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INTERSPECIFIC VARIATION IN THE CALLS OF *SPHENISCUS* PENGUINS

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ABSTRACT.—We compared the vocal repertoires of Jackass (*Spheniscus demersus*), Humboldt (*S. humboldti*), and Magellanic (*S. magellanicus*) penguins. Discriminant and cluster analyses of the bray call indicate that Jackass and Magellanic penguins are more similar to each other than either is to the Humboldt penguin, and all three are distinct from the Rockhopper penguin (*Eudyptes chrysocome*). The congruence of the vocal analyses with phylogenies based on allozyme data suggests that differences in vocalizations reflect gradual divergence over time, not character displacement. *Received 1 Jan. 1995, accepted 20 Sept. 1995.*

Vocalizations frequently are used to assess taxonomic relationships in birds and the use of song in avian systematics has been thoroughly reviewed by Payne (1986). Vocalizations also have been used to determine phylogenies of non-passerine species such as the caledrine sandpipers (Miller et al. 1988). Jouventin (1982) found calls to be the best behavioral criterion for classifying penguins. The degree of variation in calls was used to infer subspecies and species status in island populations of nine penguin taxa. Vocalizations have been shown to be of primary importance in the communication of many penguin species (Pettingill 1960, Stonehouse 1960, Boersma 1974, Spurr 1975, Jouventin 1982). Although behavior has been studied in all of the *Spheniscus* penguins, only preliminary information exists concerning their vocalizations (Boersma 1974, 1976, Eggleton and Siegfried 1979, Jouventin 1982, Scolaro 1987).

Species status and phylogenetic relationships in the genus *Spheniscus* are not clearly defined. There is insufficient detail in the fossil record to distinguish among species (Simpson 1976). Morphological studies led Clancey (1966) to classify Jackass penguins (*S. demersus*) as a subspecies of Magellanic penguins (*S. magellanicus*). O'Hare (1989) used 22 morphological characters to clearly differentiate *Spheniscus* from other penguin genera. However, he was unable to determine the taxonomic relationships among species within the genus. Utilizing data from DNA-DNA hybridization, Sibley and Monroe (1990) proposed that *S. demersus* be viewed as a superspecies containing *demersus, magellanicus,* and the Humboldt penguin (*S. humboldti*). Recent allozyme analyses suggest that

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Humboldt penguins form a distinct species, but that Jackass and Magellanic penguins are closely related (Grant et al. 1994, Thumser and Karron 1994).

We quantitatively analyzed the vocalizations of three *Spheniscus* species (Jackass, Humboldt, and Magellanic penguins) and one outgroup (Rockhopper penguins, *Eudyptes chrysocome*). Their vocal repertoires were compared to determine if species consistently differ in the acoustical structure of their calls. A resulting phylogeny was compared to an independent phylogeny based on protein polymorphisms (Thumser and Karron 1994).

METHODS

Vocalizations of 21 Humboldt penguins were recorded at the Milwaukee County Zoo in Wisconsin (February 1986–May 1987, February–March 1988), the Brookfield Zoo in Chicago, Illinois (November 1987–April 1988), and the St. Louis Zoo in Missouri (October 1988). Recordings of 12 Jackass penguins were made at the Henry Villas Zoo in Madison, Wisconsin (January–April 1988), the Knoxville Zoo in Tennessee (May 1989), and the Racine Zoo in Wisconsin (February–March 1990). Seven Magellanic penguins were recorded at the Cincinnati Zoo in Ohio (April 1988) and by Jim Klinesteker at the John Ball Zoological Gardens in Grand Rapids, Michigan (Spring 1989). Eleven Rockhopper penguins were recorded at the Cincinnati Zoo (April 1988) and the St. Louis Zoo (October 1988).

This study was performed exclusively on captive penguins. Although the majority of recorded Jackass penguins were born in captivity, most of the Magellanic, Rockhopper, and Humboldt penguins were born in the wild. The birds comprising these captive populations may have been drawn from a limited number of wild populations. However, the results from this study should be representative since seabirds usually have limited variation in their vocalizations, particularly at or below the species level (Pierotti 1987).

