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ARE THERE INDIVIDUAL AND/OR ROOST SIGNATURES IN THE ECHOLOCATION CALLS OF WILD BIG BROWN BATS (EPTESICUS FUSCUS)?

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ARE THERE INDIVIDUAL AND/OR ROOST SIGNATURES IN THE ECHOLOCATION CALLS OF WILD BIG BROWN BATS (*EPTESICUS FUSCUS*)?

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ABSTRACT

Unique individual vocal signatures are widespread among animals living in colonies such as the maternity roosts of *Eptesicus fuscus*. Such individual signatures have been identified in the echolocation calls of *E. fuscus* in the laboratory, but have not been demonstrated in the wild. By recording known wild *E. fuscus* as they emerged from their roosts at dusk, I tested the hypothesis that individual and/or roost signatures exist in the echolocation calls of wild *E. fuscus*. Analyses of calls of 176 individuals recorded at six different locations indicate that temporal and spectral features appear to contain sufficient variation to identify both roost and individual vocal signatures. Overall, bats were correctly associated with their roosts 48%, and with individual identity 14-37%, of the time. The incidence of such signatures may be significant to wild *E. fuscus* population dynamics.

Keywords: Chiroptera, wild *Eptesicus fuscus,* echolocation calls, individual and/or roost signatures.

For my parents, there were several times during the past 2 years when it would have been very easy for me to walk away. Thank you for lending me your strength when I had none.

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Chapter 1: INTRODUCTION

1.0 Vocal signatures

Unique signatures used for communication often occur in species where individuals live together in high densities (Searby and Jouventin 2004, Cortopassi and Bradbury 2006) or in conditions where olfactory and visual cues are limited (Crawford et al. 1997, Sousa-Lima et al. 2002). They are especially important under conditions where confusion among group members or individuals may result in decreased reproductive fitness or survival (Searby et al. 2004), and are logically more functional among colonial species (Leonard et al. 1997, Schmidt-French et al. 2006). Acoustic signals may have greater ranges of operation than olfactory ones, perhaps making them readily available to more receivers. Therefore, unique vocal signatures are commonly used in different situations. Vocal signatures can contain individual and/or group-specific identities (Crawford et al. 1997, Campbell et al. 2002, Searby et al. 2004, Radford 2005), prevent the misdirection of parental care (Searby and Jouventin 2004, O'Shea and Posche 2006) and aggressive behaviours (Jouventin 1982), and identify sex and age class of individuals (Sousa-Lima et al. 2002). Vocal signatures may also contain information regarding specific behaviours that can help establish and maintain territories and augment nesting and courtship behaviours (Crawford et al. 1986).

Acoustic communication signals must be variable and reliable so that a receiver obtains accurate information about the signaller or the situation. When acoustic signals are used in conjunction with visual and/or olfactory ones, signatures may be simple changes in one or two call features (Searby and Jouventin 2003). Others species, such as colonial penguins, some electric fish, and many bats that are more limited in their use of

different sensory channels produce more complex vocal signatures that vary in several elements including time and frequency (Scherrer and Wilkinson 1993, Crawford et al. 1997, Searby et al. 2004). Many bat species, including *E. fuscus*, live in dense and highly mobile colonies and it would seem therefore plausible that they would benefit from the presence of unique group or individual vocal signatures.

1.1 Variation in Chiropteran social calls

Bat social calls (calls used for a purpose other than echolocation) are variable in both time and frequency characteristics (Fenton 1994, Barclay 1999). Because of this flexibility, social calls can carry information unique to social or familial groups (Scherrer and Wilkinson 1993, Pearl and Fenton 1996) and/or contain individual signatures (Brown et al. 1983, Behr et al. 2006). There is considerable diversity of inter- and intra-specific social calls that encode situation or behaviour specific information (Pfalzer and Kusch 2003). Bat social calls are made during aggressive situations, mate attraction, screech calls, and isolation calls. Screech calls, vocalizations made by *Phyllostomus hastatuas*, appear to facilitate the recognition and maintenance of unrelated group mates before and during nightly foraging (Boughman 1997). Isolation calls are a distinct category of call, principally used by several species of colonial living bats, during mother-offspring interactions. Individually distinct calls made by pups allow mothers to identify their young during retrieval situations (Brown et al. 1983, Balcombe 1990, van Parijs and Corkeron 2002), or during a juvenile's first flight, allowing the mother and newly volant young to maintain contact (Brown et al. 1983). Interestingly, isolation calls containing individual vocal signatures made by juvenile bats actually show the frequency modulated

characteristics typical of adult echolocation sounds (Brown et al. 1983, Moss et al. 1997). As young bats mature the frequency and repetition rate increase and the duration decreases, it is hypothesized that these calls may be a precursor for echolocation calls (Brown et al. 1983, Moss et al. 1997).

1.2 Echolocation

Echolocation involves the echoes of sounds produced by an animal to collect information about its surroundings. Galambos and Griffin (1942) outlined our concept of echolocation in bats. Today we now know that it is used by a variety of organisms including toothed whales, most bats, some insectivores, and some birds (Altringham 1996). Echolocation has reached a pinnacle of sophistication with bats in the suborder Microchiroptera; their calls tend to be simple frequency-modulated sweeps that are relatively short in duration. Echolocation calls made by these bats show structured changes in both time and frequency, and are used not only as a means of orientation but also for prey and obstacle detection and discrimination (Griffin 1958). The morphology of Microchiroptera has been highly influenced by echolocation. Bats have brains adapted for processing acoustic signals (Altringham 1996) and a wide variety of ear and nose, sizes and shapes to improve the focusing of transmitted and received sound waves (Zhuang and Müller 2006). Using the physical properties of sound bats can alter the frequency and timing of their echolocation calls to determine the range and fine structural details of their targets (Simmons 1973). Big Brown bats (*Eptesicus fuscus*) are able to detect 19mm spheres at a distance of 5.1m and 4.8mm spheres at a distance of 2.9m

(Kick 1982), and *Megaderma lyra* using broadband calls are able to analyse textural depths in the range of 0.9-4.2mm (Schmidt 1988).

As specialized as echolocation calls are, prey detection and orientation may not be their only function. It is generally accepted that echolocation calls evolved from social calls, used to assess background environments by early echolocators, and may still contain enough information to additionally serve a communication function (Fenton 1984). To date scientists have identified echolocation calls that possess sufficient variation as to identify the caller's geographical location, foraging task, foraging habitat, sex, group membership, and identity.

1.3 Variation in Chiropteran echolocation calls

Differences in resource partitioning and geographic location among bat species' have lead to morphological differences and ultimately differences in their echolocation calls. Both researchers and bats (observed through playback experiments) can identify different species of bats using temporal structure patterns and frequency changes of echolocation calls (Barclay 1982, O'Farrel and Miller 1999, Barclay et al. 1998, Parsons and Jones 2000, Macias et al. 2006, Jones and Holdreid 2007). Within most aerial-feeding bats situation-specific behaviours are identifiable using their echolocation calls as they search, detect, and track prey while foraging (Griffin 1960, Faure and Barclay 1994).

Intra-specific variation in echolocation calls occurs in several species of bats. Evidence for inter-individual echolocation calls during situation specific behaviours has been identified in emergence and foraging behaviour of several species of bat. Evidence

for the presence of inter-individual signatures has been documented in the emergence and foraging echolocation calls of *Euderama maculatum*, *E. fuscus*, *Lasiurus borealis*, *L. cinereus*, and *Otomops martiensseni* (Obrist 1995, Fenton et al. 2004). Although the evidence was not as compelling, as variations in habitat were likely an interfering factor, the same occurrence of individual variation was observed in the echolocation calls of emerging *Myotis bechsteinii* (Siemers and Kerth 2006). Individuals also alter their echolocation emissions when flying with conspecifics (Habersetzer 1981, Obrist 1995). This occurrence is referred to as "jamming avoidance", and is identified when individuals flying in the same airspace make changes in both their spectral and temporal call features of their echolocation calls (Obrist 1995, Ratcliffe et al. 2004, Bartonicka et al. 2007). Bats may be individually altering their echolocation so that they may recognize their returning echo thus avoiding the misinterpretation of their surroundings or the presence/absence of prey (Bartonicka et al. 2007). It is possible that these individual call signatures are maintained even among habitats. Ratcliffe et al. (2004) demonstrated evidence for the maintenance of individual signatures. They discovered that echolocation calls from more than one individual *Tadarida brasiliensis* flying together in the same habitat varied significantly more than the echolocation calls of individual *T. brasiliensis* flying alone but in two different habitats (Ratcliffe et al. 2004).

1.4 Inter-individual variation of *Eptesicus fuscus* echolocation calls

The strongest evidence for inter-individual variation has been documented in laboratory-based studies using the echolocation calls of *E. fuscus*. Here there is enough measurable variation within the echolocation calls of individuals to contain information

about the caller's age class (juvenile vs. adult), and sex. (Masters et al. 1991, Kazial et al. 2001, Kazial and Masters 2004). Analyses of echolocation calls have revealed the presence of individual distinctive vocal signatures of captive *E. fuscus* (Masters et al. 1991, Burnett et al. 2001, Kazial et al. 2001), and the presence of a family component (presumably either learned or inherited) (Masters et al. 1995). The presence of a family-specific call signature is supported by Pearl and Fenton (1996), who found roost-specific call signatures in *Myotis lucifugus*.

Playback experiments have shown that bats can use this variation to obtain information concerning conspecifics (Kazial and Masters 2004). When female *E. fuscus* were presented with playback of echolocation calls of conspecifics, the vocalizations of the listening females were significantly different during and after playback periods (relative to pre-playback) depending upon the sex of the playback stimulus (Kazial and Masters 2004), indicating that the females may have been able to determine the sex of the caller through their vocalizations.

1.5 Statement of purpose

Bats flying in natural conditions show more call variation than bats flying in laboratory settings, as call features are altered depending on external factors such as their proximity to obstacles, environmental conditions, and the presence of other bats (Surlykke and Moss 2000). The purpose of my study was to determine if, under natural flight conditions, the echolocation calls of wild *E. fuscus* contain roost and/or individual signatures. The term colony and roost are sometimes difficult to distinguish between one another. For the purposes of this study, I shall refer to a roost as the physical structure

that bats inhabit, while the term colony describes a social grouping of the bats inside a roost. By characterizing echolocation calls, recorded from known bats as they emerge from their roost, I tested the hypothesis that it is possible to distinguish among individual wild *E. fuscus* and the maternity roosts to which they belong. Research documenting intra-individual and situation specific variation in echolocation calls has consistently identified significant differences in call duration and frequency parameters (Obrist 1995, Moss et al. 1997, Ratcliffe et al. 2004). I predicted that the echolocation calls wild *E. fuscus* would contain significant variation in both temporal and spectral features and that this variation will permit the distinction of individuals and roosts.

Chapter 2: METHODS

2.0 Field site and study species

Eptesicus fuscus are medium sized (10-21 g) forest dwelling vespertilionids that roost in tree cavities but will frequently inhabit mines, caves, and buildings. Females are seasonal residents of Fort Collins, Colorado, USA where they roost in the many buildings and other man-made structures, using them as maternity roosts during the spring and summer months.

