Doppler-shift compensation behavior by Wagner's mustached bat, *Pteronotus personatus*

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Doppler-shift compensation behavior (DSC) is a highly specialized vocal response displayed by bats that emit pulses with a prominent constant frequency (CF) component and adjust the frequency of their CF component to compensate for flight-speed induced Doppler shifts in the frequency of the returning echoes. DSC has only been observed in one member of the Neotropical Mormoopidae, a family of bats that use pulses with prominent CF components, leading researchers to suspect that DSC is a uniquely derived trait in the single species *Pteronotus parnellii*. Yet recent phylogenetic data indicate that the lineage of *P. parnellii* originates from the most basal node in the evolutionary history of the genus *Pteronotus*. DSC behavior was investigated in another member of this family, *Pteronotus personatus*, because molecular data indicated that this species stems from the second most basal node in *Pteronotus*. DSC was tested for by swinging the bats on a pendulum. *P. personatus* performed DSC as well as *P. parnellii* under identical conditions. Two other closely related mormoopids, *Pteronotus davyi* and *Mormoops megalophylla*, were also tested and neither shifted the peak frequency of their pulses. These results shed light on the evolutionary history of DSC among the mormoopids. © 2008 Acoustical Society of America. [DOI: 10.1121/1.2912436]

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I. INTRODUCTION

Doppler-shift compensation (DSC) is a highly specialized vocal behavior exhibited by selected groups of bats that rely upon a very narrowly tuned auditory system to discriminate fine acoustic details of their prey and to navigate through dense foliage (Schnitzler, 1967; Simmons, 1974; Simmons *et al.* 1979). The echolocation pulses of Doppler-shift compensating bats are distinguished by their prominent constant-frequency (CF) components. Because their auditory systems are precisely tuned to a narrow bandwidth around the CF frequency of their outgoing pulse, flight-induced Doppler shifts in the frequency of the returning echoes are canceled out by a systematic adjustments of subsequent pulse frequencies, which serves to maintain the bandwidth of the returning echo within the range of frequencies to which their ears are most sensitive. DSC has been reported for several species of the old world families Rhinolophidae and Hipposideridae (Schuller, 1980; Habersetzer *et al.*, 1984; Neuweiler *et al.*, 1987; Hiryu *et al.*, 2005), but only one mormoopid species, *Pteronotus parnellii* (Schnitzler, 1970; Schnitzler and Henson, 1980; Gaioni *et al.*, 1990; Keating *et al.*, 1994). *P. parnellii* is one of the eight members of the family Mormoopidae, two of which are in the genus *Mormoops*, and six belong to the genus *Pteronotus* (Fig. 1). Although all of the *Pteronotus* species incorporate a CF component into their pulses, *P. parnellii* is the only member of this genus to use particularly long (≈ 25 ms) CF pulses and it

is the only *Pteronotus* species believed to possess the narrowly tuned auditory system typical of other Doppler-shift compensating bats (Suga, 1989; Kossel *et al.*, 1999); thus its DSC performance has been considered a derived trait, probably unique among the mormoopids. Long CF pulses are not necessarily a prerequisite for DSC, however, as several species of hipposiderids emit short CF pulses similar in structure to the pulses uttered by the smaller *Pteronotus* species and perform DSC (Schuller, 1980; Habersetzer *et al.*, 1984; Hiryu *et al.*, 2005).

Recent phylogenetic evidence has indicated that *P. parnellii* stems from the most basal node in the *Pteronotus* lineage (Fig. 1) (Van Den Bussche and Weyandt, 2003), in which case it would be somewhat surprising if none of the more recently originated members of the genus maintained the ability to perform DSC despite continuing to use prominent CF components in their pulses. In light of this evidence, one member of the *Pteronotus* genus, *Pteronotus personatus*, becomes particularly interesting with regard to the evolution of DSC among the mormoopids because (1) this species stems from the second most basal node (so, after *P. parnellii*) in the *Pteronotus* lineage (Van Den Bussche and Weyandt, 2003) and (2) recent behavioral observations (Guillén-Servent, 2005) suggest that it forages in a manner similar to another Doppler-shift compensating neotropical bat, *Noctilio albiventris* (Roverud and Grinnell, 1985a; Kalko *et al.*, 1998). The echolocation pulses of *P. personatus* are typically 5–8 ms long and include not one but two prominent narrow bandwidth components separated by a short ~ 15 kHz downward FM sweep. Based on the above observations, we hypothesized that *P. personatus* possessed the

