a variable number of times by the same individual (Figs 2, 3). The overall structure resembled adult begging calls commonly given by breeding females when soliciting food from mates (Berg, 2011). While nestling begging often has multiple functions (Kilner and Johnstone, 1997; Leech and Leondard, 1997), it typically facilitates food acquisition that supports high rates of growth (Ricklefs, 2002). During this stage, nestling parrotlets are

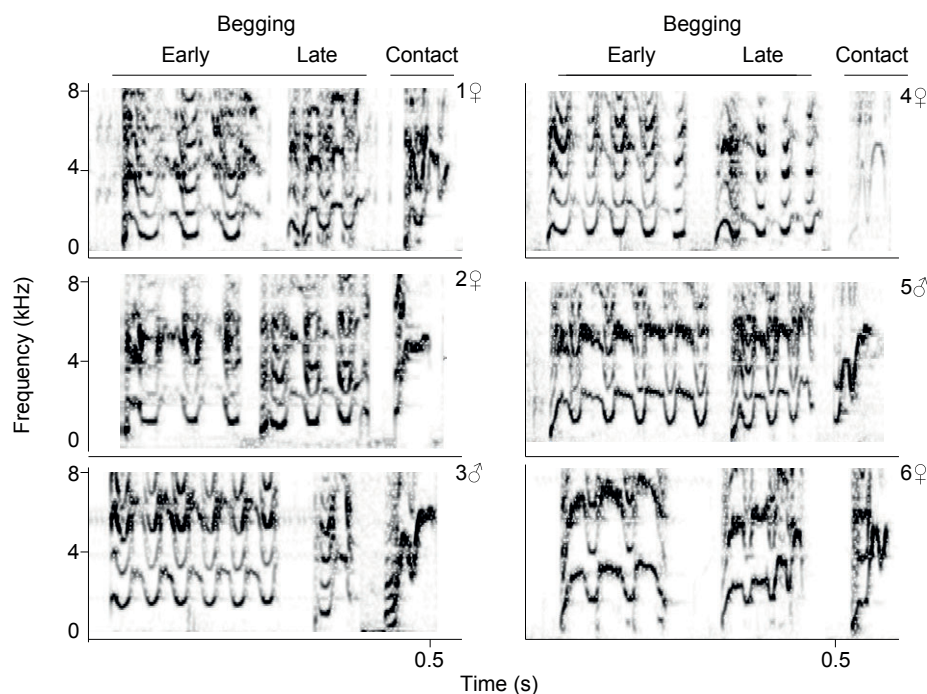


Fig. 3. Transitions between begging and contact call production. Examples of two types of middle-stage begging calls and contact calls from six siblings in one nest. The earlier begging call has a variable number of repeated large FM with no modulation of the carrier frequency. The later, or transitional, begging call often has fewer FMs and the carrier frequency is slightly inflected. The contact call has even fewer, faster FMs and is more severely inflected. Position in the hatching sequence and sex are indicated for each sibling.

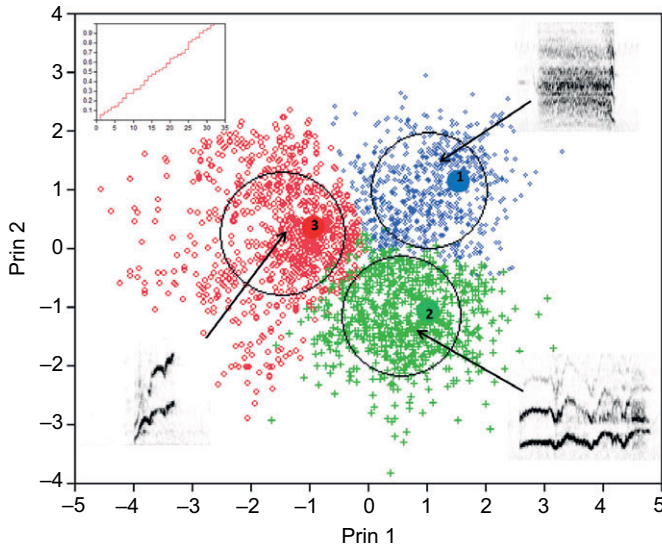


Fig. 4. Results of *k*-means cluster analysis for separation of three clusters (color and symbol coded) based on two principal components summarizing five energy-distribution measurements (duration, lower frequency, upper frequency, average derivative and average entropy) of 2517 calls from 20 nestling green-rumped parrotlets sampled at weekly intervals throughout the nestling period. Spectrograms of randomly selected calls from the center of each cluster are shown. The areas of the circles are proportional to the sample size of each cluster, with centroids indicated by numbers; non-overlapping circles indicate statistically different clusters. Cluster 1 incorporated calls from the first week in 84% of cases and was characterized by high entropy and high frequencies; cluster 2 included calls evenly distributed across the last three weeks of the nestling period (weeks 2–4, respectively: 29%, 30%, 34%) and were characterized by longer durations, lower frequencies and a repetitious FM component; cluster 3 represented the last week of nestling development (80%) and was characterized by short durations, low entropy and high average derivatives. The graph inset shows that the proportion of individuals sampled at each age was normally distributed throughout the ~30-day nestling period; the vertical axis is the cumulative probability function of being sampled at a given age (as indicated in days by the horizontal axis).