I collected data over two field seasons in the urban setting of Fort Collins (1 June – 29 July, 2004; 17 May – 8 August, 2005) in conjunction with a long term Fort Collins Bat/Rabies Project, jointly run by the United States Geological Survey (USGS), Colorado State University (CSU), and U.S. Centre for Disease Control (CDC). Over the course of the study, radio-telemetry was used to identify 142 potential roosting locations, 44 were classified as maternity roosts, and of these 23 were outfitted with Passive Integrated Transponder (PIT) tag readers. The PIT tag readers were placed over the entrance/exit points of a roost, consequently when any of the greater than 3,000 PIT tagged bats passed by a reader a unique time stamp was created identifying the bat (using a nine digit individual specific code), the time (to the one hundredth of a second), and date.

2.1 Recording sites

I selected eight recording sites within the city limits of Fort Collins (Appendix 1, Figure A.1). Selected sites had: roosts occupied by bats from May through August allowing for multiple recording opportunities, housed a minimum of approximately 30 bats, and all emergence points from the roosts were surrounded by similar open-type

habitat. Among the eight recording sites roost sizes ranged from approximately 30 to greater than 1,000 individuals.

2.2 Recording equipment and set up

I recorded the echolocation calls of bats emerging from the roost using Avisoft UltraSoundGate (USG) 116 and 416 recording systems (Specht 1998-2003) with single and multi-array condenser microphones operating with the recording program Avisoft Recorder USG on a Compaq nx9600 laptop computer, using a sampling frequency of 250 kHz. I positioned the microphone (at 45° angle from the ground) 3.7 metres away from the roosting structure and placed it on top of a 1.8 (if roost was in a single story building) or 3.0 (if roost was located in a building with more than one story) metre extender pole. This ensured that the recordings made at different roots were comparable to one another. Each night before the bats began to emerge I passed a known PIT tag by the reader three times. I documented the time of each pass so that the known PIT tag may act as a point of reference between reader and recordings when I identified individuals. I recorded consecutive 15 minute periods for an average of one and a half hours each night (6 recording periods/night). Recordings started one half hour before dusk each night and lasted until emergence was complete. Emergence was considered complete after a 10 minute period during which no bats were detected acoustically. I recorded at each roost for three consecutive nights before moving on to the next location. I maintained this cycle through out the summer. However, some sites were specifically selected if the readers indicated high levels of activity and some sites were specifically avoided if

readers indicated low levels of activity or netting was scheduled at a roost on a recording night.

2.3 Call analysis and variable extraction

Using BatSoundPro Sound Analysis 3.31b (1996-2001 Pettersson Elektronik AB), I visually identified call sequences using both the time domain and spectrogram views (Appendix 2, Figure A.1 and A.2). A call sequence had to be distinguishable from other call sequences (no overlap) and consist of five consecutive calls that are all above 10% and below 100% call amplitude in the time domain display. Calls less than 10% are too weak and calls greater than 100% are saturated providing false harmonics; in both cases, these calls do not permit extraction of accurate call information. In order to associate call sequences with a known individual, I used the unique time stamps created as PIT tagged individuals passed through the readers and matched them to times at which I recorded the call sequences. To assure accurate identification of an individual these times must have matched up to a tenth of a second. Once an individual was identified, it was assigned an individual bat and roost identification (ID). I analyzed sequences using Call Viewer 12 (Skowronski 2007). Call Viewer is a sound analysis program that uses a visual representation of recorded echolocation calls and automatically extracts 53 variables associated with call time, frequency, amplitude, and shape (Appendix 3, Table A.1)

2.4 Statistics and variable reduction

Numerical call variables extracted (Appendix 3, Table A.1) from identified call sequences were used in multiple discriminant function analyses (SPSS v.

15.0) to classify either roosts or individual vocal signatures. To avoid inflated classification accuracies when running the canonical discriminant function analyses (DFA) I maintained a maximum variable to observation ratio of 33% (Masters et al. 1995). To reduce the number of variables I discarded all variables that involved any form of amplitude measurement, as amplitude is not a robust measurement under natural free flight conditions. Any variation identified in a canonical DFA using amplitude measurements may not be representative of roost or individual identity but instead represent a bat's relative position to the microphone. Next, I omitted from the analyses any variables that contained greater than 10% of measurements with zero values. Although the absence of such a measurement may be indicative of a vocal signature, it is difficult to quantify the absence of a measurement into a DFA. To reduce the remaining variables I tested their degree of correlation using a preliminary DFA. Variables that had a correlation co-efficient of 0.750 or greater in the correlation matrix were considered to be highly correlated. For each pair of highly correlated variables I removed one from the analysis. The first variable identified in the correlated pair was always the variable removed from the analysis, unless it involved a minimum or maximum measurement in which case these were always the variables that were kept. Because I identified a different number of observations in the roost DFA and the individual DFA there were a different number of variables allowed in each respective analysis. Although the number of observations varied among the individual analyses, the number of allowable variables remained constant to ensure that individual analyses among roost locations were comparable.

2.5 Roost signature identification and analyses

A single DFA was run using 17 call variables to classify 108 randomly chosen known individual call sequences among six of the eight recording sites. As no individuals were identified at the East Laurel and 720 Peterson sites, I excluded recordings that were made at these roosts. For 100 separate DFA trials, I randomly split data into a 50:50 test to training ratio, so that the analyses were run with both known and unknown data. Subsequently I performed one-sample, one-tailed T-tests to determine if each of the correct roost classification accuracies were significantly higher than the *a priori* prediction of 16% correct classification accuracy. I calculated the *a priori* prediction by dividing the largest number of observations belonging to the same group by the total number of observations. This prediction accounted for the probability of calls being randomly assigned to the correct grouping prior to the analysis. I then performed a one-way ANOVA comparing classification accuracies among the six roosts included in the DFA.

To avoid misinterpreting individual-specific variation as roost-specific variation, each individual entered into the DFA was represented by 17 variable averages calculated from the 17 variables measured from each of the five calls in an individual sequence.

I calculated mean, standard error of the mean, and the coefficient of variation (CV) for all variables that were important (determined using absolute standardized canonical discriminant function co-efficient values > 0.361) in predicting roost membership.

2.6 Individual signature identification and analyses

I ran two sets of DFA, using the same 23 variables in each, to determine the presence of individual vocal signatures. For comparable results among the individual classification analyses, I used the same variables in each analysis. However, I identified a varying number of individuals at each roost location. Therefore, the call variables used in all individual DFA were determined through variable reduction using data from the roost with the smallest sample size (to maintain the maximum 33% variable to observation ratio). The first set of individual analyses included all individuals with two independent observations regardless of sample size.

To determine if the individual correct classification accuracies were a consequence of roost specific properties (i.e. roost size and/or roost fidelity) and not sample size, I ran a second set of DFA to identify individuals for each roost. These analyses differed from the first as each one included only nine known individuals with two separate observations per individual. Because I used individuals with two independent observations in the analyses, I included individuals recorded at five of the eight roosts during my first set of analyses, and individuals from four of the eight roosts in the second. To minimize the opportunity for unexplained variation within the echolocation calls I only compared individuals to other individuals recorded at the same roost.

As performed in the roost analysis I randomly split data into a 50:50 test to training ratio, and ran 100 trials for each individual DFA. I then ran independent one-sample, one-tailed T-tests for each roost's individual correct classification accuracies, comparing each distribution to it's corresponding *a priori* prediction. Finally, I ran two

independent one-way ANOVAs for each set of analyses to determine if there were any significant differences in individual classification accuracies among roost locations. To ensure that I measured all potential sources of individual variation, all five calls in an identified sequence were included in the individual DFA.

Chapter 3: RESULTS

3.0 Recording summary and variable reduction

Overall, I recorded 2,619 call sequences and identified 311 known individual *E. fuscus* emerging from roosts (Appendix 4, Table A.1). I used only calls from individuals identified on two independent recording nights in the analyses. This meant that I used 35% (108 individuals of a potential 311) of all identified bats when discriminating among roosts, 45% (140 out of 311) when discriminating among a varying number of individuals from each roost, and 12% (36 out of 311) when discriminating among the same number of individuals from each roost. I excluded sequences from the analyses when too few or no individuals could be identified, as a result, the 720 Peterson and East Laurel roosts were not included in any analyses. Through a process of variable analysis and a preliminary DFA (see methods), I reduced the number of variables from 53 to 17 in the roost DFA, and to 24 in all individual analyses (Appendix 3, Table A.1).

3.1 Discrimination of roosts

Using echolocation calls of emerging wild *E. fuscus* a DFA was able to distinguish among roosts (Figure 1). Overall, bats were correctly associated with roosts 48% of the time. This classification was significantly higher ($t_{99} = 32.635$, $P < 0.001$) than the 17% *a priori* prediction of randomly assigning bats to the correct roost. However, correct classification accuracies varied among roosts (Figure 2), and some roosts had significantly higher correct classification accuracies than others ($F_{5, 594} = 12.958$, $P<0.001$). In general, larger roosts achieved lower classification accuracies than smaller roosts (Figure 2). Other factors besides roost size may have contributed to the

differences in colony classification accuracies, as OFC High School and Harmony roosts were an exception to this trend.

In this study, the discriminant functions were a linear combination of the independent call variables and were important in the classification of the dependent variables (roost or individual ID). The number of discriminant function axes used in a DFA was one less than the number of grouping variables. When identifying the grouping variable, each discriminant function included a different standardized weighting of each call variable. In the Roost DFA, all five functions combined described 100% of the variation among the call variables. However, three of the five discriminant function axes (axes 1, 2, and 3) were significant in the classification (Table 1). Cumulatively these three discriminant function axes described 87% of the variation found in roost specific echolocation calls (Table 1). The variables most important in predicting successful roost membership were duration, FOmax, Time_F1, FME_F3, dF0min, and ddF010, as demonstrated by their standardized canonical discriminant function co-efficients (SCDFC) (Table 1). The DFA was most often able to identify echolocation calls from individuals residing in the OFC High School roost (Figure 2). Bats recorded emerging from the OFC High School roost had longer fundamental and harmonic durations and employed lower frequency echolocation calls (Table 2). There was a high level of variation among all roosts in regards to the call variables. However, OFC High School despite having the highest classification accuracy (Figure 2) also had the highest coefficient of variation (CV) (Table 3).

