Geographic variation in the echolocation calls of Gould's Wattled Bat *Chalinolobus gouldii*

[1]Linda Reinhold, [2]Alexander Herr, [3]Lindy Lumsden, [4]Terry Reardon, [5]Chris Corben, [6]Brad Law, [7]Patrick Prevett, [8]Greg Ford, [9]Lawrie Conole, [10]Alex Kutt, [11]Damian Milne and [12]Glenn Hoye

[1]Queensland Parks and Wildlife Service, Zoology Laboratory, 80 Meiers Road, Indooroopilly, Queensland 4068.
[2]Johnstone Centre, Charles Sturt University, Panorama Avenue, Bathurst, New South Wales 2795.
[3]Arthur Rylah Institute, Department of Natural Resources and Environment, P.O. Box 137, Heidelberg, Victoria 3084.
[4]Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, South Australia 5000.
[5]P.O. Box 2323, Rohnert Park, California 94927-2323.
[6]Research Division, State Forests of New South Wales, Beecroft, New South Wales 2119.
[7]Centre for Environmental Management, University of Ballarat, P.O. Box 663, Ballarat, Victoria 3353.
[8]University of Southern Queensland, Toowoomba, Queensland 4350.
[9]3 Gezireh Street, Pascoe Vale South, Victoria 3044.
[10]Department of Zoology and Tropical Ecology, James Cook University, Townsville, Queensland 4811.
[11]Parks and Wildlife Commission of the Northern Territory, P.O. Box 496, Palmerston, Northern Territory 0831.
[12]Fly By Night Bat Surveys, P.O. Box 271, Belmont, New South Wales 2280.

ABSTRACT

To investigate potential geographic variation in the echolocation calls of Gould's Wattled Bat *Chalinolobus gouldii*, descriptive call parameters were examined from five regions throughout Australia. Analysis of these parameters revealed a number of significant differences between the regions, especially in variables related to frequency. However, discriminant function analysis revealed extensive overlap in call parameters for the different regions. It can be concluded that, although there are differences in some parameters between regions, calls are similar overall in the five geographic regions investigated, and that the slight geographic variation which does occur does not confound the identification of *C. gouldii*.

Key words: microchiroptera, echolocation calls, regional variation, *Chalinolobus gouldii.*

Introduction

For the identification of bats by their echolocation calls, it is generally recommended that reference calls of each species be collected from a number of locations to account for geographic variation (Corben 1996; de Oliveira 1998). We define geographic variation as the existence of a consistently greater difference in calls between regions than within regions.

This study investigates the issue of geographic variation in echolocation calls for a widespread and taxonomically stable Australian bat species, Gould's Wattled Bat Chalinolobus gouldii.

The taxonomic status of C. gouldii has been stable at the species level, and currently two subspecies are recognised. Tidemann (1986) found a geographic cline in morphological variables of C. gouldii from north to south, these subspecies are the northern C. g. venatoris and the southern C. g. gouldii (Dixon 1995). A distinctive feature of C. gouldii calls is that pulses alternate in frequency (Jones and Corben 1993; Herr et al. 1997), with the higher and lower sets of pulses having consistent differences in shape (pattern of frequency change over time) (Fig. 1). This feature, combined with a broad frequency of 30 kHz, normally distinguishes this species from all others.

The purpose of this study was to investigate the variation of C. gouldii calls collected from five regions of mainland Australia and to briefly discuss the implications of using reference calls from other regions for the identification of this species.