Observations were made during breeding periods, mainly prior to and just after egg laying, since most of the calls occurred at those times. A microphone was placed inside the exhibit, but observations were made from outside the exhibit to minimize disturbance of the birds. Individuals were identified by tag color. General behavior was observed throughout the day (from 08:00 to 17:00 h CST). Notes were recorded on videotape (Hitachi HJ 5000) and by hand. Vocalizations were recorded throughout the period on a cassette recorder (Aiwa HSJ 500) using a Nakamichi (CM 100) microphone. Whenever possible the identity of the caller was noted. Peak periods of vocalization were simultaneously videotaped and tape-recorded.

The recorded calls were analyzed at 150 Hz bandwidth using a Kay 7800 Digital Sona-Graph and digitized using a Sigma Scan (1988) Program. For the bray call, the number of syllables per call, total duration of the call, sum of the inter-syllable intervals, duration of the longest syllable, and minimum, main, and maximum frequency of the longest syllable were recorded. The main frequency represented the darkest band in the sonagram of the call. These seven variables were selected to assess the acoustic structure of the bray call based on both frequency and temporal components. In addition, these parameters were selected because they could be measured precisely.

The vocalizations were analyzed using discriminant and cluster analysis in SYSTAT (Wilkinson 1990). The bray call was selected for analysis since nested ANOVA of individuals within populations within species indicated significant differences at the species level for more than one parameter (Thumser 1993). The data set included all recorded bray calls of the three *Spheniscus* penguins and the Rockhopper penguins. There were 109 calls from

TABLE 1

DISCRIMINANT FUNCTION 1 SHOWING THE CORRELATIONS BETWEEN CONDITIONAL DEPENDENT VARIABLES AND DEPENDENT CANONICAL FACTORS IN FOUR PENGUIN SPECIES

	Factor		
Variable	1	2	3
Number of syllables	−0.289	−0.710	−0.024
Total duration	0.337	−0.652	0.401
Inter-syllable interval	0.270	0.018	−0.345
Duration of longest syllable	0.622	−0.229	0.559
Maximum frequency	0.008	−0.635	−0.075
Minimum frequency	−0.145	0.464	0.083
Main frequency	−0.292	0.257	0.544
Chi-square	496.75	213.17	39.61
df	21	12	5
P	<0.001	<0.001	<0.001
Correlation	0.764	0.644	0.339

Humboldt, 77 calls from Jackass, 38 calls from Magellanic, and 106 calls from Rockhopper penguins. Each of the bray call variables was standardized by converting its values to *z*-scores prior to analysis. In discriminate analysis known groups were used to generate linear models which gave the best fit for that grouping. The data were also analyzed to determine how well the model predicts the actual groupings. Another multivariate technique, cluster analysis, was used to detect natural groupings in data with no prior expectations. In this case, Pearson's distance measures and the single-linkage method were performed by calculating the mean of each of the standardized variables for each species.

RESULTS

For the first discriminant function, Factor 1 arranged the four species primarily on the basis of the duration of longest syllable and the total duration of the call, while Factor 2 was primarily based on the number of syllables, the total duration of the call, and the maximum frequency of the longest syllable (Table 1). Overall, the analysis correctly categorized 86% of Humboldt, 82% of Jackass, 52% of Magellanic, and 79% of Rockhopper penguin calls. These vocal parameters clearly separated the *Spheniscus* penguins from the outgroup, Rockhopper penguins (Fig. 1A). Therefore, the outgroup was removed from the analysis and a second discriminant analysis was run to increase the spread among the *Spheniscus* penguins. In this discriminant function, Factor A arranged the three species primarily on the basis of syllable number and maximum frequency of the longest syllable, and Factor B was based primarily on the duration and main frequency of the longest syllable (Table 2). The analysis correctly predicted 91% of Humboldt, 71% of Jackass, and 61% of Magellanic penguin calls. Within the *Spheniscus* penguins, there was consid-

