Acoustic Signals and Speciation: The Roles of Natural and Sexual Selection in the Evolution of Cryptic Species

GARETH JONES

SCHOOL OF BIOLOGICAL SCIENCES,
UNIVERSITY OF BRISTOL,
BRISTOL, UNITED KINGDOM

I. INTRODUCTION

In 1768, Gilbert White, a pioneer of natural history studies, wrote to fellow naturalist Thomas Pennant about warblers singing near his home at Selborne: "I have now, past dispute, made out three distinct species of the willow-wrens. . . . which *constantly* and *invariably* use distinct notes" (White, 1813). White was referring to three species of warblers in the genus *Phylloscopus*—the chiffchaff, *P. collybita*, the willow warbler, *P. trochilus*, and the wood warbler, *P. sibilatrix*. The three warblers look so similar superficially, that it was not until 30 years after White's acute study of their different songs that they were described as different species. In Europe, the marsh tit, *Parus palustris*, and willow tit, *P. montanus*, are similar in appearance, but are separated easily by voice. The treecreeper, *Certhia familiaris*, and the short-toed treecreeper, *C. brachydactyla*, are almost identical to our eyes, yet their calls are quite different.

Many animal species may remain undescribed because, although they seem indistinguishable from described species by their appearance, they maintain reproductive isolation by cues that are not so obvious to our senses. To humans, many animal species look similar, yet sound different. Humans have considerable visual abilities over a wide range of light wavelengths, and animals can be classified with ease according to visual criteria. Although humans also have considerable auditory skills, especially between 500 Hz and 3 kHz, differences in acoustic signals are more difficult to quantify than differences in appearance. Vocalizations must be studied in living animals, while visual differences can be assessed by examination of museum specimens. Acoustic signals can be difficult to locate, especially

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as they are often used by animals living in niches where vision is of limited use (at night, or in dense cover, for instance, see Endler, 1992). Many animals call at frequencies beyond our range of hearing, and descriptions of vocal differences often require sophisticated bioacoustical analyses. For these reasons, acoustic differences among animals may have been neglected in comparison with visual differences. Moreover, for reasons that I shall explore later, morphology may often be conserved during evolution, while behavioral traits, such as acoustic signaling, may be more flexible. Behavioral traits often reflect the presence of species that might remain cryptic on morphological grounds alone. Given that the rates of morphological, behavioral, and genetic divergence during and after speciation are only very loosely coupled (e.g., Bruna, Fisher, and Case, 1996), it is interesting to explore under what selection pressures behavioral divergence occurs.

Species that seem similar are called cryptic species. A cryptic species is "a species the diagnostic features of which are not easily perceived; a sibling species" (Mayr, 1977). Cryptic species are often thought of as synonymous with sibling species (see Mayr's definition), though Mayr defines sibling species separately as "morphologically similar or identical populations that are reproductively isolated" (Mayr, 1977). What is not clear from the definition of a cryptic species is what is meant by the perceiver. Animals often have perception that is very different from humans, and two species that seem identical to our eyes may appear very different to the animals concerned. Birds, for example, are sensitive to ultraviolet (UV) light and may perceive colors differently from humans. Indeed, UV vision is probably important in signaling in birds (Bennett, Cuthill, and Norris, 1994), and humans are probably atypical among animals in not having sensitivity to UV (Bennett and Cuthill, 1994). Color, after all, is not absolute, and is in part the consequence of an animal's neural processing abilities. In this chapter, I will use the term cryptic in terms of human vision. The important point here is that some cryptic species probably do look similar to other, closely related species, while others may not. Without fully understanding the visual physiology of the animals concerned, crypticity can be defined only according to our visual performance. Throughout this chapter I define "appearance" as apparent form, look, or aspect (Oxford English Dictionary, 1991). "Appearance" is clearly a subjective description of an item. The quantitative description of shape is termed "morphometrics" (Rohlf, 1990). Cryptic species are often similar in morphometrics also; although morphology can be assessed relatively objectively, comparisons based on appearance may be more difficult for us to quantify. Morphology, that is, structure, is only one component of appearance.

Why do some animals look so alike to our eyes, yet are so different in their acoustic behavior? Of course, vocal differences do not always imply

speciation. In songbirds, for example, often only males sing, and regional dialects occur within species that may have little relevance to speciation (Catchpole and Slater, 1995). Nevertheless, I believe that the study of animals that use acoustic signals may shed light on speciation mechanisms, and on how natural selection and sexual selection may play quite different roles in shaping acoustic divergence in cryptic species. I also believe that the limited sensory performance of humans may result in a bias in descriptions of biological diversity. Species that are so obviously different to our eyes are more likely to get described. Many cryptic species probably remain undescribed, yet these may have considerable genetic divergence from their closest living relatives. Relationships between genetic divergence and crypticity are important in that they may allow determination of whether cryptic species evolved recently, or whether "appearance" may be conserved despite long-term separation from ancestral species. The study of cryptic species is clearly important for understanding biodiversity.