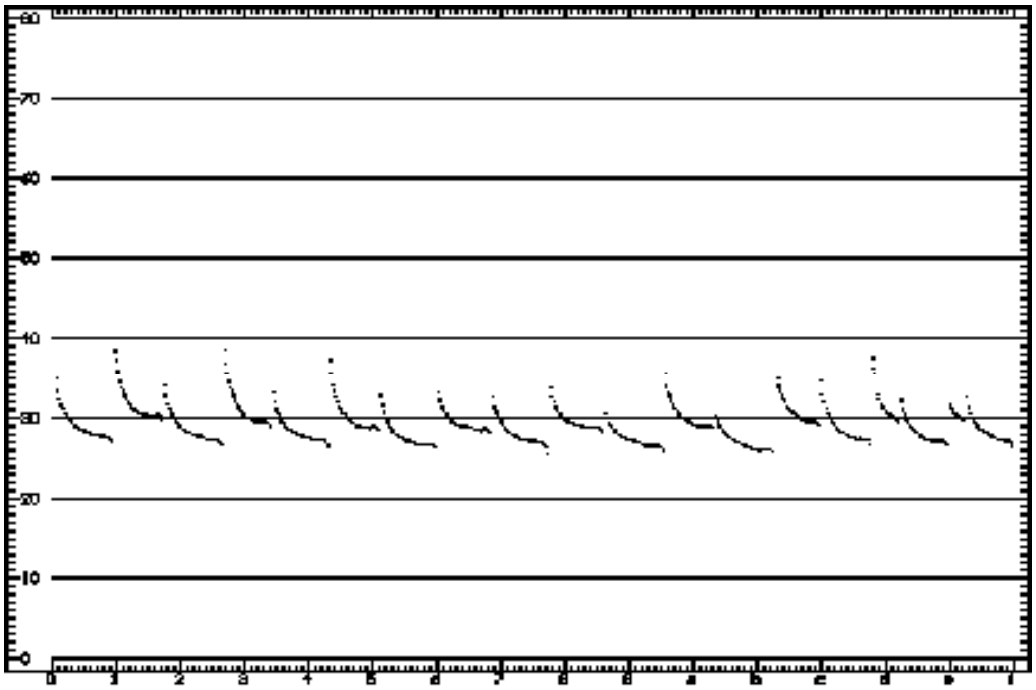

Figure 1. Commonly recorded *Chalinolobus gouldii* echolocation call showing frequency alteration. Absissa displays the time in sec, ordinate shows frequency in kHz. Display is in F7 compressed mode.

Methods

A total of 152 reference call sequences, collected from five geographical regions (Fig. 2) by the authors and other contributors, were statistically analysed. These regions comprised north-western Australia (Northern Territory and northern Western Australia); north-eastern Australia (northern and central Queensland); central eastern Australia (southern Queensland, north-eastern New South Wales and central eastern New South Wales); south-eastern Australia (southern New South Wales, Victoria and far south-eastern South Australia) and southern Australia (eastern South Australia and far western New South Wales).

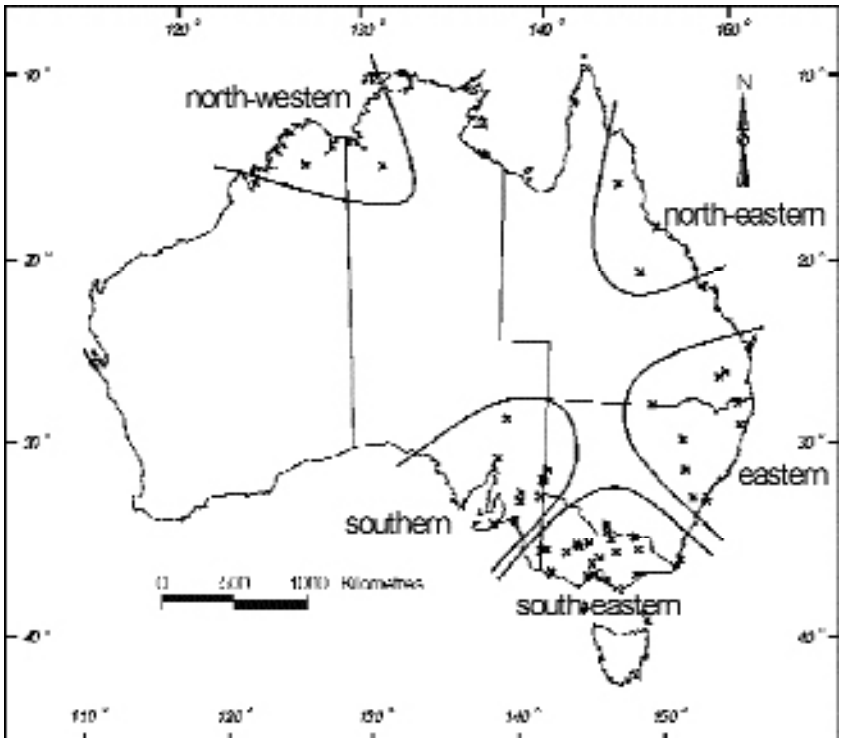

Figure 2. Geographic regions and collection sites of *Chalinolobus gouldii* reference calls.