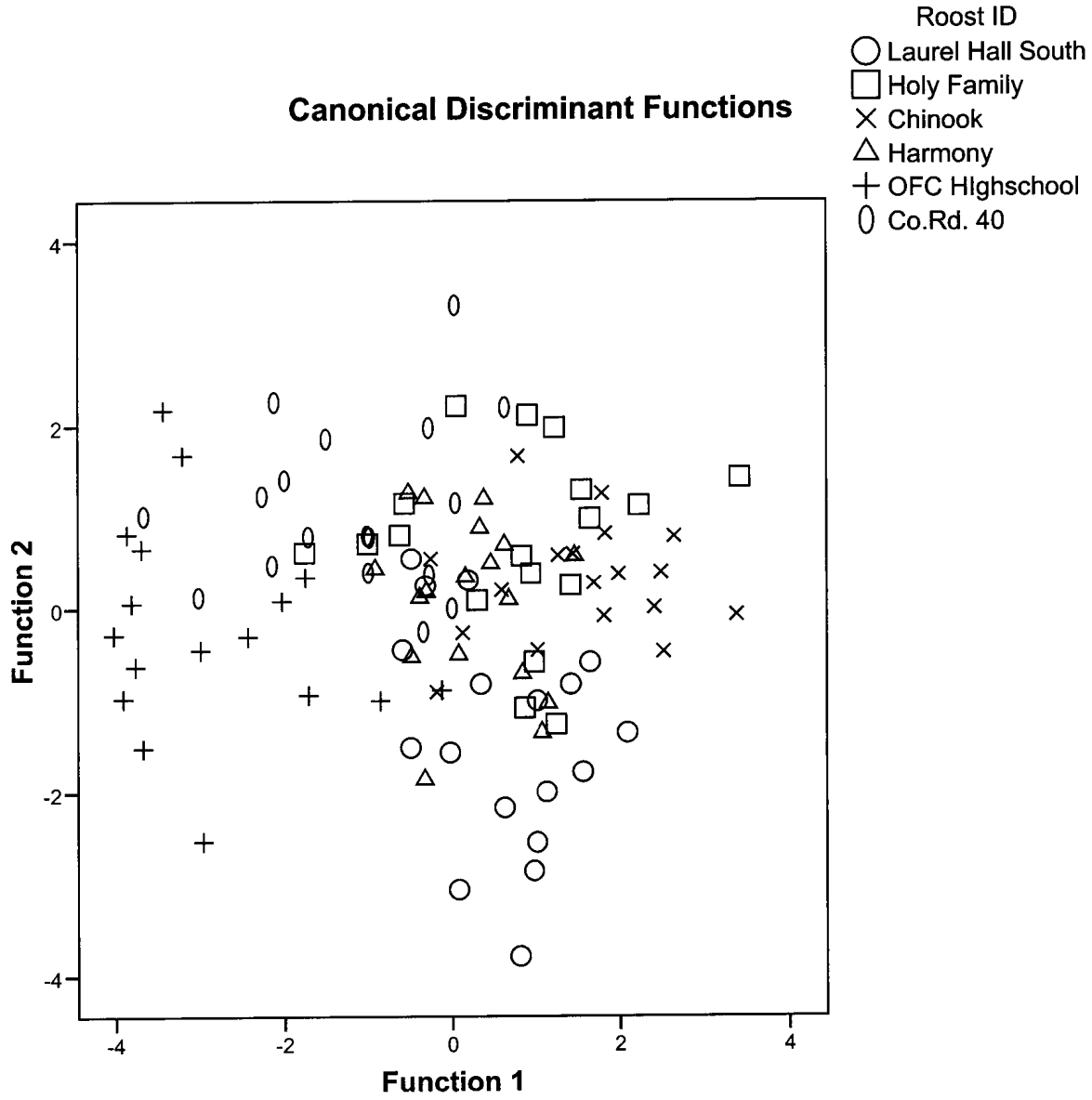

Figure 1. Plot of Standardized Canonical Discriminant Function Co-Efficients of 108 individuals based on means of 5 calls per individual, and identified by their *a priori* known roosts.

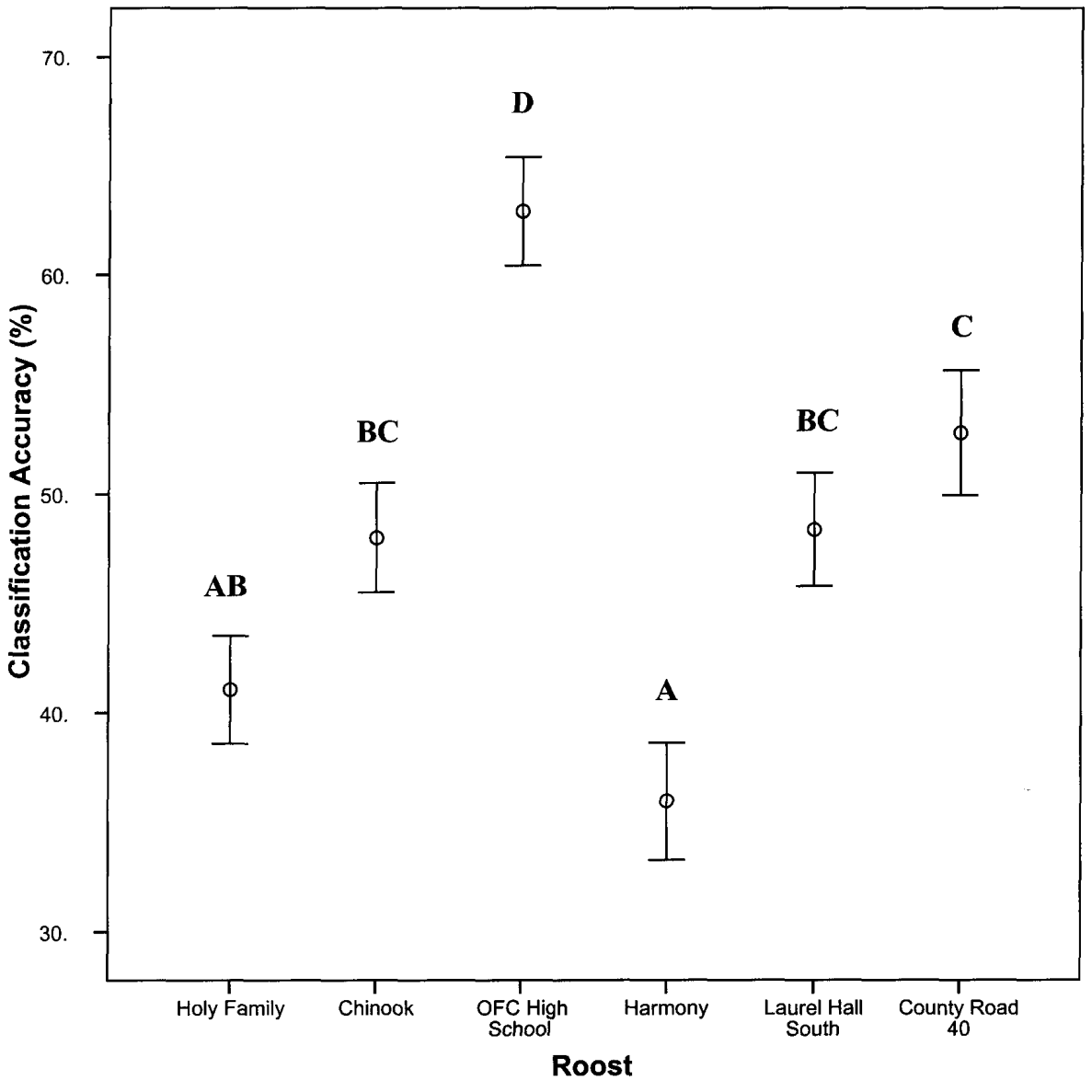

Figure 2. Mean± SE roost classification (obtained from 100 trials) accuracies significantly varied among roosts ($n_{total} = 108$, $n_{roost} = 18$). Harmony roost echolocation calls were least often correctly identified (36% ± 2.66). While, echolocation calls recorded OFC High School were most often correctly identified (63% ± 2.48). Roosts are listed in order of relative size (largest to smallest).

Table 1. *E. fuscus* roost discriminant function analysis of data from echolocation calls, including duration (DUR), the maximum frequency of the fundamental (F0_max), length of the first harmonic (Time_F1), frequency with the most energy of the first harmonic (FME_F1), minimum concavity of the fundamental (ddF0min), and concavity of the 10^{th} percentile of the fundamental (ddF010).

Discriminant Function Axis	DUR	F0_max	F1_max	Time_F1	FME_F1	ddF010	ddF0min	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	1.391	0.782	-1.178	-1.178	-0.427	0.187	0.333	2.951	56.1	0.044	208.39	85	< 0.001
Function 2	0.244	0.361	-0.630	-0.630	0.058	0.585	0.234	0.983	74.8	0.172	117.03	64	< 0.001
Function 3	1.502	0.113	0.062	0.062	-0.249	-0.399	-0.658	0.691	87.9	0.341	71.50	45	0.007
Function 4	0.936	-0.048	-1.689	-1.689	-0.674	-0.052	0.187	0.386	95.2	0.577	36.56	28	0.129
Function 5	0.048	0.414	-0.151	-0.151	0.077	0.115	0.754	0.251	100.0	0.800	14.87	13	0.316

Table 2. Call variables best at predicting roost membership when using echolocation calls of individual *E. fuscus*. N, number of individuals analyses, duration (DUR), maximum frequency of fundamental (F0_max), length of fundamental (Time_F0), length of first harmonic (Time_F1), frequency with most energy of the first harmonic (FME_F1), minimum concavity of the fundamental (ddF0min), and concavity of the 10th percentile of the fundamental (ddF010) means ± SD. CV, coefficient of variation.

		DUR (ms)		F0_max		Time_F0 (ms)		Time_F1 (ms)		FME_F1 (Hz)		ddF0min (kHz/ms/ms)		ddF010 (kHz/ms/ms)	
Colony	N	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
		4.91		67675.79		1.56		3.52		64073.35		-149.34		-50.13	
Laurel Hall South	18	±0.73	14.9	± 4606.86	6.8	± 0.85	54.5	±0.65	18.5	±3873.06	6.0	±31.59	21.2	±25.46	50.8
		6.36		71766.49		2.26		4.66		63541.66		-160.51		-19.14	
Holy Family	18	±0.93	14.6	± 5731.88	8.0	±0.60	26.5	±0.89	19.1	±2796.25	4.4	±39.47	24.6	±15.64	81.7
		6.54		72602.00		1.81		4.41		63107.64		-162.92		-27.76	
Chinook	18	±1.11	17.0	± 3896.93	5.4	±0.43	23.8	±0.79	17.9	±2886.39	4.6	±38.86	23.9	±20.13	72.5
		5.67		69894.75		1.79		4.10		62207.03		-153.11		34.27	
Harmony	18	±1.07	18.9	± 3183.66	4.6	±0.41	22.9	±0.72	17.6	±2715.02	4.4	±34.98	22.8	± 20.07	58.6
		6.88		64208.98		2.99		5.15		59993.49		-125.19		-22.15	
OFC High School	18	±1.56	22.7	± 3935.04	6.1	±0.75	25.1	±0.97	18.8	±3761.54	6.3	±28.23	22.5	±17.35	78.3
		6.12		66929.00		2.92		4.46		60839.84		-143.34		-19.38	
County Rd. 40	18	±0.65	10.6	± 4217.17	6.3	±0.50	17.1	±0.69	15.5	±4405.91	7.2	±29.20	20.4	±11.57	59.7

3.2 Discrimination of individuals

Overall, the DFA correctly identified individual bats between 13.63 and 37.26% (Figure 3) for all individual analyses using a varying number of individuals for each roost, and between 28.76% and 34.38% (Figure 4) for all individual analyses using the same number of individuals for each roost. Although classifications appeared seemingly low in some instances, all mean classification accuracies were significantly above random chance (Table 3). Individual DFA where the same number of individuals were used from each colony (Test 1) and individual DFA where a varying number of individuals were used from each colony (Test 2) differed significantly from one another for each colony (Table 4). In all cases, except for the Chinook roost, individual DFA that included the same number of individuals for each colony had higher classification accuracies (Table 3). Despite the lower classification accuracies when varying numbers of individuals were used in the analysis, the overall ranking of a roost remained the same (with the exception of Laurel Hall; Figure 3 and 4).

The variables that were best at discriminating among individuals varied among the roosts (Appendix 5, Tables A.1-A.9). However, overall DUR, F020, F050, Time_F2, FME_F2, and dF010 were best at discriminating among individuals at most if not all roosts (as indicated by SCDFC).