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FIG. 1. Phylogenetic relationships among mormoopid bats according to the maximum likelihood analyses of the concatenated nucleotide sequences of the mitochondrial ribosomal and cytochrome *b* and the nuclear RAG-2 genes. Branch lengths are proportional to the amount of evolution in substitutions per site. All nodes had 1.00 Bayesian posterior probabilities (modified from Fig. 3 of Van Den Bussche and Weyandt, 2003).

ability to perform DSC behavior, and if so we would be able to make significant inferences about the evolution of this specialized behavior among mormoopids. We tested for DSC in *P. personatus* by swinging the bats on a pendulum similar to the previous studies of DSC in *P. parnellii* (Gaioni *et al.*, 1990). We also tested in the same setting *Pteronotus davyi*, a member of the most recent radiation within *Pteronotus*, originating from an ancestor sister to *P. personatus*, and *Mormoops megalophylla*, a species in the genus sister to *Pteronotus*, in order to get information on the evolutionary history of DSC in the Mormoopidae. Here, we present the results of our analysis of the compensation performance and other vocal characteristics of these bats, and, in particular, we provide a comparative description of DSC performance by *P. personatus* in side-by-side trials with *P. parnellii* under the same conditions. Our conclusions provide insight into the origin and evolution of DSC among the Mormoopidae.

II. METHODS

The bats used in this study were captured by a harp trap as they emerged from a cave in the state of Veracruz, Mexico (Emiliano Zapata municipality, 19° 21'N 96° 42'W) just after sunset on the evening of June 8, 2005. Ten bats of each of four species of the family Mormoopidae (Fig. 1), *Pteronotus parnellii*, *Pteronotus davyi*, *Pteronotus personatus*, and *Mormoops megalophylla*, were captured and placed in individual

temporary holding cloth bags. Table I provides the representative morphometric data (body mass and forearm length) for the four species tested. All of the bats captured at this study site were females. All procedures were in accordance with the National Institutes of Health guidelines for the care and use of research animals, and were preapproved by both institutional animal care and use committees. Upon completion of the experiments, animals were released unharmed at the site where they were captured before midnight of the same evening. To test for DSC behavior, a pendulum was constructed of heavy-duty PVC irrigation pipe and placed at a distance of roughly 10 m from the entrance to the cave. The pendulum was positioned to swing toward a large concrete wall intended to serve as a target. The arm of the pendulum was 3.05 m long and swung through a maximum arc of approximately 100°, reaching a maximum velocity of 6 m/s. At this velocity, the maximum Doppler shifts in the echo frequencies ranged from 2.0 to 2.95 kHz, depending on the species-specific frequencies and bandwidths of the emitted pulses. Bats were secured facing forward in a soft foam body mold that was then secured within a hard plastic box attached to the end of the pendulum arm. Each bat was swung on the pendulum ten times, and each swing consisted of two to three forward and reverse cycles, although only the vocalization data from the first cycle of all ten swings were included in this analysis. All four of the species tested here continued to spontaneously vocalize at high rates while being restrained in the pendulum; a few individuals of the species *Pteronotus davyi* stopped calling after their first swing on the pendulum and these bats were released and replaced by other more vocal representatives of their species.

An externally polarized condenser microphone (Avisoft Bioacoustic, Berlin Germany, model CM16) facing the bat was attached to a rod extending approximately 10 cm in front of and 5 cm above the head of the bat. The frequency response of the microphone spans 10–200 kHz and is flat ± 3 dB over the range of 30–140 kHz (manufacturer's specifications). The microphone recorded both the bat vocalizations and resulting echoes reflected off the ground during the swing, which made it possible to use pulse-echo time disparities in the recording to infer the position of the pendulum arm at each vocalization. Recorded vocalizations were digitized at 250 kHz sampling rate using the Avisoft Ultrasound-Gate hardware (Avisoft Bioacoustics, model 116-200) attached to a personal computer running the accompanying AVISOFT-RECORDER software v. 2.9. Data were analyzed offline using the software AVISOFT-SASLAB PRO V. 4.3. For each

TABLE I. Body size and basic echolocation call parameters among four mormoopids.