The calls were collected using the Anabat system (Corben and O'Farrell 1999). The majority of calls were recorded directly into a computer. All calls used were reference calls (i.e. calls where the identity of the species is known by some method other than identification of the call itself). Release calls were used only where they displayed a regular pattern of search phase pulses, since these are most likely to correspond to calls recorded from free flying bats (e.g. Fenton and Bell 1981; Fullard et al. 1991; Corben 1996). The number of pulses included from each call sequence ranged from 6 to 77. The averages of 16 parameters (Table 1) were extracted from all included pulses of each call sequence. Frequency, time and slope parameters were extracted using Analook software (Corben 2000) which calculates the average values of all pulses displayed. The software does not include calculations of the pre-characteristic section, which we define as the linear section joining the knee (i.e. point of greatest change of slope) to the start of the characteristic section (i.e. the flattest part of the pulse). We define the heel as being the junction between the end of the pre-characteristic section and the start of the characteristic section. To extract measurements for the knee and the pre-characteristic section, the "modify bodies" option was used to manually select the linear section that joins the knee to the heel. To extract measurements for the heel and the characteristic section, the "modify bodies" option was used to manually select the flattest part of the pulse and parameters for the "knee" were applied to the "heel". Figure 3 displays these features.

Table 1. Variables extracted from each bat echolocation call using *Analook* software (Corben 2000).

Variable	Description
Initial slope (SINIT)	slope of the first two points at the start of a pulse;
Pre-characteristic slope (SPRE)	the slope of the linear section between the knee and the start of the characteristic section;
Characteristic slope (SCHAR)	slope of the flattest part of the pulse;
Maximum frequency (FMAX)	highest frequency of the pulse;
Frequency of the knee (FKNEE)	the frequency at the point of greatest change in slope;
Frequency of the heel (FHEEL)	the frequency at the start of the characteristic section;
Time at the knee (TKNEE)	time into the pulse at which the knee occurs;
Time at the heel (THEEL)	time into the pulse at which the heel occurs;
Average characteristic frequency (FCHAR)	frequency at the end of the flattest part of the pulse, averaged for all pulses;
Characteristic frequency, low (LOW)	as above, but for the lower set of alternating pulses only;
Characteristic frequency, high (HIGH)	as above, but for the higher set of alternating pulses only;
Time at the characteristic point (TCHAR)	time into the pulse at the characteristic point (which is the point at the end of the flattest part of the call);
End frequency (FEND)	frequency at the end of the pulse;
Duration (DUR)	total duration of pulse;
Interval (INTER)	the time from the start of one pulse to the start of the next;
Alternation (ALTER)	the difference in characteristic frequency between the higher and lower sets of pulses.

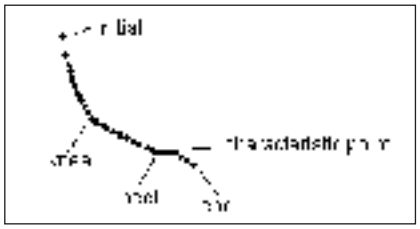

Figure 3. A typical *Chalinolobus gouldii* pulse, showing features used in measurements.

Statistical Analysis

The statistical analysis was undertaken using a two-fold approach. Firstly, ANOVAs were conducted on all 16 call parameters to compare differences between the five regions. Although the call parameters are related, results of a MANOVA could not be used because the data

were not multivariate normal, no homogeneity of variance-covariance matrices was achieved even after transformations and the design was unbalanced. Thus, univariate ANOVAs were applied and variables were transformed when necessary to achieve normality and homoscedasticity. The sensitivity for detecting differences using ANOVA for related dependent variables is less than when using a MANOVA. Thus, significance levels of 5% were deemed to be adequate to indicate differences between regions using Bonferroni posthoc tests.