able to modify the number of FMs in their begging calls, perhaps as a function of hunger (e.g. higher rates following longer intervals since the parents last fed). Given the competitive hierarchy in parrot begging behavior (Stamps et al., 1985; Krebs, 2001; Budden and Beissinger, 2009), this might be expected to generate an arms race resulting in longer-duration begging calls (Parker et al., 2002). Preliminary data indicate that, in parrotlets, the number of FM repetitions is positively related to brood size and thus the competitive

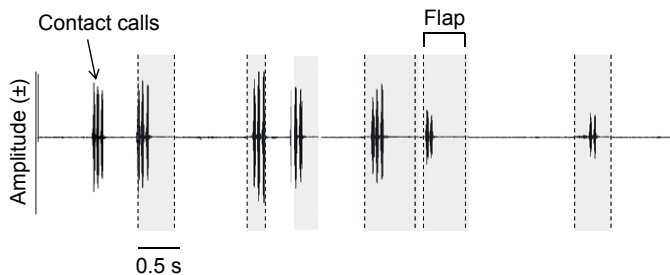


Fig. 5. Waveform of 19 contact calls (arbitrary amplitude values) given during a vertical climbing ascent of a female nestling parrotlet at 29 days post-hatch. Each gray block represents the duration of one full wing-flap cycle.

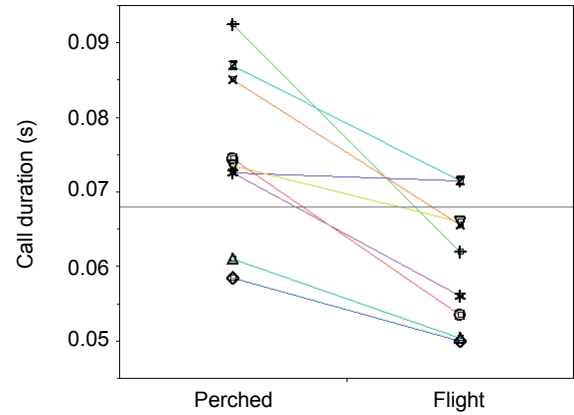


Fig. 6. Comparison between contact calls given before and after fledging. Mean contact call duration of calls given by nine nestlings from eight nests while perched inside the cavity prior to fledging *versus* contact call durations from the same individuals during virgin flights. Lines connect calls from the same individuals.

environment might encourage the incorporation of more repeats in each call, resulting in longer overall call durations [see similar findings in budgerigars (Brittan-Powell et al., 1997)]. Begging calls were often two to four times longer in duration than later-developing contact calls, and duration was positively correlated with the number of FM repetitions (Figs 3, 4). Thus, it appears that motor flexibility in response to the dynamics of the immediate environment dominates the second stage of vocal ontogeny.

#### Final stage

During the third and final stage, body mass reaches an asymptote, and nestlings become completely endothermic (Pacheco et al., 2010). The most striking structural feature of calls during this period is an abrupt decrease in duration and the addition of a strong upward rise in the fundamental frequency, resulting in an adult-like contact call. This represents acquisition of a second component of the adult repertoire. While both contact calls and prior begging calls contained FMs, we were unable to find support for the number and shape of begging-call FMs morphing into the number and shape of contact-call FMs, a salient ontogenetic pattern found in budgerigars (Brittan-Powell et al., 1997; Heaton and Brauth, 2000). Instead, the contact call structure appeared *de novo*, with incorporation of signature attributes influenced by the nestling's prior exposure to various templates provided by its social parents (Berg et al., 2012). While the incorporation of learned signature information in contact calls could aid discrimination tasks by parents as size hierarchies disappear prior to fledging, signature contact calls are probably much more important after fledging, when nestlings will use them to reunite with parents and siblings during a crucial life history transition, one characterized by high mobility and high mortality rates (Beissinger, 2008). Thus, the final stage in nestling vocal ontogeny emphasizes learning that likely prepares nestlings for future life history stages when contact calls will be used to resolve spatial cognitive problems associated with fission–fusion flock behavior and wide-ranging, social foraging and roosting networks (Wright, 1996; Bradbury et al., 2001; Buhman-Deever et al., 2008; Balsby and Bradbury, 2009; Scarl and Bradbury, 2009).