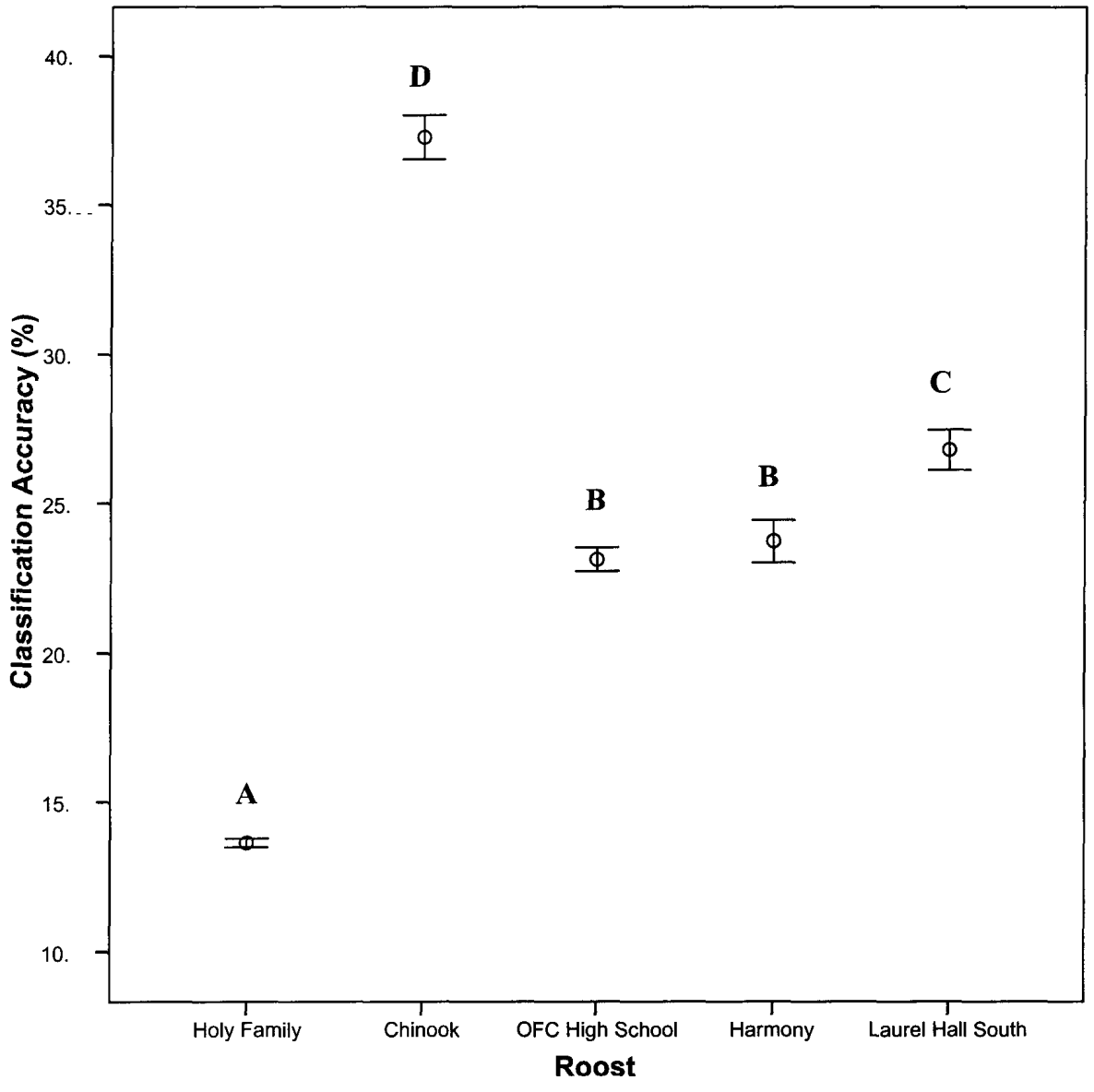

Figure 3. Mean± SE individual classification accuracies (obtained from 100 trials) varied significantly (d.f. = 4, 495, F = 211.84, P < 0.001) among roosts when using a varying number of individuals in each DFA (Holy Family n = 80, Chinook n = 10, OFC High School n = 23, Laurel Hall South n = 11, Harmony n = 8). Individuals recorded at the Holy Family $_{A}$ roost were least often correctly classified (13.64%± 0.146). While individuals recorded at the Chinook $_{D}$ roost were classified significantly more often than individuals form other roosts (37.27%± 0.739). Roosts are listed in order of relative size (largest to smallest).

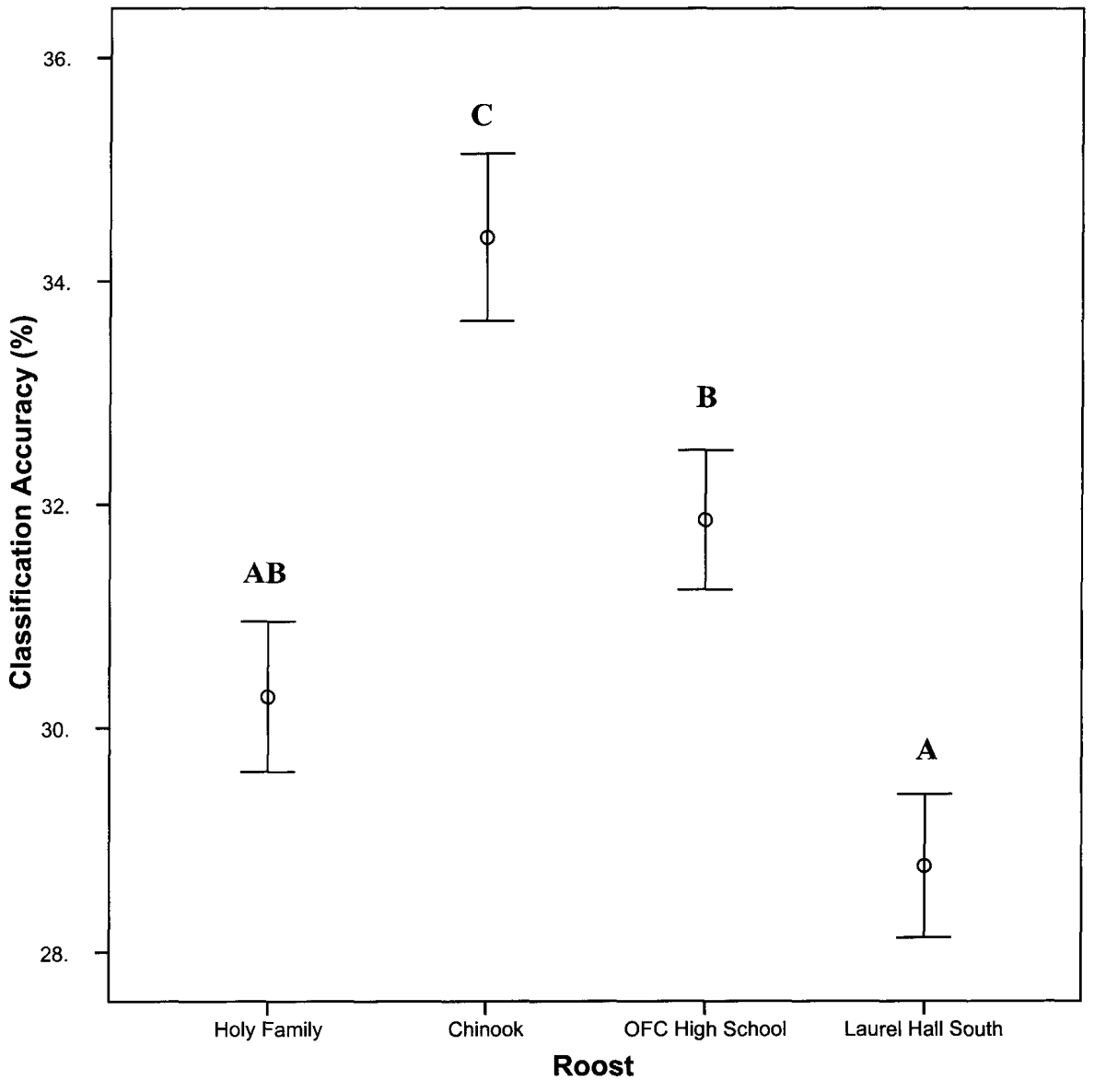

Figure 4. Mean± SE individual classification accuracies (obtained from 100 trials) varied significantly (df = 3,396, F = 12.77, P <0.001) among roosts when using the same number (n = 9) of individuals in each DFA. The Holy Family $_{AB}$ and Laurel Hall South $_{A}$ roosts statistically had the lowest individual classification accuracies (30.27% ± 0.674 and 28.76% ± 0.638 respectively). While, individuals from Chinook $_{C}$ roost were correctly classified significantly more often than individuals from other roosts (34.39%±0.746). Roosts are listed in order of relative size (largest to smallest).

Table 3. Individual DFA classification accuracies, when using a varying number of individuals and when using the same number of individuals for each colony, were significantly higher than the highest possible chance of randomly correctly classifying individuals. Random chance is calculated by dividing the largest number of observations belonging to the same group by the total number of observations. This prediction accounts for the probability of calls being randomly assigned to the correct grouping prior to the analysis.

Colony	Test	# of Individuals included in Analysis	Classification Mean±SE (%)	Random Chance of Correct Classification (%)	t	d.f.	P value
Holy Family	1	9	30 ±0.674	11	28.38	99	<0.001
	2	88	13 ±0.146	1	10.117	99	<0.001
Chinook	1	9	34 ±0.746	11	31.281	99	<0.001
	2	10	37 ±0.7388	11	35.109	99	<0.001
OFC High School	1	9	31 ±0.624	11	32.779	99	<0.001
	2	23	23 ±0.410	3	48.422	99	<0.001
Laurel Hall South	1	9	28 ±0.639	11	27.663	99	<0.001
	2	11	24 ±0.716	5	33.125	99	<0.001
Harmony	1	N/A	N/A	N/A	N/A	N/A	N/A
	2	8	27 ±0.671	14.0	13.347	99	<0.001

1- Individual DFA where the same number of individuals were used from each roost
2- Individual DFA where a varying number of individuals were used from each roost

Table 4. Individual DFA using the same number of individuals for each roost was significantly different from DFA classification accuracies calculated from the same roosts but using a larger number of individuals.

Colony	Test 1 Classification Mean ± SE (%)	Test 2 Classification Mean ± SE (%)	t	d.f.	P value
Holy Family	30 ±0.67	13 ±0.14	10.16	198	<0.001
Chinook	34 ±0.74	37 ±0.73	-2.193	198	0.029
OFC High School	31 ±0.62	23 ±0.41	11.496	198	<0.001
Laurel Hall South	28 ±0.63	23 ±0.71	2.206	198	0.029

1- Individual DFA where the same number of individuals were used from each roost
2- Individual DFA where a varying number of individuals were used from each roost

Chapter 4: DISCUSSION

4.0 Roost and Individual Signatures

Using the echolocation calls of wild E. *fuscus,* a DFA was able to identify roost membership. The roost DFA had an overall classification accuracy of 48%, but in some roosts classification accuracies were as high as 63% (Figure 2). Group signatures have previously been identified among roosts of *Myotis lucifugus* (Pearl and Fenton 1996). There are individual fitness benefits to maintaining group faithfulness in colonial living bats (Kunz 1982), including reduced thermoregulation costs at maternity colonies and information transfer pertaining to reliable foraging and hibernations sites (Altringham 1996, Pp 155). *E. fuscus* are faithful to maternity roosts (Brigham and Fenton 1986), and use a fission-fusion roosting pattern, utilising a number of smaller roosts but maintaining the same group membership among these sites (Willis and Brigham 2004). Roost signatures are important for maintaining group cohesion (e.g. *Phyllostomus hastatus,* Boughman 1997), and *E. fuscus* of Fort Collins could use roost vocal signatures in echolocation calls to maintain a stable group membership among the maternity roosts.