Cryptic species that differ in olfactory characters also occur, though little research seems to have been conducted on such animals, probably because humans have a relatively poor sense of smell. Two species of Australian scorpionflies are almost identical in morphology, but fail to interbreed in the laboratory because the male sex pheromones differ between the cryptic species (Bornemissza, 1966). I predict that many cryptic species of animals with different olfactory characters that are used as secondary sexual signals will be described in the future, but this chapter will concentrate on cryptic species that differ in vocalizations. Animals that communicate largely by acoustic signals include bats, anurans, and many insects and birds. Vision may not be an effective means of signaling in low levels of ambient light (e.g., Martin, 1990; Endler, 1992; Römer, 1993), and I expect acoustic cryptic species to be more prevalent in nocturnal taxa, and in species that live in dense foliage or turbid water, where vision is of restricted use. Vocal signals are also important for orientation and prey capture by echolocation in bats (Griffin, 1958), and in this chapter I use signals in a broader definition than that normally used by behavioral ecologists (e.g., Dawkins and Krebs, 1978), where signaling is synonymous with communication. Bat echolocation calls are traditionally referred to as signals, even though their use is primarily in orientation and prey capture, rather than in communication (though see Fenton, 1985, for the role of echolocation calls in communication).

I will use a case study on pipistrelle bats to illustrate how much hidden biodiversity may remain unrecognized. It was long assumed that the pipistrelle, *Pipistrellus pipistrellus,* was one species. The pipistrelle is believed to be the most widespread European bat, and is probably the most common (Stebbings and Griffith, 1986; Stebbings, 1988). Studies on echolocation calls showed that pipistrelles belong to one of two "phonic types," however

(Jones and Parijs, 1993). Subsequent work, summarized later, confirmed that vocal differences were not the consequence of jamming avoidance or habitat-specific echolocation, but that the phonic types are cryptic species with considerable genetic divergence. The pipistrelle example shows how natural selection may shape vocal divergence in echolocating bats. Why the differences in echolocation calls, yet the remarkable similarities in appearance and morphology? I argue that there may be strong selective pressures for acoustic divergence in echolocating animals where call frequency may influence echo strength from acoustic targets of different sizes, and hence prey selection. In this respect, echolocating bats may differ from most insects, anurans and birds, where female choice for elaborate vocalizations may be a stronger force promoting acoustic divergence. I consider whether divergence in the echolocation calls of cryptic bat species can evolve by sympatric speciation.

I discuss whether intersexual selection for acoustic characters may by itself result in sympatric speciation in nonecholocating animals. The bird group containing the largest number of species is the passerines, where acoustic signals have reached their greatest elaboration, so the importance of acoustic signaling in speciation certainly deserves closer attention. I argue that sexual selection, rather than natural selection, is the prime force in shaping acoustic divergence in nonecholocating animals. I consider whether cryptic species are recently evolved species, or whether they are as genetically distinct as "conventional" species, and conclude by discussing the relevance of the genetic constitution of cryptic species to our understanding of biodiversity.

II. A Case Study: Britain's Most Common Bat is Two Species

A. Echolocation Calls

The pipistrelle has long been known to have a diverse range of echolocation calls. Calls may change within individuals in relation to foraging situation, reflecting behavioral adaptation (e.g., Kalko and Schnitzler, 1993; Kalko, 1995), but substantial variation in call frequency also exists between individuals. Miller and Degn (1981) interpreted individual variation in echolocation calls as having an antijamming function. They hypothesized that, when bats flew in groups, they altered the dominant frequency of their calls so that their own echoes had less chance of being confused with echoes from the calls of conspecifics. This hypothesis was based on observational data, yet pipistrelles flying in the laboratory do not appear to change the frequency of their calls when flying in groups compared with when flying

alone (G. Jones, unpublished data). Thus, jamming avoidance cannot explain between-individual variation in the echolocation calls of pipistrelles. Jamming is avoided not by frequency shifting, but perhaps by each bat having a distinctive call and associated personalized frequency response (see Suga, Niwa, Taniguchi, and Margoliash, 1987), and by neural filters that limit the bats' responses to echoes that arrive only within particular time windows that correspond to expected return times from the individual's own calls (Pollak and Casseday, 1989).

