INTRODUCTION

The echolocation calls of free-flying microchiropteran bats have proven to be extremely useful for studying the ecology and behavior of these otherwise elusive animals. For decades, systems that detect echolocation calls have been used for various purposes, some of which require assigning calls to particular species or species groups. Bat detectors have been used to address both basic and applied issues, including questions about the relative abundance of different species in an area, the diversity of species and how diversity varies geographically, the ecological or morphological structure of bat communities, the presence of rare or endangered species, the use or selection of particular foraging habitats by different species, and the determination of critical habitats. There is also the potential for identifying cryptic species by their echolocation calls (e.g., Jones

GEOGRAPHIC VARIATION IN THE ECHOLOCATION CALLS OF BATS: A COMPLICATION FOR IDENTIFYING SPECIES BY THEIR CALLS

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Variation in the echolocation calls of bats in different populations of the same species (i.e. geographic variation) may complicate attempts to use echolocation to identify free-flying bats to species. Such variation occurs in several species. Variation in echolocation calls is correlated with variation in body size and is also associated with differences in habitat type on a local level. Factors such as environmental conditions (e.g., relative humidity), prey types (e.g., size and defense mechanisms), foraging behavior (e.g., gleaning versus aerial hawking), and the presence of other bats may also translate into variation at larger geographic scales. We suggest ways to account for potential call variation when using a reference library to identify bats to species.

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and van Parijs 1993).

Early studies using bat detectors indicated that different species of bats use different echolocation calls, thus suggesting that free-flying individuals could be identified on the basis of their calls (Ahlén 1981; Fenton and Bell 1981). However, one problem in using echolocation calls to identify species of bats is that the characteristics of the calls of each species (e.g., duration, bandwidth, frequency, harmonic structure) vary. To address questions requiring species identification, researchers rely on the fact that variation in call structure is generally greater among species than within species. Within many bat communities, the calls of some species are distinctive (Fig. 1A), and even though there is variation within each species, calls can be assigned unambiguously to particular species. In most cases, however, the call characteristics of various species overlap, because on average the calls of different species are very similar to one another, or because there is greater variation in the calls of each species (Fig. 1B). Assigning calls to a particular species is less certain under these circumstances. Calls that fall within the zone of overlap (Fig. 1B) may be assigned to a species with a probability less than one, with the probability increasing the closer the combination of traits is to the mean value for that species. By definition, there is error involved in making such classifications. Often, multivariate statistical techniques such as discriminant function analysis (DFA) or neural networks are used to determine the probability of assignment (e.g., Jones et al. 2000; Parsons and Jones 2000; Vaughan et al. 1997).

Variation in the echolocation-call characteristics of a particular species of bat clearly increases the uncertainty of identifying calls of unknown, free-flying individuals to that species. Such variation occurs for a number of reasons, including variation within a population related to characteristics of the individual, such as age, gender, and body mass (Betts 1998; Brigham et al. 1989; Jones et al. 1992; Jones and Ransome 1993; Obrist 1995). Some of these factors may cause variation in calls from different locations (e.g., body mass), while others will not (e.g., age and gender), unless population structure varies (e.g., Barclay 1991). The purpose of this review is to consider geographic variation, especially among populations within a species, and to make recommendations as to how to account for it when conducting studies that depend on identifying bats by their echolocation calls.

By geographic variation we mean variation in the structure of echolocation calls of individuals of the same species recorded in different locations. It is a potential problem if call characteristics from one population are used to identify the calls of unknown individuals from another population (Law et al. 2002). Most studies rely on a set of calls from known individuals (a reference library) to establish the characteristics used to identify unknown bats recorded by the monitoring system.

Suppose there are two species in our study area and we base species identification on the call characteristics determined from another location (Fig. 2). Suppose further that the characteristics of the calls of the two species are distinct, but call characteristics from individuals in our study populations differ from those in the reference library. Such geographic variation could result in the inability to identify some calls and misidentification of others, depending on the amount of geographic variation (Fig. 2).