Masters et al. (1995) demonstrated a familial component to echolocation calls, demonstrating that the calls of mother and offspring are more similar in structure to one another than to an unrelated individual. However, there was no strong correlation between siblings, indicating that echolocation calls may have some plasticity and learning may play a role in the formation of their structure (Masters et al. 1995). This idea is supported by Boughman (1997), who suggested that group specific calls require a long-term association of individuals through roost fidelity. Lower classification accuracies

may be a result of roost fidelity. Many of the roosts that had lower classification accuracies were located near roosts where bats had recently been excluded or roosts that were not actively monitored. Anecdotal evidence suggests that immigration between roosts occurs when bats are excluded from their roosts. That is when PIT tagged bats were excluded from a monitored roost they began to appear at nearby roosts where they previously had not been observed. It is possible that the roots that were accessible by bats from other colonies had higher immigration rates than colonies that were isolated. This could have an effect on a colony vocal signature, as bats that have recently immigrated would not have the time to modify their echolocation calls to match those of their new roost mates.

The number of bats living in a given roost may cause another source of variation in roost signatures. The roosts included in this study housed approximately 30 to thousands of bats (See Figure 2 for order of roost size). Large roosts that housed many individuals, such as Harmony, had significantly lower roost classification accuracies than smaller roosts, such as County Road 40 (Figure 2). Lower roost classification may be a result of the roosting structure. *E. fuscus* are forest-living species that inhabit tree cavities (Kurta and Baker 1990). However, *E. fuscus* is a common urban bat, frequently roosting in artificial structures. Despite the differences in the physical structures of artificial and natural roosts, *E. fuscus* maintain similar roosting behaviour. It has been suggested that urban *E. fuscus* treat a building as if it were the forest, and independent roosting areas within the building as individual trees (Brigham, personal comm.). This could explain why the larger roosts had lower classification accuracies. It is possible that one large roosting structure is inhabited by more than one colony of *E. fuscus*. Although

the bats use the same point of emergence when exiting or entering a roost, in large buildings they many be roosting in smaller independent groups.

Despite evidence for roost signatures I found high variation among call variables that were most important at predicting roost membership (as indicated by CV values; Table 2). Obrist (1995) when identifying behavioural specific echolocation calls within a species observed similarly high CV values that suggested individual variation. Call variation among the Fort Collins *E. fuscus*, indicate that echolocation calls that contain roost signatures may additionally contain individual signatures.

Individual vocal signatures found in the echolocation clicks of Oilbirds (*Steatornis caripensis,* Steatornithidae) suggest that adult birds could be an artefact of natural morphological variation (Suthers and Hector 1988). Individual signatures, found mostly in the isolation calls of mother-offspring interactions, are present in the social calls of some species of colonial living bats (Brown et al. 1983, Balcombe 1990, van Parijs and Corkeron 2002, Pfalzer and Kusch 2003, Behr et al. 2006). Echolocation calls most likely evolved from social calls and retain sufficient variation to additionally carry unique information (Fenton 1984) including individual identity. A DFA of echolocation call features found some evidence for an individual component in the echolocation calls of wild *E. fuscus.* Individual classification accuracies varied among roosts, ranging from 14% to 37% (analyses using a varying number of individuals/roost) and 29% to 34% (analyses using the same number of individuals/roost). My results support this hypothesis, as do those of Masters et al. (1995), Burnett et al. (2001), and Kazial et al. (2001). However, although I obtained individual classification accuracies that were significantly higher than random (Table 3), indicating the possibility of unique

signatures, they were considerably lower than those found by Burnett et al. (2001) and Kazial et al. (2001). Differences in recording conditions are a probable explanation the differences in classification results. Burnett et al. (2001) and Kazial et al. (2001) determined the individual identities using trained bats whose echolocation calls were recorded in an anechoic chamber. Bats flying under laboratory-controlled conditions have significantly different echolocation calls (shorter call duration and higher frequencies) than those flying under natural conditions (Surlykke and Moss 2000). I was able to control for variation due to technical artefact by following similar recording protocol used by Pearl and Fenton (1996). I recorded echolocation calls only during periods of emergence, the microphone was placed at a consistent distance, height, and angle to the point of emergence, and only calls of similar signal-to-noise ratio were used, minimizing the effect of distance and direction of the bat relative to the microphone. However, I was unable to control for environmental conditions such as temperature, wind speed, or humidity that can result in the frequency variation of echolocation calls (Guillen et al. 2000). Additionally, the position of a bat relative to the microphone results in differences in the recorded echolocation call parameters, specifically call amplitude. Both varying environmental conditions and the position of a bat relative to the microphone will have an effect on a bat's echolocation call, and may make it difficult to identify slight changes in echolocation call structure that would help researchers confidently identify individual signatures.

Siemers and Kerth (2006) identified individual differences in the echolocation calls of wild *Myotis bechsteinii*, but were unable to obtain high classification accuracies or identify a robust call parameter that described individual identity. Interestingly,

classification of individuals was higher when only a single call sequence from each individual (opposed to using more than one sequence per individual) was included in the DFA, demonstrating that echolocation calls are uniform within a sequence, but differ considerably between sequences. My analyses also included more than one sequence per individual while attempting to discriminate among individuals and found similarly low levels of classification accuracies. Unlike Siemers and Kerth (2006), I included individual DFA sequences recorded from the same individual on different nights (as I strictly recorded emergence calls). The lower individual classification accuracies that I obtained were more likely due to differences in recording conditions (i.e. the afore-mentioned environmental conditions and bat to microphone position) between nights.

Echolocation calls are context-specific and are subject to variation depending on the flight situation (Obrist 1995, Surlykke and Moss 2000, Siemers and Kerth 2006). Individual variation may not be present in echolocation calls during emergence, as it is not necessary for individuals to identify their own echoes in that flight situation. Under low levels of illumination under laboratory conditions the California leaf-nosed bat (*Macrotus californicus*) relies on visual cues to locate prey (Bell 1985). The eyesight of many bats appears to be as good as that of *M. californicus* or that of small nocturnal mammals, and that eyesight may play a large role in orientation (Altringham 1996 Pp 107-108). As *E. fuscs* leave their roosts they may be flying in "auto-pilot" already familiar with their surroundings and using their vision to navigate. Bats may be using echolocation at the roost as they emerge as a "warm-up" and not for orientation, instead adjusting their calls to the present environmental conditions (Skowronski, personal comm.). It is likely that vocal signatures would exist in the other types of echolocation

calls (i.e. foraging calls). In foraging situations, *E. fuscus*, forages independently from one another but in the same habitat as conspecifics and sympatric species, where it becomes important for a bat to recognize its own returning echo.

4.1 Future Work

There are many potential sources of variation in the echolocation calls of wild *E. fuscus*, including roost fidelity and size. Using immigration and emigration data of individual bats among roosts I would like to create a fidelity index that may offer further insight into the differences in roost classification.

It is also interesting to note that there may be roost specific call variables or "accents". To ensure that DFA results were comparable to one another when identifying individuals the same call variables were used in each analysis. However, preliminary analysis indicates that important call variables for discrimination among individuals may be different for each roost location. For example, call duration and minimum frequency may be the most important call variables when identifying individuals at roost A, but at roost B frequency with most energy and maximum frequency are more important. Identification of the important call variables (or accents) at each roost would likely increase the success of correctly identifying wild *E. fuscus* individuals under natural flight conditions while still using our current recording systems and techniques.

4.2 Conclusion

1. I found evidence for roost and individual vocal signatures in the echolocation calls of wild *E. fuscus*.

2. There are several potential sources of variation in the echolocation calls of wild *E. fuscus* . This variation can result in the presence of more than one signature within the same echolocation call.

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APPENDIX 1: Location of study sites within the city limits of Fort Collins, Colorado, USA.

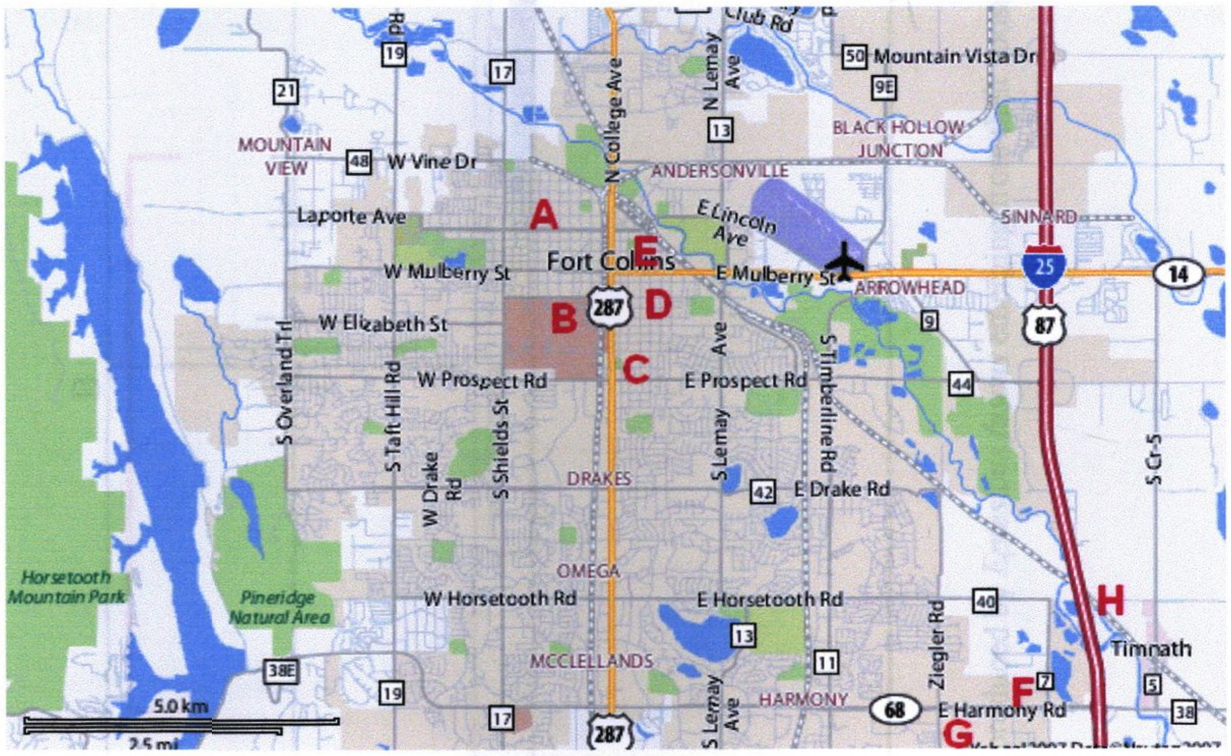

Figure A.1. Recordings of individual *E. fuscus* were made at 8 independent locations within the city limits of Fort Collins, CO (**A** = Holy Family, **B** = Laurel Hall South, **C**= OFC High School, **D** = 720 Peterson, **E** = East Laurel, **F** = Chinook, **G** = Harmony, **H** = County Road 40).

APPENDIX 2: Call sequence identification.