#### Reasons for short contact-call durations

The dramatic metamorphosis in call durations between the middle begging stage and the final contact call stage surely reflects the need

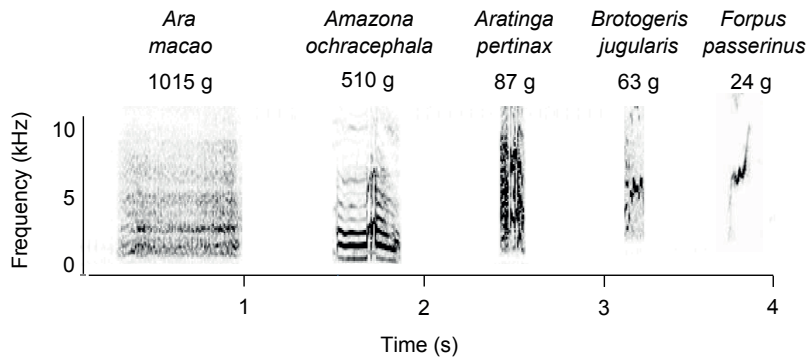


Fig. 7. Spectrograms of representative flight calls from five sympatric parrot species recorded at Masaguara, Venezuela (2006–2009), with body mass (g) provided by Dunning (Dunning, 2008).

to produce the very short duration contact calls typical of adults. There are several ways that shortening of late-nestling calls might be related to later flight. First, short call durations in both adults and fledglings may be partially explained by respiratory constraints associated with wing-beating during flight. Wingbeat frequency is inversely related to body size across bird species (Nudds et al., 2004), and contact-call durations are positively related to body size in sympatric parrot species (Fig. 7). Green-rumped parrotlets are the smallest of the New World parrots (25 g) (Juniper and Parr, 1998; Dunning, 2008), have short-duration wing-beat cycles ( $0.054 \pm 0.012$  s; mean  $\pm$  s.d.) and predictably short duration contact calls ( $0.077 \pm 0.012$  s) (Berg et al., 2011) and synchronize call production with flapping phases of intermittent flight (Fig. 1). The latter characteristic suggests an advantage to calling while flapping. Because downstroke phases are often synchronized with expiratory phases (Tucker, 1968; Boggs, 1997; Tobalske and Dial, 1994), and vocal production requires a build-up of positive expiratory pressure at the sub-syringeal aperture (Suthers, 1997; Cooper and Goller, 2004; Ohms et al., 2012), contact call emission may be more economical during flapping phases and less so during non-flapping phases when inspiration is likely more economical. In support of this explanation, nestling call durations showed an abrupt decrease shortly after they began using wings to aid locomotor displacements inside the nest cavity. Because wingbeat frequencies during WAIR were lower than that recorded for full flight, calls during this activity were shorter than begging calls, but not as short as the calls fledglings later exhibited when adopting the much higher wingbeat frequencies of their virgin flights. The demonstrated contraction of contact calls in two steps during late development may thus arise from the linkage of call production with the moderate wingbeat frequencies seen in WAIR locomotion and the even higher wingbeat frequencies associated with fledglings' virgin flights.

A second and complementary explanation for short-duration contact calls in late-stage nestling parrotlets is that wing flapping could be beneficial to effective vocal communication. Contact call production was synchronized with wing-flaps during WAIR before actual wing-powered flight developed and thus before major flight-related respiratory or muscular constraints (Fig. 5). According to this explanation, the high levels of expiratory pressure generated by wing-powered flight might then provide individuals with a fortuitous and energetically inexpensive way of amplifying their high frequency calls. As a general rule, small sound-producing animals are limited to higher frequencies and lower amplitudes than otherwise equivalent large animals (Bradbury and Vehrencamp, 2011). Higher frequencies attenuate energy more quickly. Small parrots might thus achieve greater ranges of detection by committing the energy available for sound production into a short-duration, high-amplitude call instead of a long-duration, low-amplitude one.

Similar considerations have been proposed for short-duration, high-frequency, high-amplitude calls of bats during forward flapping flight (Speakman and Racey, 1991; Jones, 1994; Lancaster et al., 1995; Wong and Waters, 2001; Holderied and von Helversen, 2003). However, captive experiments using wind tunnels and telemetry, or free-flying individuals and camera–microphone arrays, are needed to verify the suggested physiological couplings in birds, before determining why parrotlets develop short-duration contact calls.

#### Perched versus in-flight calling

Nestling and adult parrotlets, and, in fact, most parrots so far studied, often exchange contact calls when perched. If contact-call durations in flight are due to constraints generated by respiratory–wingbeat linkages, why don't these species use longer duration contact calls when the physiological constraints are relaxed? We believe this occurs because the signature role of contact calls is of paramount importance in both contexts: if parrotlets were to use different call durations and structures depending on context, the individual signature functions of the calls would be lost (Bradbury and Vehrencamp, 2011). When a signature call is to be used in multiple contexts, as appears to be the case in most parrots so far studied (Bradbury, 2003; Cortopassi and Bradbury, 2006; Buhman-Deever et al., 2008; Adams et al., 2009; Balsby and Bradbury, 2009; Balsby and Adams, 2011; Berg et al., 2011), we would expect the most constrained context to define the call structure. If parrotlet body size defines their appropriate wingbeat frequencies, and respiration, wing beating and call emission are physiologically linked, nestling parrotlets may need to develop short-duration contact calls to maintain the same signature information both in flight and when perched. It will be interesting to see whether this proposed causal sequence is supported as the vocal ontogeny of other parrot species is examined.