While geographic variation is most commonly thought of on a large scale, an analogy being the dialects of humans and birds, there is relatively little information regarding this for the echolocation calls of bats. A large part of the problem is a lack of consistency in recording techniques and equipment among studies. There are, however, considerable data regarding smaller-scale geographic variation and variation caused by other factors that may result in large-scale geographic differences. We thus examine these sorts of variation, make predictions

![Figure 1: Variation in two characteristics of echolocation calls within and between two species of bats. A: Variation within each species, depicted as the shaded circles, is relatively small and calls can be unambiguously assigned to one species or the other. B: The mean values for the two call variables are the same as in A, for each species, but there is greater within-species variation. This means that there is overlap among the calls of the two species. Calls with characteristics in the zone of overlap cannot be assigned to a particular species with 100% certainty.](image)

![Figure 2: Potential problems associated with geographic variation in the characteristics of the echolocation calls of two species of bats. Characteristics from the reference library for each species (shaded circles) are distinctive, but differ from the characteristics of the calls from the study populations (open circles). Because of this, some calls will be unidentifiable (they do not fall within either reference set), and others will be misidentified (they fall within the reference set of the other species).](image)
about how they may translate to larger geographic scales, and examine the evidence that such large scale, interpopulation variation exists.

**Small-Scale Variation in Echolocation Calls**

Insectivorous bats vary the design of their echolocation calls depending on the task they are performing and the type of information they require. Calls change in structure as a bat shifts from searching for prey to approaching and attacking it (e.g., Kalko 1995; Kalko and Schnitzler 1993). Bats also vary call design depending on the nature of the habitat (but see Pavey et al. 2001 for a situation where variation was not present). Calls from a particular species are often higher in frequency, broader in frequency sweep, and shorter in duration the more cluttered the habitat is, the smaller the gap in a forest is, or in general the closer the bat is to vegetation (e.g., Barclay et al. 1999; Kalko and Schnitzler 1993; Obrist 1995; Schnitzler and Kalko 2001; Siemers and Schnitzler 2000; Figs. 3 and 4). This has been attributed to the need for bats to avoid overlap between the outgoing echolocation pulse and the returning echo (Kalko and Schnitzler 1993), and therefore, the need to use shorter calls when bats are closer to targets. In addition, in more open habitats, longer, lower-frequency calls are more efficient for longer-range prey detection (Barclay and Brigham 1991).

The height at which a bat is flying also influences the design of its echolocation calls. For several species, individuals flying low nearer the ground produce shorter, higher-frequency calls, perhaps for similar reasons to the correlations noted above (Jensen and Miller 1999; Zbinden 1989; Fig. 5).

Thus, at a local level, bats foraging in different locations may differ in the echolocation calls they emit, thereby generating variation in call characteristics in a population. Such variation presumably occurs for the same reasons between more distant locations if the foraging habitats differ, or if individuals forage at different heights. This might occur between populations if the species occupies a range of habitat types (e.g., forests and grasslands), or if prey distributions differ in different locations and the optimal foraging habitat for bats thus varies.

**Other Factors Influencing Echolocation Call Variation**

Echolocation calls vary among species of insectivorous bats in relation to body size (e.g., Barclay and Brigham 1991; Heller and von Helfersen 1989). This may be for purely physical reasons (large vocal chords can not produce high frequency sounds), or for adaptive reasons associated with prey size and detection (e.g., Barclay and Brigham 1991; Guillén et al. 2000). Within a species, a similar correlation between echolocation call characteristics and body size occurs (Barclay et al. 1999; Guillén et al. 2000; Fig. 4). In *Miniopterus schreibersii*, individuals with longer wings have lower frequency calls (Jacobs 1999). Body condition (e.g., body mass relative to forearm length) also appears to influence call characteristics in some species (Guillén et al. 2000; Jones et al. 1994), but not others (Russo et al. 2001). If body size or condition varies geographically, as occurs in some species (Parsons 1997; Patriquin 2001), then call design may also vary geographically, for example with latitude, although to our knowledge no explicit data exist regarding this.

Environmental factors also influence the echolocation calls of bats. For example, high relative humidity attenuates high-frequency sound more than low frequency sound (Griffin 1971), and there may thus be an advantage to producing lower-frequency calls in areas of high humidity. Indeed, such a correlation exists for at least one species (Guillén et al. 2000).