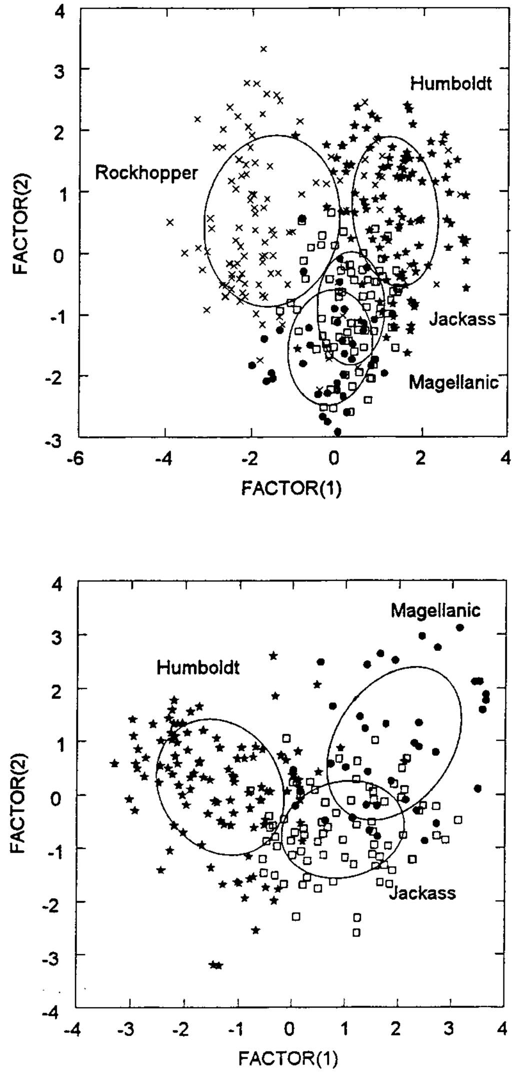

FIG. 1. (A) A scatterplot of the similarity between the bray calls of three *Speniscus* species (Jackass, Magellanic, and Humboldt) and an outgroup (Rockhopper penguins). (B) A scatterplot of the similarity between the bray calls of the three *Spheniscus* species. Both scatterplots have ellipses around 50% of the data points for Humboldt (dark star), Jackass (open square), Magellanic (dark circle), and Rockhopper (cross) penguins.

TABLE 2

DISCRIMINANT FUNCTION 2 SHOWING THE CORRELATIONS BETWEEN CONDITIONAL DEPENDENT VARIABLES AND DEPENDENT CANONICAL FACTORS IN THREE *SPHENISCUS* SPECIES

Variable	Factor A	Factor B
Number of syllables	0.711	−0.020
Total duration	0.311	0.319
Inter-syllable interval	−0.136	−0.274
Duration of longest syllable	−0.225	0.735
Maximum frequency	0.441	−0.065
Minimum frequency	−0.257	0.074
Main frequency	−0.041	0.423
Chi-square	287.22	60.23
df	14	6
P	<0.001	<0.001
Correlation	0.804	0.491

erable overlap in the vocalizations of Magellanic and Jackass penguins (Fig. 1B). In fact, 37% of the Magellanic penguin calls were incorrectly classified as Jackass penguin calls, and 12% of Jackass penguin calls were incorrectly classified as Magellanic penguin calls. By contrast, there was less similarity in the bray call parameters of Humboldt and Jackass penguins (Fig. 1B). Only 6% of the Humboldt penguin calls were incorrectly classified as Jackass penguin calls and 17% of the Jackass penguin calls were incorrectly classified as Humboldt penguin calls. Humboldt and Magellanic penguins were the least similar in their calls (Fig. 1B). In both species 3% of their calls were incorrectly classified as the other species. These results were supported by the cluster analysis shown in Table 3 and Figure 2. The distance matrix and tree show the Magellanic and Jackass penguins clustering closely together, the Humboldt penguins more distant, and the Rockhopper penguins the most distant.