Secondly, the call parameters were subjected to a principal component analysis (PCA) following z-transformations to reduce the number of variables to four uncorrelated components, which accounted for 87% of the variability in the data. A component was accepted if its eigenvalue was not less than one. These components were then subjected to a canonical discriminant analysis (CDA) using the five regions as a priori groupings. This was to determine the discriminative ability of the components over the different regions.

Results

A qualitative examination of calls revealed that the key identification feature of alternating pulses holds in call sequences examined from a wide range of localities.

The calls of C. gouldii showed significant differences for 8 of the 16 call parameters measured (Table 2). The average characteristic frequency of call sequences ranged from 26.8 kHz to 31.9 kHz. After separating the alternating pulses, characteristic frequency of the low pulses ranged from 25.7 kHz to 31.3 kHz, and the high pulses from 27.9 kHz to 34.1 kHz. The difference in characteristic frequency between alternating pulses ranged from 0.3 kHz to 3.3 kHz.

Differences between north-western Australia and the other regions accounted for most of the between-region variation in call parameters (Table 2). The north-western region had higher LOW, FEND, FCHAR and FHEEL, but lower ALTER, than the other regions. The Bonferroni test also identified the southern region as being different from the south-eastern region for SCHAR, TKNEE and ALTER, and from the eastern region for SCHAR.

Table 2. Means and standard deviations for the call parameters of *Chalinolobus gouldii* by geographic region. Significance values are calculated using univariate ANOVAs. Regional differences as identified by the Bonferroni posthoc test (5% level) are shown in the final column with ≠ representing regions that are significantly different from each other. n is the number of call sequences from each region.

Variable	1 north-west (n = 6)	2 north-east (n = 15)	3 central east (n = 29)	4 south-east (n = 77)	5 south (n = 25)	Pr>f	Regional differences
SINIT, OPS	229.4±142.1	261.5±129.6	312.4±101.3	308.1±103.4	295.7±123.8	0.288	nil
SPRE, OPS	33.0 ± 5.3	38.4 ± 7.3	41.8 ± 10.9	39.6 ± 4.9	41.8 ±8.3	0.046	nil*
SCHAR, OPS	6.4 ± 1.9	7.6 ± 3.2	11.0 ± 4.6	10.4 ± 3.6	7.7 ± 3.8	0.000	3≠5; 4≠5
FMAX, kHz	38.8 ± 4.9	38.7 ± 5.7	40.6 ± 4.3	40.7 ± 4.0	39.8 ± 5.2	0.470	nil
FKNEE, kHz	33.0 ± 0.8	31.9 ± 0.9	31.9 ± 1.1	31.7 ± 0.9	32.1 ± 1.4	0.037	1≠4
FHEEL, kHz	31.5 ± 0.6	30.0 ± 0.9	30.1 ± 1.0	30.1 ± 0.9	30.1 ± 1.2	0.020	1≠2; 1≠3; 1≠4; 1≠5
TKNEE, ms	1.6 ± 0.8	1.7 ± 0.7	2.0 ± 0.5	2.1 ± 0.5	1.7 ± 0.4	0.002	4≠5
THEEL, ms	3.8 ± 0.8	4.3 ± 0.6	4.3 ± 0.9	4.1 ± 0.6	3.8 ± 0.6	0.074	nil
FCHAR, kHz	31.1 ± 0.7	29.6 ± 0.8	29.5 ± 1.0	29.5 ± 1.0	29.7 ± 1.3	0.009	1≠2; 1≠3; 1≠4; 1≠5
LOW, kHz	30.6 ± 0.8	28.8 ± 0.8	28.8 ± 1.1	28.7 ± 1.0	29.0 ± 1.3	0.001	1≠2; 1≠3; 1≠4; 1≠5
HIGH, kHz	31.6 ± 0.9	30.7 ± 0.9	30.7 ± 1.1	30.7 ± 0.9	30.7 ± 1.5	0.303	nil
TCHAR, ms	6.5 ± 0.9	7.1 ± 0.9	7.0 ± 1.3	6.8 ± 1.1	6.7 ± 1.1	0.688	nil
FEND, kHz	31.1 ± 0.6	29.4 ± 0.9	29.1 ± 1.0	29.2 ± 1.0	29.5 ±1.4	0.001	1≠2; 1≠3; 1≠4; 1≠5
DUR, ms	6.8 ± 1.1	7.4 ± 1.1	7.4 ± 1.4	7.1 ± 1.1	7.0 ± 1.1	0.481	nil
INTER, ms	161.0 ± 48.4	148.0 ± 36.6	138.1 ± 53.6	135.8 ± 32.0	143.4 ± 38.6	0.482	nil
ALTER, kHz	1.1 ± 0.4	1.9 ± 0.6	1.8 ± 0.5	2.0 ± 0.5	1.6 ± 0.7	0.000	1≠2; 1≠3; 1≠4; 4≠5