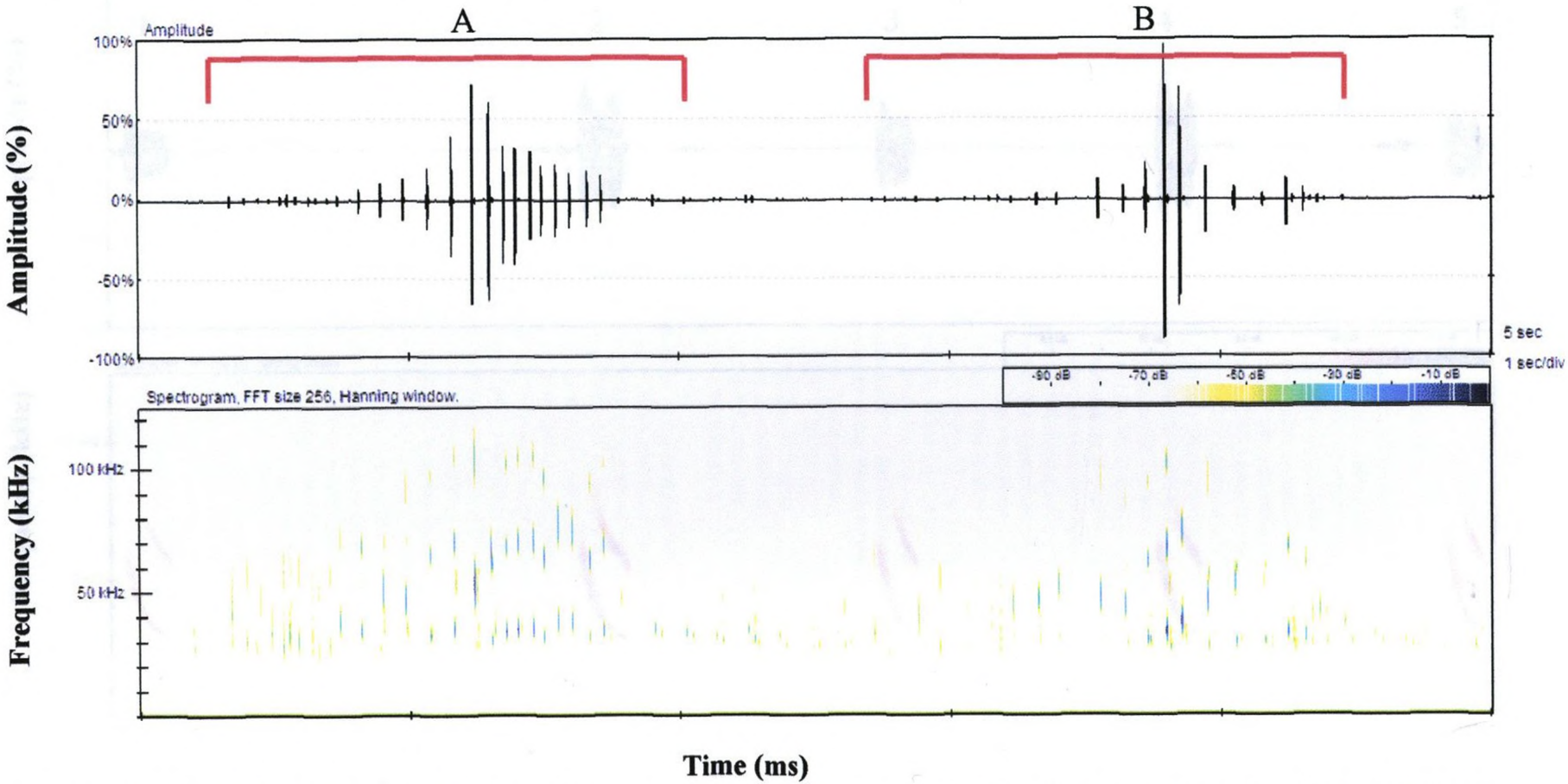

Figure A.2. Two independent echolocation call sequences (A and B) produced by *Eptesicus fuscus* emerging from their roost.

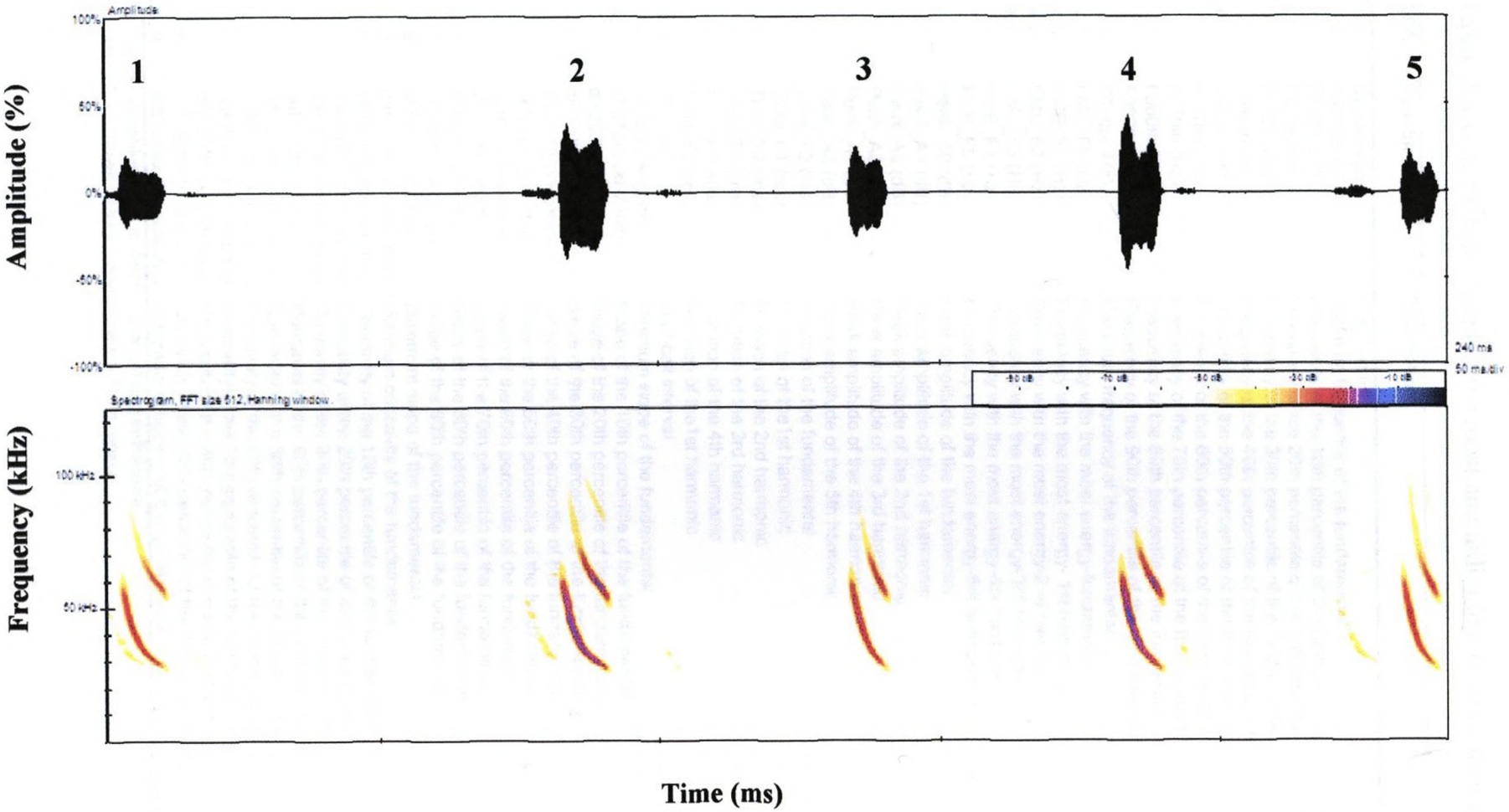

Figure A.3. An individual call sequence is made up of 5 consecutive calls recorded from an known *E. fuscus* individual.

APPENDIX 3: Call variables used in DFA.

Table A.1. Call variables used in the roost and individual discriminant function analyses.

DFA	Variable	Description
a, b	Duration (ms)	Call Duration
a, b	F0min (Hz)	Minimum frequency of the fundamental
	F010th (Hz)	Frequency of the 10th percentile of the fundamental
a	F020th (Hz)	Frequency of the 20th percentile of the fundamental
	F030th (Hz)	Frequency of the 30th percentile of the fundamental
	F040th (Hz)	Frequency of the 40th percentile of the fundamental
a	F050th (Hz)	Frequency of the 50th percentile of the fundamental
	F060th (Hz)	Frequency of the 60th percentile of the fundamental
	F070th (Hz)	Frequency of the 70th percentile of the fundamental
	F080th (Hz)	Frequency of the 80th percentile of the fundamental
	F090th (Hz)	Frequency of the 90th percentile of the fundamental
a, b	F0max (Hz)	Maximum frequency of the fundamental
a, b	FME_F0 (Hz)	Frequency with the most energy-fundamental
a, b	FME_F1 (Hz)	Frequency with the most energy- 1st harmonic
a, b	FME_F2 (Hz)	Frequency with the most energy-2nd harmonic
a, b	FME_F3 (Hz)	Frequency with the most energy-3rd harmonic
	FME_F4 (Hz)	Frequency with the most energy-4th harmonic
	FME_F5 (Hz)	Frequency with the most energy-5th harmonic
	Peak_A0 (dB)	Peak amplitude of the fundamental
	Peak_A1 (dB)	Peak amplitude of the 1st harmonic
	Peak_A2 (dB)	Peak amplitude of the 2nd harmonic
	Peak_A3 (dB)	Peak amplitude of the 3rd harmonic
	Peak_A4 (dB)	Peak amplitude of the 4th harmonic
	Peak_A5 (dB)	Peak amplitude of the 5th harmonic
b	Time_F0 (ms)	Duration of the fundamental
b	Time_F1 (ms)	Duration of the1st harmonic
a	Time_F2 (ms)	Duration of the 2nd harmonic
	Time_F3 (ms)	Duration of the 3rd harmonic
	Time_F4 (ms)	Duration of the 4th harmonic
	Time_F5 (ms)	Duration of the1st harmonic
a, b	ICI (ms)	Inter-call interval
a, b	dF0min (kHz/ms)	Minimum slope of the fundamental
a	dF010th (kHz/ms)	Slope of the 10th percentile of the fundamental
	dF020th (kHz/ms)	Slope of the 20th percentile of the fundamental
	dF030th (kHz/ms)	Slope of the 30th percentile of the fundamental
	dF040th (kHz/ms)	Slope of the 40th percentile of the fundamental
	dF050th (kHz/ms)	Slope of the 50th percentile of the fundamental
a	dF060th (kHz/ms)	Slope of the 60th percentile of the fundamental
	dF070th (kHz/ms)	Slope of the 70th percentile of the fundamental
a	dF080th (kHz/ms)	Slope of the 80th percentile of the fundamental
a	dF090th (kHz/ms)	Slope of the 90th percentile of the fundamental
a, b	dF0max (kHz/ms)	Maximum slope of the fundamental
a, b	ddF0min (kHz/ms/ms)	Minimum concavity of the fundamental
a, b	ddF010th (kHz/ms/ms)	Concavity of the 10th percentile of the fundamental
b	ddF020th (kHz/ms/ms)	Concavity of the 20th percentile of the fundamental
	ddF030th (kHz/ms/ms)	Concavity of the 30th percentile of the fundamental
	ddF040th (kHz/ms/ms)	Concavity of the 40th percentile of the fundamental
	ddF050th (kHz/ms/ms)	Concavity of the 50th percentile of the fundamental
a	ddF060th (kHz/ms/ms)	Concavity of the 60th percentile of the fundamental
	ddF070th (kHz/ms/ms)	Concavity of the 70th percentile of the fundamental
a	ddF080th (kHz/ms/ms)	Concavity of the 80th percentile of the fundamental
a, b	ddF090th (kHz/ms/ms)	Concavity of the 90th percentile of the fundamental
a, b	ddF0max (kHz/ms/ms)	Maximum concavity of the fundamental

a- the 24 variables used to discriminate among individuals

b - the 17 variables used to discriminate among roosts

APPENDIX 4: Recording dates.