A bat's foraging style and type of prey may also influence the echolocation calls it uses. Some species are able to glean insects from vegetation or the ground, as well as capture insects in the air (e.g., *Myotis oxytos*; Faure and Barclay 1994). In many gleaning species, prey detection is accomplished using sounds produced by the prey, and/or visual cues, in addition to echolocation. Gleaning also involves flying in closer proximity to clutter than...
does aerial hawking. It is thus not surprising that species that use both foraging styles vary their calls depending on their foraging behavior (Faure and Barclay 1994). *Myotis evotis*, for example, uses quieter calls and does not produce a feeding buzz when gleaning. If conspecifics in different locations forage in different ways, perhaps because prey resources differ, for example, then the echolocation calls they use may also differ.

Prey characteristics may affect the type of echolocation calls used by bats (Leippert et al. 2002). Some prey, such as some moths, can detect the echolocation calls of bats, and this defense may have favored the evolution of specific echolocation features such as high or low frequency, low intensity, or short duration (Fenton and Fullard 1979). Characteristics of different prey communities within the geographic range of a bat species may thus have resulted in geographic variation in echolocation calls. Because the frequency of a sound and its corresponding wavelength also influence the strength of the returning echo from targets of different size (Hartley 1989), the size of prey available in an area could also potentially favor certain echolocation call designs. Specifically, the need to detect smaller prey should favor the use of higher-frequency calls. If so, and prey size varies over a bat species' range, echolocation calls might also vary geographically.

Finally, the presence of conspecifics and other species may influence the type of echolocation calls individuals use. Individuals of several species modify their calls in the presence of conspecifics (Obrist 1995). At a minimum, this will increase the amount of variation in call characteristics in a particular location and thus make species identification more difficult.

**Geographic Variation at a Larger Scale**

Although few studies have specifically assessed geographic variation in bat echolocation, there is evidence that the echolocation call characteristics of some species of bats do vary geographically across the species' ranges. Among various rhinolophid and hipposiderid bats that use constant frequency (CF) echolocation calls, considerable geographical variation occurs in the CF frequency (e.g., Francis and H. abersetzer 1998; Guillén et al. 2000; Heller and von H. elversen 1989; Figs. 6 and 7). Variation in body size does not provide a satisfactory explanation in many of these cases. However, among the subspecies of hoary bat (*Lasiurus cinereus*), body size does correlate with significant geographic variation in echolocation call characteristics (Barclay et al. 1999; Fig. 4). There is also geographic variation in call structure in both species of New Zealand bats, and this variation may be related to body size or subspecies differences, although data are limited (Parsons 1997). In other cases, geographic variation in call structure has been documented in various species, but no functional explanation has been determined (Muray et al. 2001; Thomas et al. 1987).

**Consequences of Geographic Variation and Recommendations**

Despite limited study, there is evidence that the echolocation calls of a number of species vary geographically and there are both physical and adaptive reasons to expect such variation. Although in some cases, geographic variation may not be great enough to cause
problems in identification (Murray et al. 2001), we believe that studies using echolocation calls to identify individuals to species need to take geographic variation into consideration. If the reference calls used to identify unknown calls to species come from locations other than the study area, there is the potential for a mismatch between the reference calls and those actually used by the same species in the study area (Fig. 2). If so, at best the ability to accurately identify calls to species will be reduced. The number of unidentifiable calls may increase because they fall outside the set of values determined from the reference library. Assigning a probability that an unknown call belongs to a particular species may also be inaccurate. At worst, misidentification will occur (Fig. 2).

To maximize the accuracy of species identification, we recommend the following:

- The library of reference echolocation calls from known individuals should be drawn from individuals captured and recorded in the study area.
- When this is not possible, reference calls should come from locations as close to the study area as possible, and from areas that have the same foraging habitats as the study area.
- Reference calls should come from individuals from the subspecies that is present in the study area, and from individuals that are the same body size and condition.
- Reference calls should be recorded from free-flying bats of both genders in the variety of habitats to be studied. Ideally, reference calls should be matched to unknown calls from the same habitat.
- Reference calls should be taken from a significant number of individuals so as to incorporate the variation inherent in the population.
- The recording and analysis equipment and technique used for the reference calls should be the same as those used for the unknown calls.

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LITERATURE CITED


Section 4: Resources, Research and Study