#### ACKNOWLEDGEMENTS

S. Vehrencamp, E. Adkins-Regan and I. Lovette reviewed earlier drafts. C. Blohm and the late T. Blohm provided hospitality and access to wild parrotlet populations; V. Sanz D'Angelo provided mentoring; J. Acosta and S. Delgado provided logistical assistance; A. Charpentier, S. Delgado, M. Halley, J. Hernandez, A. Medina, R. Okawa, R. Ordoñez, N. Sly and E. Sylva provided field assistance; C. Benton, T. Chatterjee, S. Delgado, L. Hou, S. Iacovelli, N. Lavin and C. Masco helped with audio-video analysis; B. Land and K. Cortopassi helped with acoustic analysis. Research protocols were approved by the Institutional Animal Care and Use Committee of Cornell University (No. 07-0124) and the Ministerio del Poder Popular para el Ambiente in Venezuela (No. 3437). Examples of audio-video files can be found online at <http://macaulaylibrary.org/video/470662> to <http://macaulaylibrary.org/video/470669>.

#### FUNDING

K.S.B. was supported by the National Science Foundation [Grant OISE-0825675], Andrew and Margaret Paul Fellowship, Charles Walcott Graduate Fellowship,

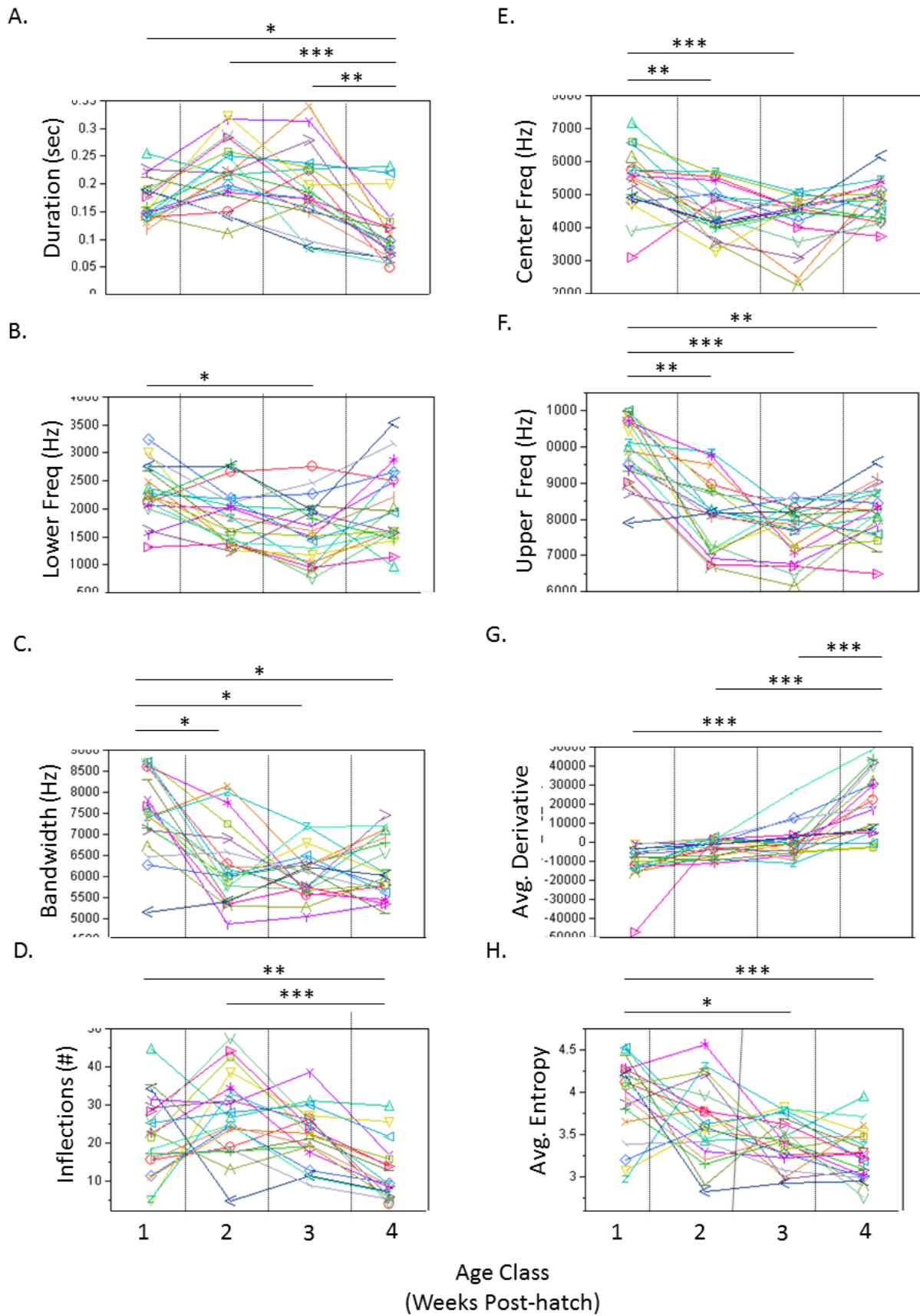


Animal Behavior Society, Cornell's Graduate School, Latin American Study Program and the National Geographic Society. S.R.B. was supported by NSF IBN-0113173. J.W.B. was supported by NSF IBN-02-29271. Shure Microphone Corporation donated microphones.