	<i>M. megalophylla</i>	<i>P. parnellii</i>	<i>P. personatus</i>	<i>P. davyi</i>
Weight (g)	14.9 $\pm$ 1.2	14.6 $\pm$ 1.7	7.4 $\pm$ 1.2	7.4 $\pm$ 1.1
Forearm length (mm)	55.8 $\pm$ 1.4	57.0 $\pm$ 1.3	42.7 $\pm$ 1.2	44.0 $\pm$ 2.0
CF2 ^a frequency (kHz)	53.9 $\pm$ 0.88	64.9 $\pm$	85.1 $\pm$ 1.3	73.6 $\pm$ 2.0
Duration (ms)	5.1 $\pm$ 1.1	19.7 $\pm$ 6.2	4.8 $\pm$ 0.9	4.9 $\pm$ 0.6
Bandwidth (kHz)	6.5 $\pm$ 1.9	10.7 $\pm$ 1.8	15.1 $\pm$ 1.5	16.2 $\pm$ 1.8

Since the echolocation calls of *M. megalophylla* do not exhibit a true CF, values presented as CF2 frequency measurements refer to the frequency of the loudest portion of their quasi-CF echolocation calls (F_{peak}) for this species, and are provided as a point of comparison across the genus. Calls were recorded from stationary bats.

bat, spectral parameters of the echolocation pulses, including the average value of the CF component of the dominant second harmonic (CF2), the bandwidth of the terminal FM (tFM) component, and for *P. personatus* and *P. davyi*, the average value of the terminal CF (tCF2) component, were taken from a spectrogram created with a 1024-point fast Fourier transform (FFT). Since the echolocation pulses of *Mormoops megalophylla* may exhibit a quasi-CF component (in search phase) but never a true CF component, we defined the frequency of the loudest portion of its vocalization as identified in the magnitude power spectrum as the peak frequency of the second harmonic (F_{peak}), and the maximum (highest starting) and minimum (lowest ending) frequencies (F_{max} and F_{min}) were defined as the frequencies of the upper and lower bandwidths of the power spectrum surrounding the F_{peak} of the vocalization at -40 dB relative to the loudness of the peak frequency. For the purpose of comparisons across species, we speculate that the quasi-CF component represented by F_{peak} in *M. megalophylla*'s search phase calls is ancestral to the CF2 component that characterizes the echolocation calls of the entire *Pteronotus* genus, and therefore this measurement is the most appropriate value to compare to the CF2 of the three *Pteronotus* species. For the tFM sweep bandwidth, we measured the bandwidth of the power spectrum extending below the CF2 at -40 dB relative to the loudness of the CF2. For temporal analyses, pulse durations and intervals were measured from a 256-point FFT. For automated measurements of pulse duration and intervals, the thresholds for pulse onset and offset were defined as the time points at which the rising and falling amplitudes of the pulse passed a value -20 dB relative to the peak pulse amplitude. Initial pulse values prior to being swung on the pendulum were calculated from 1 min of pulses (typically about 100 pulses) recorded from the restrained bat while the pendulum hung straight down in a stationary position. For each of the species described here, echolocation pulses consisted of multiple prominent harmonics; however, the second harmonic was the dominant harmonic for all four bats, and therefore the measurements presented for comparison were taken from the second harmonic. Pulse amplitudes on the pendulum varied from 85 to 115 dB SPL. Statistical analyses were performed using the commercial statistical software package SIGMASTAT V. 3.1 (Systat Software, Inc.). For statistical comparisons, either a paired t-test or a nonparametric one-way repeated measure ANOVA was used to assess the significance of changes in pulse parameters between data sets. Data are presented as means $\pm$ SD unless indicated otherwise.

To quantify each species' DSC behavior, ten bats of each of the four species were swung on the pendulum ten times. For each of the ten swings, the CF2 (or for *M. megalophylla* the peak frequency) of the last pulse occurring prior to when the pendulum was released was compared to the lowest recorded CF2 values taken from pulses emitted while the pendulum was traveling at its fastest velocity, i.e., near the half-way point of the forward swing, as the bat passed closest to the ground. From these measurements, the maximum observed change in CF2 frequency on each swing was determined for each animal.

FIG. 2. Spectrograms of representative echolocation pulse sequences for the four species of bats in flight near the site of capture, including examples of (A) *Pteronotus personatus*, (B) *Pteronotus davyi* flying alongside *Pteronotus parnellii*, and (C) *Mormoops megalophylla* flying past the corner of a large brick wall.