Table A.2. Recording dates and number of individuals identified during the 2004 and 2005 field season.

Location	Total No. Individuals Identified	Date of Recording	Total No. Individuals Identified	Date of Recording
OFC High School	40	3-Jun-04	28	1-Jun-05
		4-Jun-04		6-Jun-05
		11-Jun-04		7-Jun-05
		14-Jun-04		8-Jun-05
		21-Jun-04		9-Jun-05
		22-Jun-04		10-Jun-05
		23-Jun-04		14-Jun-05
		13-Jul-04		
		14-Jul-04		
Laurel Hall South	10	8-Jun-04	15	15-May-05
		9-Jun-04		25-May-05
		10-Jun-04		27-May-05
		8-Jul-04		31-May-05
		9-Jul-04		21-Jun-05
		19-Jul-04		23-Jun-05
		20-Jul-04		24-Jun-05
				28-Jul-05
				29-Jul-05
Holy Family	42	24-Jun-04	107	18-May-05
		28-Jun-04		19-May-05
		29-Jun-04		20-May-05
		2-Jul-04		23-May-05
		15-Jul-04		24-May-05
		16-Jul-04		26-May-05
				18-Jul-05
				22-Jul-05
				27-Jul-05
				12-Aug-05
720 Peterson	0	30-Jun-04	N/A	
		1-Jul-04		
		7-Jul-04		
		27-Jul-04		
		28-Jul-04		
County Road 40	18	2-Jun-04	N/A	
		25-Jun-04		
		5-Jul-04		
		6-Jul-04		
		11-Jul-04		
		12-Jul-04		
		21-Jul-04		
Chinook	N/A		31	13-Jun-05
				15-Jun-05
				16-Jun-05
				17-Jun-05
				20-Jun-05
				12-Jul-05
				19-Jul-05
Harmony House	N/A		20	22-Jun-05
				27-Jun-05
				28-Jun-05
				29-Jun-05
1213 East Laurel	N/A		0	5-Jul-05
				6-Jul-05
				11-Jul-05

APPENDIX 5: Individual DFA axis significance and important call variables for analyses using varying number of individuals and the same number of individuals.

Table A.3.*E. fuscus* individual discriminant function analysis of using a varying number of individual echolocation calls recorded at Holy Family roosts. Including duration (DUR), maximum frequency of the fundamental (F0_max), frequencies of the 20th and 50th percentile of the fundamental (F020 and F050), length of the second harmonic (Time_F2), frequency with the most energy of the second harmonic (FME_F2), slope of the 10th and 80th percentile of the fundamental (dF010 and dF080). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F0_max	F020	F050	Time_F2	FME_F2	dF010	dF080	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	0.101	0.020	-0.490	1.260	-0.139	-0.153	0.741	0.163	1.257	15.5	0.002	5189.51	2088	< 0.001
Function 2	0.130	-0.226	0.461	0.332	0.519	0.522	-0.838	0.188	1.059	28.5	0.004	4440.59	1978	< 0.001
Function 3	1.453	-0.405	0.251	0.667	-0.528	-0.525	-0.286	-0.160	0.924	39.9	0.005	3865.36	1870	< 0.001
Function 4	0.618	1.262	0.726	-0.822	0.016	0.219	0.854	0.074	0.654	47.9	0.015	3343.36	1764	< 0.001
Function 5	-0.646	-0.498	0.909	-0.834	-0.673	-0.204	0.280	0.031	0.602	55.3	0.025	2942.19	1660	< 0.001
Function 6	0.587	0.612	0.229	-1.247	-0.200	0.341	1.048	0.041	0.489	61.3	0.040	2566.41	1558	< 0.001
Function 7	-0.908	-0.246	-0.349	0.025	1.235	0.943	0.239	-0.018	0.470	67.1	0.060	2248.89	1458	< 0.001
Function 8	1.644	0.163	0.349	-0.748	1.434	1.204	0.252	-0.437	0.321	71.1	0.087	1941.66	1360	< 0.001
Function 9	-0.233	0.011	0.213	-0.544	0.540	0.092	0.064	-0.706	0.302	74.8	0.116	1719.56	1264	< 0.001
Function 10	0.017	0.372	-0.334	-0.269	0.565	0.052	0.722	-0.372	0.267	78.1	0.151	1509.27	1170	< 0.001
Function 11	0.451	0.359	-0.640	0.932	-1.792	-1.317	0.121	-0.296	0.255	81.2	0.191	1320.69	1078	< 0.001
Function 12	0.828	0.006	0.907	-0.003	-1.146	-0.850	0.287	0.019	0.204	83.7	0.239	1139.67	988	0.001
Function 13	-1.650	0.126	-1.035	0.484	0.919	0.756	0.480	0.874	0.185	86.0	0.288	941.44	900	0.018
Function 14	0.325	-0.007	-0.190	0.195	0.268	0.415	0.119	0.016	0.176	88.2	0.342	855.98	814	0.149
Function 15	-0.338	0.527	0.864	-0.768	-0.136	-0.586	0.237	0.771	0.169	90.3	0.402	726.66	730	0.528
Function 16	0.307	0.338	0.163	0.526	-0.699	-0.410	0.416	-0.477	0.137	92.0	0.470	602.21	648	0.901
Function 17	0.195	0.059	-0.359	-0.262	-0.055	0.337	-0.394	0.059	0.123	93.5	0.534	499.77	568	0.982
Function 18	-0.775	0.051	-0.962	-0.306	1.264	1.026	0.077	-0.010	0.112	94.9	0.600	407.12	490	0.997
Function 19	0.167	0.131	-0.226	-0.111	0.006	-0.208	0.419	-0.196	0.109	96.2	0.667	322.84	414	1.000
Function 20	0.326	-0.218	0.425	0.595	-0.551	-0.322	0.115	0.216	0.082	97.2	0.740	240.47	340	1.000
Function 21	0.335	-0.037	-0.036	0.237	-0.449	-0.128	-0.050	-0.113	0.071	98.1	0.800	177.75	268	1.000
Function 22	-0.181	-0.170	-0.128	0.074	0.059	0.005	0.027	-0.325	0.064	98.9	0.857	123.44	198	1.000
Function 23	0.011	-0.051	-0.010	0.611	-0.637	-0.620	0.212	0.344	0.050	99.5	0.911	73.92	130	1.000
Function 24	-0.650	0.136	-0.063	0.067	0.426	0.402	-0.061	0.437	0.045	100.0	0.957	34.87	64	0.999

Table A.4. *E. fuscus* individual discriminant function analysis of data using a varying number of individual echolocation calls recorded at Chinook roosts. Including duration (DUR), frequency of the 50th percentile of the fundamental (F050), length of the second harmonic (Time_F2), slope of the 10th, 60th, 70th, and 80th percentile of the fundamental (dF010, dF060, dF070, and dF080). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F050	Time_F2	dF010	dF060	dF070	dF080	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	-2.952	-1.131	1.653	1.274	1.244	0.038	0.191	4.136	33.5	0.002	525.10	216	< 0.001
Function 2	0.066	-0.281	0.717	-0.276	-0.357	0.313	1.160	3.414	61.2	0.008	392.57	184	< 0.001
Function 3	1.882	0.453	0.071	-1.157	-0.256	0.181	-0.057	1.856	76.2	0.035	272.31	154	< 0.001
Function 4	0.252	-1.005	1.464	0.907	-0.747	1.268	-0.283	0.803	82.7	0.099	187.30	126	< 0.001
Function 5	-1.285	-1.064	1.080	-0.129	-0.558	0.283	0.617	0.683	88.2	0.179	139.56	100	0.006
Function 6	0.000	0.918	0.576	-0.812	1.187	0.012	-0.306	0.568	92.8	0.301	97.37	76	0.05
Function 7	0.794	0.122	-0.377	-0.189	1.081	0.444	-1.048	0.507	96.9	0.471	60.96	54	0.24
Function 8	0.063	0.529	0.292	-0.880	0.328	0.451	0.299	0.258	99.0	0.710	27.74	34	0.767
Function 9	-1.246	0.418	0.926	0.167	0.193	0.316	0.102	0.119	100.0	0.893	9.13	16	0.908

Table A.5.*E. fuscus* individual discriminant function analysis of data using a varying number of individual echolocation calls echolocation calls recorded at Laurel Hall South roosts. Including duration (DUR), maximum frequency of the fundamental (F0_max), frequency of the 20th and 50th percentile of the fundamental (F020 and F050), length of the second harmonic (Time_F2), frequency with the most energy in the second harmonic (FME_F2), slope of the 10th and 60th percentile of the fundamental (dF010 and dF060), and concavity of the 10th, 70th , and 90th percentile of the fundamental (ddF010, ddF070, and ddF090). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F0max	F020	F050	Time_F2	FME_F2	dF010	dF060	ddF010	ddF070	ddF090	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	-2.640	0.037	0.048	1.528	0.790	0.698	-0.062	1.075	0.495	0.246	-0.279	5.001	27.6	0.004	490.70	240	< 0.001
Function 2	-1.528	1.959	0.880	-2.500	1.904	1.284	2.427	-1.285	0.024	-0.676	1.106	3.857	47.5	0.014	382.06	207	< 0.001
Function 3	3.437	-0.414	0.631	0.848	-2.273	-1.917	0.778	-0.947	-1.022	1.386	-0.363	3.195	65.1	0.038	292.92	176	< 0.001
Function 4	-0.843	-0.406	-0.086	0.846	0.483	0.212	0.578	0.122	0.028	-0.573	0.176	1.762	75.2	0.095	210.65	147	< 0.001
Function 5	-2.342	0.192	-1.343	0.983	1.256	1.063	1.674	0.844	-0.233	0.868	0.278	1.197	84.2	0.177	154.83	120	0.018
Function 6	1.261	0.284	0.660	-0.315	-1.427	-0.897	-0.081	-0.717	-0.962	-0.460	0.591	0.884	90.7	0.313	103.82	95	0.252
Function 7	-1.867	0.069	-0.480	0.610	2.545	1.305	-0.264	0.957	0.057	0.817	-0.696	0.779	94.2	0.487	64.33	72	0.728
Function 8	-0.729	-0.415	0.439	-1.242	2.480	1.574	0.014	-1.304	0.015	0.008	0.153	0.513	97.4	0.635	40.67	51	0.849
Function 9	1.114	0.003	1.179	0.543	-0.455	-0.298	0.289	0.738	-0.529	0.725	1.133	0.254	99.0	0.807	19.24	32	0.963
Function 10	1.566	0.013	-0.095	0.410	-1.464	-0.834	-0.708	0.177	0.278	0.123	0.012	0.156	100.0	0.920	7.48	15	0.943