*not significant, see results

The variables SPRE and THEEL did not comply with the homogeneity of variance assumption even after transformation. However, their significance levels for ANOVA differences between sites were 0.046 and 0.074 respectively. The differences in SPRE were deemed not to be significant since the critical F-value must be larger to be significant when non-compliance with the assumptions occurs (Coakes and Steed 1996).

The variables measuring the timing of pulses (e.g. DUR and INTER) and the initial parts of pulses were similar in all regions. The variables describing the slope and frequency of the characteristic section displayed highest differences (Table 2).

Four components accounting for 87% of the variability in the data were extracted by the PCA. Component one (explaining 42.4% variability) consisted of six frequency descriptors, namely FCHAR, FHEEL, FEND, HIGH, LOW and FKNEE. The second component (explaining 21.2% variability) was comprised of the four variables TKNEE, SINIT, FMAX, THEEL and the negatively related INTER. SCHAR contributed negatively to the third component (explaining 16.7% variability), which also included TCHAR and DUR. The fourth component (explaining 6.7% variability) consisted of only one variable, SPRE.

The discriminant function analysis using the four PCA components resulted in a 49% misclassification rate. The component scores do not separate clearly from each other, and the group centroids are in close proximity (Fig. 4). These factors indicate low power of the discriminant function to separate the five regions.

Discussion

Analysis of 16 call parameters suggests the existence of geographic variation, particularly between calls from the north-west and other areas, but the level of variation is not considered an impediment to call identification.

The Bonferroni posthoc test identified the calls from six pairs of regions as being distinctly different for at least one variable. In particular, the north-western group had greater variation between localities than within localities, therefore displaying geographic variation. However, the means of all parameters were