Representative recordings of echolocation pulses emitted while the bats were in free flight were obtained by positioning a microphone 1 m above ground, facing upwards at a 45° angle, approximately 10 m away from the cave opening. Although each of the four species tended to follow a slightly different trajectory upon emerging from the cave, they all stayed with 2–3 m off the ground as they passed over our recording equipment.

III. RESULTS

A. A comparison of the echolocation pulse structures of all four species

Figure 2 illustrates the orientation sounds emitted by the four species of bats included in this study in free flight as they exited the cave. Since previous reports have provided reliable descriptions of the echolocation pulse structures of flying *Pteronotus davyi*, *Pteronotus personatus*, and *Pteronotus parnellii* (Griffin and Novick, 1955; Novick and Vaisnys, 1964; O'Farrell and Miller, 1997; Ibanez *et al.*, 1999; Macias and Mora, 2003), we restrict our description here to the vocal behavior of these animals on the pendulum. *P. parnellii* was included in the study because its DSC behavior on a pendulum is already well documented and thus could serve as a point of reference between these and prior experiments (Gaioni *et al.*, 1990; Keating *et al.*, 1994). We have included additional details regarding the vocal characteristics of *Mormoops megalophylla* because the vocal characteristics of this species are sparsely represented in the literature. Table I provides a comparison of basic call features for all four species of bats.

FIG. 3. Representative power spectra of individual calls emitted by (A) *P. personatus* and (B) *P. davyi* (B) in flight versus [(C) and (D)] when restrained. Both bats emit short (5 ms) calls characterized by brief initial and terminal CF components. In both species, the second harmonic is the dominant harmonic component of the call, but often both lower and higher harmonic components are also detectable in recordings. Insets show calls represented in power spectra. Note that the CF is substantially reduced in both species when held stationary, but a prominent peak representing the CF was still distinguishable in the power spectrum for (C) *P. personatus* but not in (D) *P. davyi*, where the FM component of the call was emphasized relative to the rest of the call.

1. *Mormoops megalophylla*

In flight [Fig. 2(c)], *M. megalophylla* was observed emitting quasi-CF-FM pulses of approximately 6.2 ± 1.2 ms ($n=250$ pulses). When approaching obstacles such as nearby trees and buildings [as seen in Fig. 2(c)], *M. megalophylla* was observed to increase pulse bandwidth by increasing the starting frequency ($F_{\max}$) and decreasing the ending frequency ($F_{\min}$) while also shortening the pulse duration. These broadband calls began with steeply sloping downward FM sweeps which became shallowly frequency modulated in the center of the pulse for a brief period of 1–2 ms before resuming a second rapid drop in frequency, thus producing an S-shaped pulse with a peak intensity centered in the middle of the pulse. Restrained *M. megalophylla* slightly emitted shorter pulses (5.0 ± 1.1 ms, 6.5 ± 1.9 kHz bandwidth; $n=1000$ pulses from ten bats) than those observed in open flight. The F_{peak} of the narrow bandwidth pulses emitted in flight (54.5 ± 0.85 kHz, $n=250$ pulses) closely corresponded with the central F_{peak} recorded from restrained bats on the pendulum (53.9 ± 0.88 kHz, $n=1000$ pulses), which

indicated that a change in pulse bandwidth was not normally accompanied by a change in the peak frequency of the pulse in this species.

2. *Pteronotus parnellii*, *Pteronotus personatus*, and *Pteronotus davyi*

The echolocation pulses of each of the *Pteronotus* species were essentially identical to previous descriptions in the literature (Griffin and Novick, 1955; Novick and Vaisnys, 1964; O'Farrell and Miller, 1997; Ibanez et al., 1999; Macias and Mora, 2003). Echolocation pulses of restrained *P. parnellii* averaged 19.7 ± 6.2 ms long ($n=1000$) and had a mean CF2 value of 64.9 ± 0.9 kHz. The pulses of *P. personatus* displayed an average duration of 4.8 ± 0.9 ms ($n=1000$). In flight [Figs. 2(a) and 3(a)], the pulses of *P. personatus* exhibited two separate CF components; an initial CF (CF2) and a terminal CF (tCF2), separated by a brief downward FM sweep. For stationary *P. personatus*, the initial CF2 prior to being swung on the pendulum averaged 85.1 ± 1.3 kHz and the tCF2 averaged $70.0 \text{ kHz} \pm 1.2$ kHz. On the pendulum,