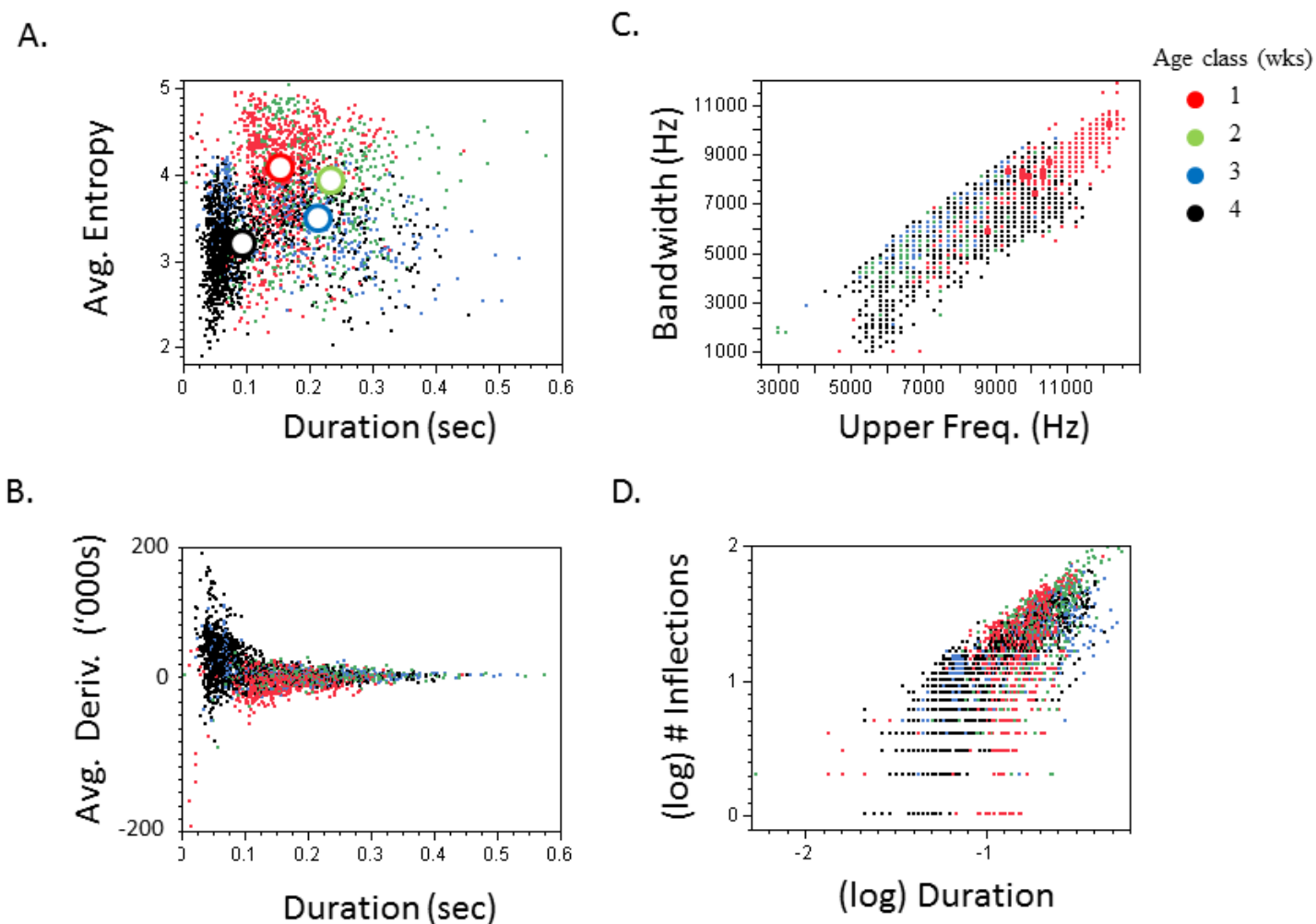
## REFERENCES

- Adams, D. M., Balsby, T. J. S. and Bradbury, J. W. (2009). The function of double chees in orange-fronted conures (*Aratinga canicularis*; Psittacidae). *Behaviour* **146**, 171-188.
- Alberts, J. R. (1985). Ontogeny of social recognition: an essay on mechanism and metaphor in behavioral development. In *The Comparative Development of Adaptive Skills: Evolutionary Implications* (ed. E. S. Gollin), pp. 95-136. New York, NY: Erlbaum.
- Baker, M. C., Baker, M. S. A. and Gammon, D. E. (2003). Vocal ontogeny of nestling and fledgling black-capped chickadees *Parus atricapilla* in natural populations. *Bioacoustics* **13**, 265-296.
- Baldwin, J. M. (1896). A new factor in evolution. *Am. Nat.* **30**, 441-451.
- Balsby, T. J. S. and Adams, D. M. (2011). Vocal similarity and familiarity determine response to potential flockmates in orange-fronted conures (Psittacidae). *Anim. Behav.* **81**, 983-991.
- Balsby, T. J. S. and Bradbury, J. W. (2009). *Vocal matching by orange-fronted conures (Aratinga canicularis)*. *Behav. Processes* **82**, 133-139.
- Beckers, G. J. L., Nelson, B. S. and Suthers, R. A. (2004). Vocal-tract filtering by lingual articulation in a parrot. *Curr. Biol.* **14**, 1592-1597.
- Beissinger, S. R. (2008). Long-term studies of the green-rumped parrotlet (*Forpus passerinus*) in Venezuela: hatching asynchrony, social system and population structure. *Ornithol. Neotrop.* **19**, 73-83.
- Berg, K. S. (2011). Development and function of vocal signatures in green-rumped parrotlets (*Forpus passerinus*). PhD dissertation, Cornell University, Ithaca, NY, USA.
- Berg, K. S., Delgado, S., Okawa, R., Beissinger, S. R. and Bradbury, J. W. (2011). Contact calls are used for individual mate recognition in free-ranging green-rumped parrotlets (*Forpus passerinus*). *Anim. Behav.* **81**, 241-248.
- Berg, K. S., Delgado, S., Cortopassi, K. A., Beissinger, S. R. and Bradbury, J. W. (2012). Vertical transmission of learned signatures in a wild parrot. *Proc. Biol. Sci.* **279**, 585-591.
- Boggs, D. F. (1997). Coordinated control of respiratory pattern during locomotion in birds. *Am. Zool.* **37**, 41-53.
- Bradbury, J. W. (2003). Vocal communication in wild parrots. In *Animal Social Complexity: Intelligence, Culture, and Individualized Societies* (ed. F. B. M. de Waal and P. L. Tyack), pp. 293-316. Cambridge, MA: Harvard University Press.
- Bradbury, J. W. and Vehrencamp, S. L. (2011). *Principles of Animal Communication*. New York: Sinauer and Associates.
- Bradbury, J. W., Cortopassi, K. A. and Clemmons, J. R. (2001). Geographical variation in the contact calls of orange-fronted parakeets. *Auk* **118**, 958-972.
- Brittan-Powell, E. F., Dooling, R. J. and Farabaugh, S. M. (1997). Vocal development in budgerigars (*Melopsittacus undulatus*): contact calls. *J. Comp. Psychol.* **111**, 226-241.
- Budden, A. E. and Beissinger, S. R. (2009). Resource allocation varies with parental sex and brood size in the asynchronously hatching green-rumped parrotlet (*Forpus passerinus*). *Behav. Ecol. Sociobiol.* **63**, 637-647.
- Buhrman-Deever, S. C., Hobson, E. A. and Hobson, A. D. (2008). Individual recognition and selective response to contact calls in foraging brown-throated conures, *Aratinga pertinax*. *Anim. Behav.* **76**, 1715-1725.