TABLE 3

PEARSON'S DISTANCE MATRIX SHOWING THE DISTANCE BETWEEN FOUR PENGUIN SPECIES BASED ON SEVEN VARIABLES OF THE BRAY CALL

Species	1	2	3
1 Humboldt Penguin			
2 Jackass Penguin	1.304		
3 Magellanic Penguin	1.436	0.260	
4 Rockhopper Penguin	1.462	1.664	1.509

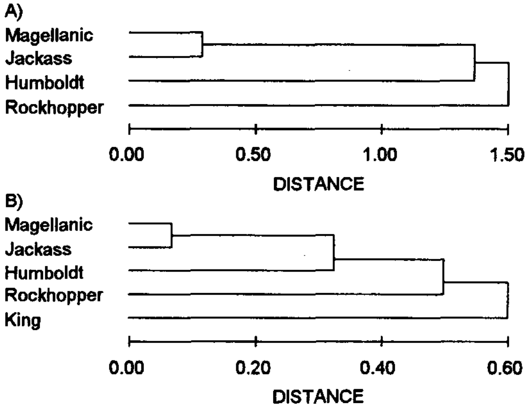

FIG. 2. (A) A tree using the single-linkage method based on Pearson's distances of parameters of the bray call. (B) UPGMA tree based on modified Rogers distance of allozyme data (Thumser and Karron 1994).

DISCUSSION

Overall, the *Spheniscus* penguins have retained a complement of calls that are similar in structure and function (Thumser 1993). The bray call is used to establish a territory and to advertise availability for pairing. The bird stands with its head pointing up and calls while slowly flapping its wings. This was the only call which showed sufficient species-level variation for phenetic analysis.

The analyses of selected vocal parameters of the bray call clearly distinguish Humboldt from both Jackass and Magellanic penguins. However, discriminant and cluster analyses often could not distinguish between the Magellanic and Jackass penguin calls. This may reflect the evolutionary relationships among the species or may have resulted from other factors. Since Humboldt and Magellanic penguins occur sympatrically in South America, another possible explanation for the differences in their breeding calls is character displacement. By contrast, the similarity of South American Magellanic and African Jackass penguin calls is unlikely to result from convergence.

In order to determine whether character displacement has occurred it is necessary to know which vocal characters are ancestral. It is difficult to root a tree based on vocalizations and determine the most ancestral species because vocalizations can be subject to strong selection. However, a comparison of allozyme variation enhances these results because protein markers are subject to weaker and different selective forces than those influencing behavioral traits. Trees based on allelic characters are also more easily rooted using outgroup taxa.

The allozyme data were consistent in two different studies (Grant et al. 1994, Thumser and Karron 1994). Grant et al. (1994) based their analysis on 15 polymorphic loci in 75 captive (Humboldt, Magellanic) and wild (Jackass, Rockhopper, and Macaroni [*Eudyptes chrysolophus*]) penguins. Thumser and Karron (1994) studied nine polymorphic loci in 165 captive (Jackass, Humboldt, Rockhopper, King [*Aptenodytes patagonicus*]) and wild (Humboldt, Magellanic) penguins. In both studies, Jackass and Magellanic penguins were very closely related and Humboldt penguins clearly formed a distinct species. There is a striking similarity of phenetic trees based on the allozyme data and the vocal analysis presented here (Fig. 2). Cladistic analysis of the allozyme data confirmed that *Spheniscus* penguins form a monophyletic group (Grant et al. 1994, Thumser and Karron 1994). These findings suggest that differences in vocalizations between the Humboldt and Magellanic penguins are not due to character displacement, but rather reflect gradual genetic divergence of separate evolutionary lineages. Although the Humboldt and Magellanic penguins occur sympatrically, they have lower genetic identities and greater vocal differences than the more closely related Jackass and Magellanic penguins.

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