FIG. 4. Spectrograms of sequences of pulses emitted during the initial 0.75 s of the forward swing of the pendulum by (A) *Mormoops megalophylla*, (B) *Pteronotus davyi*, and (C) *Pteronotus parnellii*. To serve as a reference, a dotted line is placed in each graph just above the CF2, or for *Mormoops* the peak frequency, of pulses emitted before the swing began. The second and third harmonics are visible in all three panels.

the initial CF2 was maintained while the tCF2 became much shorter [Fig. 3(c), inset], although evidence of the tCF2 remained prominent in the magnitude power spectra [Fig. 3(c)].

In free flight, the echolocation pulses of *P. davyi* were also observed to include a second, CF2 component at the end

of the pulse that fell roughly 10 kHz below the initial CF2 [Fig. 2(b)]; however, the tCF2 component was completely absent from the spectrograms of pulses recorded from restrained *P. davyi* on the pendulum [Fig. 3(d), inset] and a tCF2 was not consistently visible in the power spectra [Fig. 3(d)]. Prior to being swung on the pendulum, the echolocation pulses of restrained *P. davyi* averaged 4.9 ± 0.6 ms ($n = 1000$) and the initial CF2 averaged 73.6 ± 2.0 kHz.

B. Doppler-shift compensation behavior on the pendulum

Figures 4 and 5 present the representative examples of call sequences emitted by each of the four species of bats while swinging forward on the pendulum. Entire forward swings lasted approximately 1.5 s, but for the sake of clarity we show here only brief sections of the swing corresponding to the period of maximum acceleration (the first 0.75 s). The four species studied differed in their vocal responses on the pendulum, and, in particular, in the average change in the CF2 or F_{peak} frequency while swinging on the pendulum (Fig. 6). As mentioned, although the echolocation pulses of *Mormoops megalophylla* did not include a true CF component, a prominent F_{peak} is clearly visible in the power spectrum (Fig. 7), which we used here for purposes of comparing *Mormoops megalophylla* to the other mormoopids. All four bats responded to forward pendulum swings with rapid bursts of calls (Fig. 4). Of the four bats tested, *P. personatus*

FIG. 5. Spectrogram of a sequence of pulses emitted during the initial forward swing of the pendulum by (A) *Pteronotus personatus*. In panel B, pulse CF2 frequency data from five complete swings from five different bats, each represented by a different symbol, are shown to illustrate the typical pattern of changes in pulse frequency. Estimated echo frequencies during pendulum swings shown in panel B (solid line) are based on the empirically determined speed of the pendulum and the average CF2 frequency of the bats before the swing began. Time 0–1.5 s represents the time course of the forward swing and the time period from 1.5 to 3.0 s represents the backwards swing. Observe that since the bats rapidly raised their pulse CF2 frequencies between 1.0 and 1.5 s while still moving forward, they were likely focusing their attention on the receding ground beneath them rather than the intended forward target. Since most of the pulse CF2 frequencies recorded during the backward swing were higher than the initial CF2, it may be concluded that the bats partly compensated for negative changes in echo frequency. Panel C illustrates the average pulse emission rate of the same five bats (five swings per bat) during the pendulum swings divided into 250 ms time bins.

FIG. 6. (A) Box plot of the average difference in the peak or CF2 frequency (or F_{peak} for *M. megalophylla*) of pulses uttered at the midway point of the forward pendulum swing relative to their average frequency at the start of the swing. Only *P. parnellii* and *P. personatus* displayed a statistically significant shift (*) in mean pulse frequency when swung on the pendulum. The solid line denotes the mean, the box denotes the first standard deviation, the whiskers denote the 10th/90th percentiles, and the black dots denote the 5th/95th percentiles. $N=100$ (ten forward swings from each of ten bats). (B) Here, the average frequency differences are expressed as a percentage of the estimated maximum Doppler shift based on the initial CF2 frequency for each bat. *P. personatus* showed the highest relative compensation, but *P. parnellii* exhibited on average a much less variable compensation performance than *P. personatus*.