- Cooper, B. G. and Goller, F. (2004). Multimodal signals: enhancement and constraint of song motor patterns by visual display. *Science* **303**, 544-546.
- Cortopassi, K. A. and Bradbury, J. W. (2006). Contact call diversity in wild orange-fronted parakeet pairs, *Aratinga canicularis*. *Anim. Behav.* **71**, 1141-1154.
- Dial, K. P. (2003). Wing-assisted incline running and the evolution of flight. *Science* **299**, 402-404.
- Dunning, J. B. (2008). *Handbook of Avian Body Masses*, 2nd edn. Boca Raton, FL: CRC.
- Düttmann, H., Bergmann, H. H. and Engländer, W. (1998). Development of behavior. In *Avian Growth and Development* (ed. J. M. Starck and R. E. Ricklefs), pp. 223-240. New York: Oxford University Press.
- Farabaugh, S. M. and Dooling, R. J. (1996). Acoustic communication in parrots: laboratory and field studies of budgerigars, *Melopsittacus undulatus*. In *Ecology and Evolution of Acoustic Communication in Birds* (ed. D. E. Kroodsma and E. H. Miller), pp. 97-117. Ithaca, NY: Cornell University Press.
- Farabaugh, S. M., Linzenbold, A. and Dooling, R. J. (1994). Vocal plasticity in budgerigars (*Melopsittacus undulatus*): evidence for social factors in the learning of contact calls. *J. Comp. Psychol.* **108**, 81-92.
- Finlay, B. L. and Darlington, R. B. (1995). Linked regularities in the development and evolution of mammalian brains. *Science* **268**, 1578-1584.
- Gottlieb, G. (1991). Experiential canalization of behavioral development: theory. *Dev. Psychol.* **27**, 4.
- Heaton, J. T. and Brauth, S. E. (2000). Telencephalic nuclei control late but not early nestling calls in the budgerigar. *Behav. Brain Res.* **109**, 129-135.
- Hoese, W. J., Podos, J., Boetticher, N. C. and Nowicki, S. (2000). Vocal tract function in birdsong production: experimental manipulation of beak movements. *J. Exp. Biol.* **203**, 1845-1855.
- Holderied, M. W. and von Helversen, O. (2003). Echolocation range and wingbeat period match in aerial-hawking bats. *Proc. Biol. Sci.* **270**, 2293-2299.
- Janik, V. M. and Slater, P. J. B. (2000). The different roles of social learning in vocal communication. *Anim. Behav.* **60**, 1-11.
- Jones, G. (1994). Scaling of wingbeat and echolocation pulse emission rates in bats: Why are aerial insectivorous bats so small? *Funct. Ecol.* **8**, 450-457.
- Juniper, T. and Parr, M. (1998). *Parrots: A Guide to Parrots of the World*. New Haven, CT: Yale University Press.
- Kilner, R. and Johnstone, R. A. (1997). Begging the question: are offspring solicitation behaviours signals of need? *Trends Ecol. Evol.* **12**, 11-15.
- Kondo, N. and Watanabe, S. (2009). Contact calls: information and social function. *Jpn. Psychol. Res.* **51**, 197-208.
- Krebs, E. A. (2001). Begging and food distribution in crimson rosella (*Platyercus elegans*) broods: why don't hungry chicks beg more? *Behav. Ecol. Sociobiol.* **50**, 20-30.
- Kroodsma, D. E. (1996). Ecology of passerine song development. In *Ecology and Evolution of Acoustic Communication in Birds* (ed. D. E. Kroodsma and E. H. Miller), pp. 97-117. Ithaca, NY: Cornell University Press.