Table A.6. *E. fuscus* individual discriminant function analysis of data using a varying number of individual echolocation calls recorded at OFC High School roosts. Including duration (DUR), frequency of the 20th and 50th percentile of the fundamental (F020 and F050), slope of the 10th, 60th, and 80th percentile of the fundamental (dF010, dF060, and dF080). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F020	F050	dF010	dF060	dF080	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	-1.738	0.072	0.659	1.668	-0.436	0.946	4.683	32.3	0.000	751.749	484	< 0.001
Function 2	-1.679	-1.277	0.682	1.163	1.736	-1.626	2.189	47.4	0.001	596.241	441	< 0.001
Function 3	1.117	-1.786	1.987	0.478	-0.996	-0.587	1.758	59.5	0.004	492.433	400	0.001
Function 4	-1.070	2.284	0.142	0.488	1.492	0.439	1.173	67.6	0.011	401.648	361	0.069
Function 5	2.216	-1.402	1.592	-0.802	-1.206	0.376	0.953	74.1	0.024	332.158	324	0.365
Function 6	0.434	-1.440	1.366	0.642	0.833	-0.002	0.911	80.4	0.048	272.292	289	0.752
Function 7	-0.655	0.697	-2.055	0.418	-1.216	0.601	0.757	85.6	0.091	214.344	256	0.970
Function 8	-0.641	0.970	0.445	0.404	0.905	0.378	0.513	89.2	0.160	163.924	225	0.999
Function 9	0.600	1.981	-1.213	0.785	-0.728	0.777	0.383	91.8	0.242	126.842	196	1.000
Function 10	-0.396	0.351	-0.164	-0.153	-0.131	-0.212	0.272	93.7	0.335	97.844	169	1.000
Function 11	0.744	-0.149	-0.241	0.351	1.350	-1.254	0.246	95.4	0.426	76.295	144	1.000
Function 12	0.849	-0.860	1.993	-1.264	-0.175	1.239	0.192	96.7	0.531	56.623	121	1.000
Function 13	0.595	-0.620	1.976	0.158	0.771	0.011	0.180	97.9	0.633	40.923	100	1.000
Function 14	-0.572	-2.552	2.233	0.852	0.798	0.545	0.106	98.7	0.747	26.109	81	1.000
Function 15	0.117	0.656	-0.553	0.907	-0.888	0.705	0.068	99.1	0.826	17.088	64	1.000
Function 16	0.503	-0.864	0.027	-0.310	-0.259	-0.156	0.047	99.4	0.882	11.238	49	1.000
Function 17	-0.159	0.475	0.642	-0.322	0.349	0.388	0.037	99.7	0.923	7.143	36	1.000
Function 18	-0.194	1.540	-0.584	-0.130	0.160	-0.429	0.023	99.9	0.958	3.860	25	1.000
Function 19	0.131	1.561	-1.593	0.584	-0.735	-0.191	0.013	100.0	0.980	1.806	16	1.000
Function 20	0.062	0.003	-0.160	0.136	-0.438	0.416	0.006	100.0	0.993	0.626	9	1.000
Function 21	-0.077	1.142	-0.439	-0.182	0.048	-0.026	0.010	100.0	0.999	0.999	4	1.000
Function 22	0.047	-0.428	-0.084	-0.081	-0.978	0.544	0.000	100.0	1.000	0.962	1	1.000

Table A.7.*E. fuscus* individual discriminant function analysis of data using a varying number of individual echolocation calls recorded at Harmony roosts. Including duration (DUR), frequency of the 20th and 50th percentile of the fundamental (F020 and F050), slope of the 10th and 60th percentile of the fundamental (dF010 and dF060), and concavity of the 70thpercentile of the fundamental (ddF070). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F020	F050	dF010	dF060	ddF070	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	-0.550	-1.143	1.910	-0.198	2.649	1.195	4.136	42.1	0.010	284.660	168	< 0.001
Function 2	0.308	0.452	0.518	-0.787	0.852	-0.517	3.414	20.2	0.043	195.320	138	0.001
Function 3	1.168	-1.454	2.057	1.027	0.007	0.455	1.856	13.7	0.109	137.340	110	0.040
Function 4	-0.537	-0.485	2.310	1.573	1.533	0.671	0.803	8.4	0.224	92.870	84	0.238
Function 5	1.413	-0.102	0.446	0.804	0.381	0.889	0.683	6.1	0.368	62.040	60	0.403
Function 6	-1.393	0.190	0.085	0.721	0.720	0.542	0.568	5.7	0.538	38.410	38	0.451
Function 7	-0.604	-0.045	0.528	-0.452	-0.461	-0.318	0.507	3.8	0.772	16.010	18	0.592

Table A.8. *E. fuscus* individual discriminant function analysis of data using the same number of individual echolocation calls recorded at Holy Family roosts. Including duration (DUR), frequency of the 20th and 50th percentile of the fundamental (F020 and F050), length of the second harmonic (Time_F2), and slope of the 10th and 60th percentile of the fundamental (dF010 and dF060). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F020	F050	Time_F2	dF010	dF060	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	0.670	-2.305	2.006	0.447	0.684	-0.891	10.552	46.8	0.000	256.92	184	< 0.001
Function 2	2.198	-0.059	0.897	-1.306	-1.451	1.544	4.571	67.0	0.002	183.51	154	0.052
Function 3	-0.351	-1.031	-0.896	2.464	1.716	-0.935	2.970	80.2	0.012	131.98	126	0.340
Function 4	1.432	1.153	-0.394	-2.179	0.086	-0.226	1.768	88.2	0.049	90.62	100	0.738
Function 5	1.518	3.012	-0.459	-1.719	0.161	-0.087	1.071	92.8	0.135	60.07	76	0.910
Function 6	-0.092	0.917	0.225	1.973	-0.836	-0.803	0.842	96.5	0.280	38.23	54	0.949
Function 7	-1.455	-1.406	1.038	0.829	0.813	0.861	0.409	98.3	0.515	19.90	34	0.974
Function 8	0.536	0.537	-0.727	-0.66	0.292	-0.251	0.378	100.0	0.726	9.62	16	0.886

Table A.9. *E. fuscus* individual discriminant function analysis of data using the same number of individual echolocation calls recorded at Chinook roosts. Including duration (DUR), minimum frequency of the fundamental (F0min), frequency of the 20th and 50th percentile of the fundamental (F020 and F050), length of the second harmonic (Time_F2), and slope of the 10th and 60th percentile of the fundamental (dF010 and dF060). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F0min	F020	F050	Time_F2	FME_F2	dF010	dF060	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	-6.871	0.942	-1.184	-3.238	5.144	4.311	2.908	1.205	12.304	46.5	0.000	300.60	184	< 0.001
Function 2	1.452	-1.064	1.330	1.029	-0.643	-0.125	-0.49	-1.619	6.595	71.5	0.001	215.20	154	0.001
Function 3	0.765	-0.566	0.359	0.152	0.358	0.207	-0.254	-0.106	2.754	81.9	0.011	148.29	126	0.085
Function 4	0.044	0.457	-0.147	-1.136	0.968	0.667	1.479	-0.376	1.693	88.3	0.042	104.63	100	0.356
Function 5	2.899	2.002	0.234	0.039	-2.022	-1.199	-0.418	0.574	1.434	93.7	0.113	7.93	76	0.611
Function 6	-0.331	-0.977	-0.525	1.355	0.335	0.306	-0.218	1.311	0.798	96.7	0.275	42.59	54	0.869
Function 7	1.849	1.108	0.132	-0.826	-0.189	0.121	-0.626	-0.046	0.590	99.0	0.495	23.23	34	0.918
Function 8	0.135	0.196	0.249	-0.566	0.065	0.166	0.688	0.542	0.272	100.0	0.786	7.93	16	0.951

Table A.10.*E. fuscus* individual discriminant function analysis of data using the same number of individual echolocation calls recorded at Laurel Hall South roosts. Including duration (DUR), frequency of the 20th and 50th percentile of the fundamental (F020 and F050), length of the second harmonic (Time_F2), frequency with the most energy in the second harmonic (FME_F2), minimum slope of the fundamental (dF0min), slope of the 10th and 60th percentile of the fundamental (dF010 and dF060), and concavity of the 70th and 90th percentile of the fundamental (ddF070 and ddF090). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F020	F050	Time_F2	FME_F2	dF0_min	dF010	dF060	ddF070	ddF090	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	2.180	-1.912	4.206	-1.155	-1.137	1.881	0.715	0.102	0.632	1.083	12.456	52.7	0.000	266.46	184	< 0.001
Function 2	2.310	1.261	-0.109	-0.728	-0.297	-0.992	2.994	-1.901	3.680	-1.904	5.375	75.4	0.004	180.68	154	0.07
Function 3	-0.603	-1.942	3.274	-2.632	-1.680	-1.174	2.159	1.744	2.357	-0.846	2.803	87.3	0.027	119.55	126	0.645
Function 4	-0.593	0.989	-1.142	0.831	1.320	-0.634	0.611	-0.392	0.522	-0.510	0.943	91.3	0.102	75.47	100	0.968
Function 5	0.633	-0.714	0.567	0.106	0.450	1.418	-0.450	0.650	-1.713	3.097	0.813	94.7	0.197	53.55	76	0.976
Function 6	0.022	-0.119	0.115	-0.517	-0.406	0.838	0.193	0.826	0.300	0.459	0.600	97.3	0.358	33.91	54	0.985
Function 7	-2.198	-1.679	1.411	2.052	2.139	-0.156	-0.669	0.786	0.710	-0.816	0.369	98.8	0.573	18.41	34	0.987
Function 8	0.018	0.384	-1.896	1.635	0.837	1.156	1.645	-2.149	0.348	1.628	0.276	100.0	0.784	8.05	16	0.947

Table A.11. *E. fuscus* individual discriminant function analysis of data using the same number of individual echolocation calls recorded at OFC High School roosts. Including duration (DUR), minimum frequency of the fundamental (F0min), frequency of the 20^{th} and 50^{th} percentile of the fundamental (F020 and F050), frequency with the most energy in the second harmonic (FME_F2), slope of the 60^{th} and 80^{th} percentile of the fundamental (dF060 and dF080), minimum concavity of the fundamental (ddF0min), and concavity of the 30^{th} percentile of the fundamental (ddF030). Call variables most important in predicting successful group membership were identified using the absolute largest Standardized Canonical Discriminant Function Co-Efficients for significant functions.

Discriminant Function Axis	DUR	F0min	F020	F050	FME_F2	dF060	dF080	ddF0min	ddF030	Eigenvalue	Cummulative %	Wilk's λ	χ^2	d.f.	P value
Function 1	1.993	-1.753	-0.549	1.597	1.888	3.613	-2.318	-1.761	2.147	6.513	31.8	0.000	285.339	184	< 0.001
Function 2	2.703	1.032	-2.486	3.146	-0.031	-2.573	-0.438	-0.624	0.298	5.730	59.7	0.001	218.791	154	< 0.001
Function 3	-0.842	0.538	-3.500	2.055	1.292	-2.022	0.812	0.849	-1.042	2.712	72.9	0.009	155.957	126	0.036
Function 4	0.062	2.940	-6.050	1.212	-0.012	-2.114	1.261	-0.133	-1.261	2.191	83.6	0.033	112.675	100	0.182
Function 5	-0.214	0.579	-3.798	6.172	0.341	-0.245	3.662	-0.453	-0.632	1.646	91.7	0.105	74.383	76	0.531
Function 6	0.195	0.420	0.737	0.964	0.779	0.448	0.799	0.279	-0.563	0.973	96.4	0.278	42.268	54	0.876
Function 7	1.036	-1.025	2.935	-1.024	0.349	-1.922	0.907	-0.325	-0.085	0.574	99.2	0.548	19.843	34	0.975
Function 8	0.791	-0.761	-0.299	0.381	-0.075	-1.220	0.418	-0.200	-0.220	0.159	100.0	0.863	4.871	18	0.996