displayed the greatest shifts in its CF2 on the pendulum, on average lowering their frequencies by 2.75 ± 0.99 kHz, which means that they compensated for approximately 94% of the maximum Doppler shifts appearing in the echo. Figure 5(b) shows the pattern of changes in *P. personatus*' CF2 value throughout the entire swing for five swings representing five different bats. In Fig. 5(b), it can be seen that the bats rapidly lowered their CF2 values early in the swing, but also started to rapidly raise their CF2 values in the second half of the forward part of the swing, which indicates that the bats were turning their attention to echoes deflected off the ground (which was receding) rather than echoes deflected off the intended forward target. From Fig. 5(b), it can also be observed that *P. personatus* raised and on average held the CF2 values of their calls above their starting CF2 values for the duration of the backwards swing, suggesting that these bats at least partly compensated for negative shifts in echo frequency. Figure 5(c) plots the average call rate over the time course of the complete swing for the same five bats; *P. personatus* rapidly increased call rate during the forward swing, but call rate held steady at or below the initial rate during the return swing.

As expected, *P. parnellii* also performed DSC well, on average lowering the frequency of their CF2 by 1.79 kHz, which represented a compensation level of approximately 88%. *P. davyi* exhibited a small change in CF2 on the pendulum, on average lowering their frequencies by 709 ± 969 Hz (change not significant, $P > 0.05$). *M. megalophylla* made significant changes in pulse structure and bandwidth (described below) but we did not find a significant change in the F_{peak} of their pulses when swinging on the pendulum; the average F_{peak} changed only slightly (220 ± 974 Hz) and insignificantly ($P > 0.05$; Fig. 6).

C. Changes in pulse bandwidth

During the forward swing *M. megalophylla* increased pulse rate, shortened pulse durations, and increased the bandwidth of its pulses by increasing F_{max} and decreasing F_{min} (Fig. 7). *M. megalophylla* increased F_{max} by adding a very steep downward FM sweep to the beginning of its pulse, and decreased F_{min} by exaggerating the FM portion that is

present in all of its pulses [Fig. 7(a)]. F_{max} increased from an average of 56.3 ± 1.0 kHz at the beginning of the swing to 60.8 ± 3.3 kHz at the midway point, and F_{min} decreased from 49.8 ± 1.7 to 44.4 ± 1.5 kHz. This amounted to an approximate threefold increase in bandwidth (Fig. 7, panels C and D), which was also accompanied by a relative increase in the loudness of the higher third and fourth harmonics of the pulses relative to the dominant second harmonic (Fig. 7, panel B).

In the case of *P. personatus*, both the initial CF2 portion of the pulse and CF2 were shifted down in frequency during the forward swing of the pendulum [Figs. 8(b) and 8(c)]. Notably, the tCF2 was not lowered as much as the initial CF2 component, but instead went down by an average of 1.85 kHz [75% compensation, Figs. 8(c) and 8(d)], meaning that unlike *M. megalophylla*, *P. personatus* ultimately reduced the bandwidth of its pulses while swinging on the pendulum. By comparison, *P. parnellii* significantly increased the bandwidth of the tFM component of their pulses while swinging forward on a pendulum; tFM increased from 10.7 ± 1.8 kHz before the swing to 14.6 ± 2.0 kHz (pairwise comparison, Signed-Rank test, $P < 0.01$, $n = 100$) at the midway point of the forward swing, and returned to an average of 11.4 ± 1.8 kHz at the midway point of the backwards swing.

Although less pronouncedly, our results indicated that *P. davyi* also manipulated the bandwidth of the FM components on the pendulum. Prior to swinging, *P. davyi*'s tFM bandwidth averaged 16.2 ± 1.8 kHz (median of 15.8 kHz), and during the swing it slightly increased to 16.7 ± 1.9 kHz (median of 17.4 kHz), while on the return swing was reduced to 15.1 ± 1.9 kHz (median of 14.8 kHz). The mean data for before swinging and during the forward swing were not significantly different from one another ($P > 0.05$), but both data sets were significantly broader than the mean tFM bandwidth of pulses emitted on the return swing ($P < 0.01$).