- Lancaster, W. C., Henson, O. W., Jr and Keating, A. W. (1995). Respiratory muscle activity in relation to vocalization in flying bats. *J. Exp. Biol.* **198**, 175-191.
- Leech, S. M. and Leonard, M. L. (1997). Begging and the risk of predation in nestling birds. *Behav. Ecol.* **8**, 644-646.
- Leonard, M. L. and Horn, A. G. (2006). Age-related changes in signaling of need by nestling tree swallows (*Tachycineta bicolor*). *Ethology* **112**, 1020-1026.
- Marler, P. (2004). Bird calls: their potential for behavioral neurobiology. *Ann. N. Y. Acad. Sci.* **1016**, 31-44.
- Moravec, M. L., Striedter, G. F. and Burley, N. T. (2006). Assortative pairing based on contact call similarity in budgerigars, *Melopsittacus undulatus*. *Ethology* **112**, 1108-1116.
- Nelson, D. A. (1997). Social interaction and sensitive phases for song learning: a critical review. In *Social Influences on Vocal Development* (ed. C. T. Snowdon and M. Hausberger), pp. 7-22. Cambridge, UK: Cambridge University Press.
- Nowicki, S. and Searcy, W. A. (2005). Song and mate choice in birds: how the development of behavior helps us understand function. *Auk* **122**, 1-14.
- Nudds, R. L., Taylor, G. K. and Thomas, A. L. R. (2004). Tuning of Strouhal number for high propulsive efficiency and stroke amplitude relate and scale with size and flight speed in birds. *Proc. Biol. Sci.* **271**, 2071-2076.
- Ohms, V. R., Beckers, G. J. L., ten Cate, C. and Suthers, R. A. (2012). Vocal tract articulation revisited: the case of the monk parakeet. *J. Exp. Biol.* **215**, 85-92.
- Pacheco, M. A., Beissinger, S. R. and Bosque, C. (2010). Why grow slowly in a dangerous place? Postnatal growth, thermoregulation, and energetics of nestling green-rumped parrotlets (*Forpus passerinus*). *Auk* **127**, 558-570.
- Parker, G. A., Royle, N. J. and Hartley, I. R. (2002). Begging scrambles with unequal chicks: interactions between need and competitive ability. *Ecol. Lett.* **5**, 206-215.
- Podos, J. (1996). Motor constraints on vocal development in a songbird. *Anim. Behav.* **51**, 1061-1070.
- Redondo, T. (1991). Early stages of vocal ontogeny in the magpie (*Pica pica*). *J. Ornithol.* **145**-163.
- Ricklefs, R. E. (2002). Sibling competition and the evolution of brood size and development rate in birds. In *The Evolution of Begging: Competition, Cooperation and Communication* (ed. J. Wright and M. L. Leonard), pp. 283-301. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Saunders, D. A. (1983). Vocal repertoire and individual vocal recognition in the short-billed white-tailed black cockatoo, *Calyptorhynchus funereus latirostris* Carnaby. *Aust. Wildl. Res.* **10**, 527-536.
- Scarl, J. C. and Bradbury, J. W. (2009). Rapid vocal convergence in an Australian cockatoo, the galah *Eolophus roseicapillus*. *Anim. Behav.* **77**, 1019-1026.
- Sewall, K. B. (2009). Limited adult vocal learning maintains call dialects but permits pair-distinctive calls in red crossbills. *Anim. Behav.* **77**, 1303-1311.
- Sharp, S. P. and Hatchwell, B. (2006). Development of family specific contact calls in the long-tailed tit *Aegithalos caedatus*. *Ibis* **148**, 649-656.
- Speakman, J. R. and Racey, P. A. (1991). No cost of echolocation for bats in flight. *Nature* **350**, 421-423.