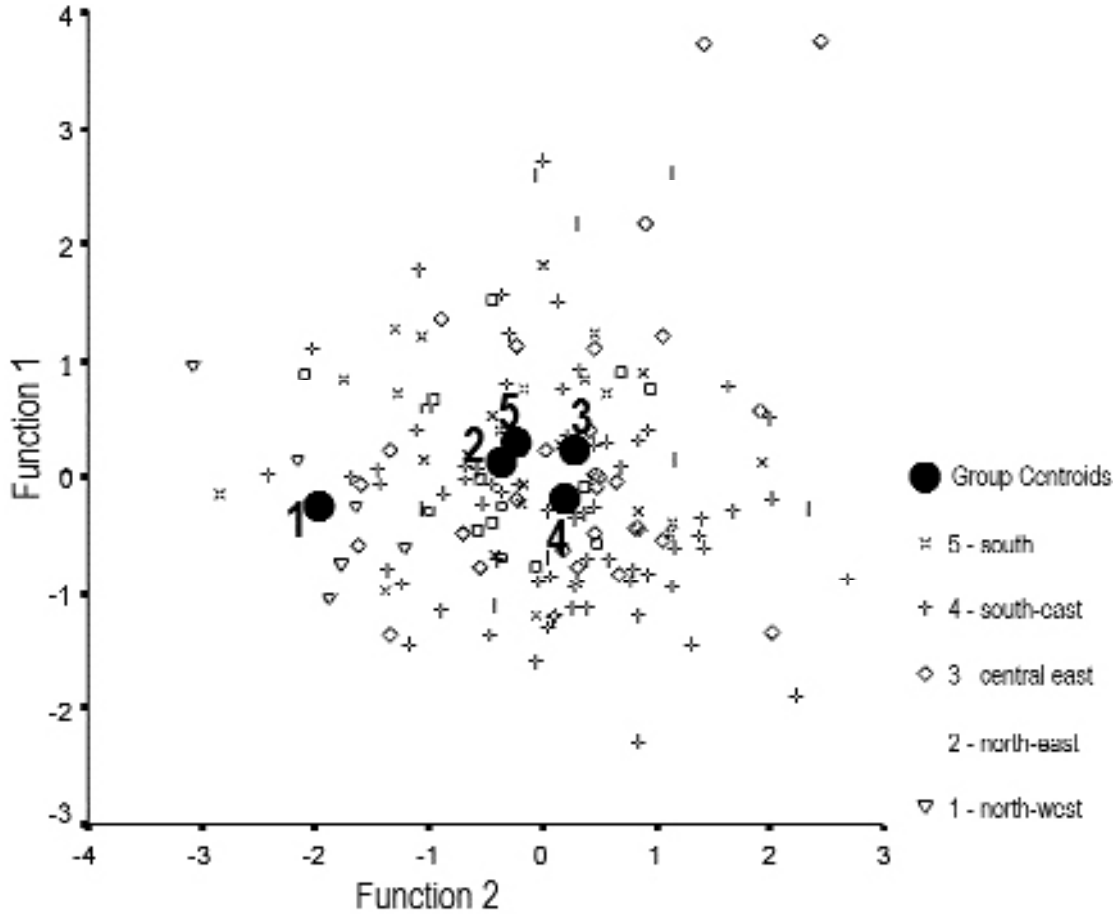

Figure 4. Group centroids (mean scores) and scores of the PCA components using the first two discriminant functions and the five regions as a *priori* groupings.

within one standard deviation between most of the regions and all regions overlapped in eight parameters (Table 2).

The multivariate analysis showed a high misclassification rate for calls from the five regions. However, both the north-western and north-eastern groups only had one misclassified call each. There was no separation pattern related to the five regions (Fig. 4). Separation was no clearer using the third function. The multivariate analysis was not very useful in detecting patterns of variation, perhaps as a result of uneven sample sizes. Patterns of variation in the groups with the largest sample sizes could have dominated the analysis and therefore skewed the output.

Woodside and Taylor (1985) reported a minimum pulse frequency of 35.7 kHz for C. gouldii, which is much higher than found in this study and other studies (Fullard et al. 1991: 29.6 kHz; Jones and Corben 1993: 29.7 kHz; Herr 1998: 30.7 kHz). This difference is likely to be a reflection of recording bat calls inside enclosures, where bats rarely emit the search phase calls typical of open habitats (Corben 1996). The bats recorded by Woodside and Taylor (1985) had not settled into search phase calls, which is apparent by the short pulse duration (1.3 ms versus 7 ms in this study).

C. gouldii calls are variable, with the characteristic frequency of pulses differing by several kHz. However, the identification of this species is aided by the frequency alternation of consecutive pulses (Figure 1).

The calls used in this study were recorded in a variety of surroundings. It is likely that the vegetation structure of the release areas in the various localities differed, resulting in different degrees of clutter. These differences in clutter may have added to the variability of some of the call parameters. Despite such additional variation, the calls from the regions overlapped considerably. It can be concluded that the identification of field recordings of C. gouldii is not confused by geographic variation within the species.

Other species occurring in the same area pose a greater problem with the echolocation call identification of C. gouldii. If a call sequence is brief or of poor quality, search phase calls of the Inland Broad-nosed Bat Scotorepens balstoni and the attack phase of freetail bats Mormopterus spp. can superficially resemble the calls of C. gouldii (Reinhold and Prevett 2001). However, a search phase call sequence of C. gouldii which is of sufficient length to show the regular alternations in pulse frequency is unlikely to be mistaken for any other species.

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