IV. DISCUSSION

The DSC behavior occurs in bats that have narrowly tuned yet extremely sensitive auditory systems. In general, the use of CF-type echolocation pulses is one aspect of the

FIG. 7. Changes in the acoustic structure of echolocation pulses emitted by *M. megalophylla* while swinging forward on the pendulum. Panel A shows a representative collection of pulses taken at different succeeding time points during a forward swing; call a was taken before the swing began, calls b and c were emitted early in the swing, and call d is representative of calls emitted while the swing was moving at its highest velocity. The power spectra for two of these pulses (calls a and d) are shown in (B) to demonstrate the increase in bandwidth and the increase in the loudness of the higher frequency harmonic components of the pulse. (C) and (D) are the box plots of the mean peak, minimum (ending), and maximum (starting) frequencies of the pulses ($n=100$).

specialized sensorial strategies employed by bats that fly and hunt within dense vegetation (Schnitzler and Kalko, 2001; Neuweiler, 2003), but CF pulses and DSC behavior have also been associated with fishing behavior in some bats (Roverud and Grinnell, 1985a, 1985b). While relatively little is known about the foraging behaviors of any Mormoopidae, an examination of the auditory systems of several mormoopids (but not including *P. personatus*) concluded that *P. parnellii* might be the only mormoopid possessing an auditory system so finely tuned that it would benefit from DSC behavior (Kossel et al., 1999). Recent well resolved phylogenetic data indicating that *P. parnellii* and *P. personatus* stem, respectively, from the two most basal nodes at the base of the

FIG. 8. Changes in acoustic structure of echolocation pulses emitted by *P. personatus* while performing DSC on a pendulum. The pulses in A were selected from the sequence shown in Fig. 5 (A). (B) Power spectra of calls a and c shown in panel A demonstrate the bat lowering its CF2 frequency as well as shifting the entire pulse bandwidth downward. (C) and (D) are the box plots of the frequencies of the CF2 and CF component of the pulses for all ten *P. personatus* tested ($n=100$) before and during the pendulum swing.

Pteronotus lineage provided an opportunity to address questions of when DSC may have appeared during the evolution of the mormoopids. Our observation that *P. personatus* performs DSC is consistent with a conclusion that DSC behavior may have been an ancestral characteristic in the *Pteronotus* lineage.

While the two members of the genus *Mormoops*, *M. blainvillii* (Kossel et al., 1999) and *M. megalophylla*, do, in fact, use narrowband pulses in open flight (own observations), these bats appear to control the sound of their voices in a manner more similar to FM bats in that they transition to using short broadband pulses while approaching targets. Alternatively, these bats appear distinct from other FM bats in the way they produce almost symmetrical increases and decreases in the beginning and ending frequencies of their pulses, respectively, and in that the peak energy of the pulse is maintained in the center of the pulse's bandwidth, whereas other FM bats only increase the initial frequency while keep-

ing the ending frequency almost invariable (Kalko and Schnitzler, 1998). This dependence on a central peak frequency in *Mormoops* undoubtedly played a role in the evolution of the CF pulse structure in the *Pteronotus* lineage. This bat seems to forage in more open space than other mormoopids (Guillén-Servent, 2005). The evolution of enhanced sensitivity to the narrowband component of the signal could have triggered the adaptation to forage near vegetation aided by narrow frequency analysis echolocation in the sister lineage, *Pteronotus*, in a process similar to what has been suggested for other bats (Guillén-Servent and Ibáñez, 2007), and not necessarily through an ontogenetic accident as suggested by other authors (Kossl *et al.*, 1999). However, given the evidence that *M. megalophylla* uses broadband echolocation pulses, it is not surprising that this bat did not exhibit a vocal response similar to DSC. That *Mormoops* does not perform DSC suggests that the behavior evolved after the separation between the *Mormoops* and *Pteronotus* lineages.