- Stamps, J., Clark, A., Arrowood, P. and Kus, B. (1985). Parent-offspring conflict in budgerigars. *Behaviour* **94**, 1-39.
- Starck, J. M. and Ricklefs, R. E. (ed.) (1998). *Avian Growth and Development*. New York: Oxford University Press.
- Stolson, S. H. and Beissinger, S. R. (1997). Hatching asynchrony, brood reduction, and food limitation in a neotropical parrot. *Ecol. Monogr.* **67**, 131-154.
- Suthers, R. A. (1997). Peripheral control and lateralization of birdsong. *J. Neurobiol.* **33**, 632-652.
- Suthers, R. A. and Zollinger, S. A. (2004). Producing song: the vocal apparatus. *Ann. N. Y. Acad. Sci.* **1016**, 109-129.
- Suthers, R. A., Thomas, S. P. and Suthers, B. J. (1972). Respiration, wing-beat and ultrasonic pulse emission in an echo-locating bat. *J. Exp. Biol.* **56**, 37-48.
- Tchernichovski, O., Mitra, P. P., Lints, T. and Nottebohm, F. (2001). Dynamics of the vocal imitation process: how a zebra finch learns its song. *Science* **291**, 2564-2569.
- Tobalske, B. W. and Dial, K. P. (1994). Neuromuscular control and kinematics of intermittent flight in budgerigars (*Melopsittacus undulatus*). *J. Exp. Biol.* **187**, 1-18.
- Tucker, V. A. (1968). Respiratory exchange and evaporative water loss in the flying budgerigar. *J. Exp. Biol.* **48**, 67-87.
- Vleck, C. M. and Bucher, T. L. (1998). Energy metabolism, gas exchange and ventilation. In *Avian Growth and Development* (ed. J. M. Starck and R. E. Ricklefs), pp. 89-111. New York: Oxford University Press.
- Waltman, J. R. and Beissinger, S. R. (1992). Breeding behavior of the green-rumped parrotlet. *Wilson Bull.* **104**, 65-84.
- Wong, J. G. and Waters, D. A. (2001). The synchronisation of signal emission with wingbeat during the approach phase in soprano pipistrelles (*Pipistrellus pygmaeus*). *J. Exp. Biol.* **204**, 575-583.
- Wright, T. F. (1996). Regional dialects in the contact call of a parrot. *Proc. Biol. Sci.* **263**, 867-872.





**Fig. S1.** Means of eight spectrographic measurements of 2517 calls from 20 nestling green-rumped parrotlets sampled at weekly intervals throughout the nestling period. Connecting line color and unique symbols represent individual nestlings. Horizontal lines connect age classes whose pooled means are significantly different from each other (Tukey–Kramer highly significant difference). \* $P < 0.05$ , \*\* $P < 0.001$ .



**Fig. S2.** Relationships between different spectrographic attributes of 2517 calls of 20 nestling green-rumped parrotlets and color-coded for the four weeks of development. (A) Duration *versus* average entropy revealed significant clustering of age groups; development was characterized by increasingly lower entropy, and calls from the fourth week clustered along low entropy values and short durations. (B) Duration *versus* average derivative indicates that the fourth week has a higher derivative, as well as a potential tradeoff between durations over which modulation of the carrier frequency is constrained; increasingly longer durations show diminishing marginal departure either way from neutral derivative scores. (C) Bandwidth was mainly achieved by increasing upper frequencies as opposed to reducing lower frequencies. (D) Duration showed a positive log-linear relationship with the number of inflections.