While swinging forward on the pendulum, *Pteronotus davyi* maintained a prominent CF throughout the swing and yet made only minor changes to its CF2 frequency and tFM bandwidth. From our results, we conclude that *P. davyi* does not exhibit DSC behavior on the pendulum, although it remains possible that *P. davyi* may yet perform the behavior under more natural conditions. If, however, we accept the conclusion that *P. davyi* does not or cannot perform DSC, then it is reasonable to conclude that the DSC behavior may have been lost at some point along the evolutionary lineage leading to *P. davyi*. The most parsimonious hypothesis would be that the foraging strategy adopted by *P. davyi* relies upon a more broadly tuned auditory system and, like most FM bats, can tolerate modest Doppler effects. *P. davyi* similarly behaves to other FM bats in the shortening of the FM and the ending narrowband tail of the pulses when approaching targets or flying near background clutter, but differs from them in the multiharmonic nature of the pulses and in keeping an almost fixed bandwidth and maintaining a short CF element at the beginning of the pulses during all the phases of the echolocation behavior (Ibarra-Alvarado and Guillén-Servent, 2005). Questions remain on the functional meaning of the short CF element and the physiological base for the tolerance to Doppler shifts in it. We would hypothesize that this bat's auditory system exhibits tuning characteristics similar to those reported for *P. quadridens* and *P. macleyi*, which use echolocation pulses with similar design. Likewise, we would predict that the auditory system of *M. megalophylla* is similar to that of *M. blainvillii*. No prominent narrow peaks of highly enhanced sensitivity to narrow frequency bands, such as those present in *P. parnellii*, appear in the audiograms of any of these species (Kossl *et al.*, 1999). The putative loss of DSC in the lineage leading to *P. davyi* adds further puzzle to the different success of narrow frequency echolocation in the Old World (Hipposiderids and Rhinolophids have radiated in some 150 species using this echolocation system) versus the New World tropics [only one species in the mormoopid family (Neuweiler, 2003)]. Secondary loss of the capacity in the most recently evolved lineages of the Mormoopidae points to a possible limit to evolutionary diversification of bats using this sonar system imposed by differences between the two

biogeographical realms in the ecological space available for realizing the foraging strategy associated with narrow frequency analysis echolocation.

The observation that *P. personatus* not only performs DSC but that it was the only mormoopid tested that decreased rather than increased the overall pulse bandwidth while swinging forward on the pendulum suggests that this bat may be under some pressure to maintain both the initial CF and tCF portions of its pulses within narrow ranges of acoustic sensitivity. In some situations, increased call bandwidth may be a by-product of increased call intensity, and since we observed increases in call intensity during the forward swing for all four species, this may explain the observed increases in bandwidth in those species that did so. Both horseshoe bats (Tian and Schnitzler, 1997) and the mustached bat *P. parnellii* increase the bandwidth of the tFM component of their pulses by lowering the $F_{\min}$ while approaching the targets, which raises several questions about why *P. personatus* would not do so. This may imply that their auditory system is finely tuned to the bandwidth of the second, lower CF, or it may reflect a more benign mechanical constraint associated with producing this particular pulse structure. DSC behavior could be part of the adaptations that this bat uses for the particular foraging behavior over water (Guillén-Servent, 2005), when it uses echolocation pulses with a prominent narrowband tail often lacking the initial CF and most of the FM sweep (Guillén-Servent, unpublished data). Further studies on the behavior, ecology, and auditory physiology of *P. personatus* may hold the answers to these questions.

Finally, we address the unique sequence of changes in pulse structure that *P. personatus* appeared to use as it performed DSC (Fig. 8, panel A). The long CF pulses used by horseshoe bats (for example, *Rhinolophus ferrumequinum*), the lesser bulldog bat (*Noctilio albiventris*), and the mustached bat *P. parnellii* (Fig. 2, panel B) include initial upward FM sweeps, and as these bats lower their pulse frequency during DSC the initial upward sweep is maintained throughout the DSC behavior. Although not as prominent, the echolocation pulses of *P. personatus* sometimes included short upward FM sweeps (see call "a" of Fig. 8, panel A), but during their initial response to the Doppler-shifted echoes on the pendulum, the acoustic structure of the pulses changed with the appearance of an initial downward sweep that swept down to a plateau representing a slightly lower initial CF2 than the previous pulse (compare calls "a" and "b" of Fig. 8, panel A). As the pendulum reached its peak velocity, the initial downward sweeps disappeared as the bat reestablished its normal pulse structure at a lower CF2 frequency (call "c" of Fig. 8, panel A). The appearance of this transitional pulse shape (call "b" of Fig. 8, panel A) during DSC performance is interesting not just because it reflects a pattern of pulse adjustments not seen in other Doppler-shift compensating bats but also because of how closely it resembles some of the transitional pulse shapes used by *M. megalophylla* on the pendulum. This sequence of transitions in pulse structure exhibited by *P. personatus* on the pendulum could be interpreted as a reflection of the evolutionary history of CF pulse types in the mormoopids. Thus, at least from a behavioral

standpoint, *P. personatus* would seem to be endowed with some vocal characteristics that probably were present in the ancient common ancestor with *M. megalophylla* and others that can be traced to the more recent common ancestor with *P. parnellii* and the other *Pteronotus*.

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