The echolocation calls emitted by pipistrelles searching for prey are described as "FM/CF." Thus, they have a broadband frequency-modulated (FM) sweep terminating in an almost (or quasi-) constant-frequency (CF) tail (Jones and Parijs, 1993; Fig. 1). Most of the energy in the call is concentrated in the CF tail, so the peaks of power spectra (frequencies containing most energy) from sound analyses correspond to the frequency of the CF tail. The frequency-modulated part of the call is probably adapted to range determination, while the CF tail may be effective for long-distance echolocation, or for the detection of "glints" from insect wingbeats (e.g.,
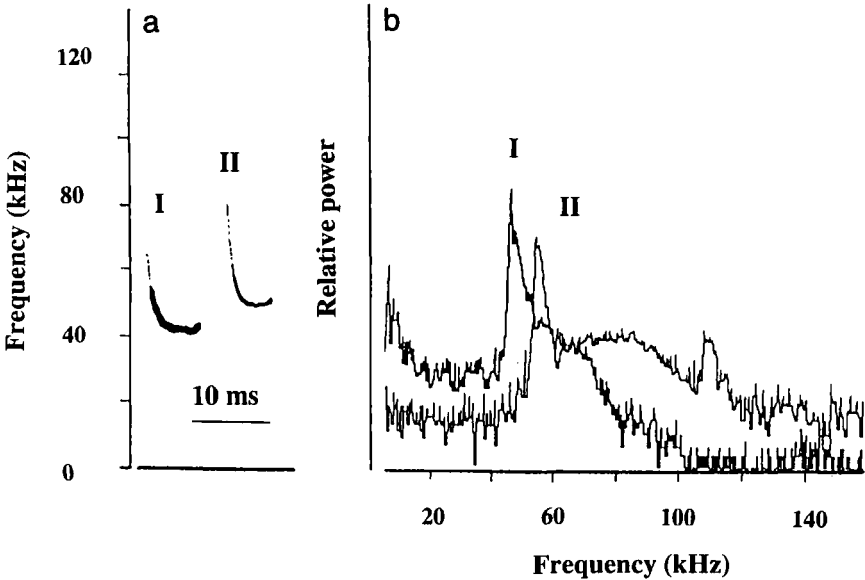

FIG. 1. Search phase echolocation calls of the two phonic types of pipistrelle bats. (A) Sonagrams. (B) Power spectra. Note how most energy in the calls is in the terminal CF tail. Call I had a frequency containing most energy (FMAXE) of 46 kHz, and was emitted by a "45-kHz" pipistrelle. Call II had a FMAXE of 54 kHz, and was emitted by a "55-kHz" pipistrelle. Redrawn from Jones and Parijs (1993) with permission from The Royal Society.

Neuweiler, 1990). When the bats are searching for insects in open spaces, they emit "search-phase" echolocation calls (Griffin, Webster, and Michael, 1960), like those described previously. If the bats fly in more cluttered situations, call duration is shortened, the CF tail is less pronounced, and the calls become more broadband (Kalko and Schnitzler, 1993). Broadband calls will give greater spatial resolution in clutter (Simmons, Fenton, and O'Farrell, 1979), and the shortening of the calls allows the bats to avoid overlap between outgoing pulse and incoming echo as the bats approach targets (Kalko and Schnitzler, 1993; Kalko, 1995).

Pipistrelles in Switzerland emit search phase calls with CF tails between 41 and 62 kHz that form two separate clusters in a multivariate analysis of call parameters (Zingg, 1990). The two groups of pipistrelles showed very little overlap in the terminal frequencies of their echolocation calls: one group showed a median end frequency of 44.8 kHz, the other 57.5 kHz. Zingg interpreted this difference in terms of habitat selection by pipistrelles—when the bats flew in one type of habitat they used one sort or signal, switching to another call type in a different situation.

Jones and Parijs (1993) showed that pipistrelle calls fell into two frequency bands in Britain (Fig. 1). One call type had frequencies of most energy averaging 46 kHz, the other close to 55 kHz. The bats are referred to as the "45-kHz" and "55-kHz" phonic types for simplicity hereafter. In summer, female bats usually form maternity colonies where they give birth and rear their young. Every maternity colony studied contained bats of only one phonic types (Fig. 2). Because volant juveniles of both sexes were recorded leaving maternity roosts, it was clear that the call differences of the magnitude observed were not caused by sex or age effects, as seen in some other bat species (e.g., Jones and Ransome, 1993; Jones and Kokurewicz, 1994). Males, the smaller sex, emit calls that have peak frequencies on average about 2 kHz higher than females in both phonic types of pipistrelle (Park, Altringham, and Jones, 1996), but between-sex differences were small compared with differences between phonic types. Habitat specificity of echolocation calls, as suggested by Zingg (1990), could be rejected, since a bimodal distribution of call frequencies was maintained when bats of both phonic types were released into similar habitats. The released bats always used the same frequencies as those of roostmates flying from their maternity colonies (Jones and Parijs, 1993; Fig. 3). The phonic types exist in sympatry over much of Britain and Europe, so the possibility of them being geographical races or subspecies was dismissed (Jones and Parijs, 1993).

Interpulse intervals of 45-kHz bats are longer (82.25 ± 10.48 (SD) ms, $N = 125$) than those of 55-kHz pipistrelles (76.60 ± 10.49 ms, $N = 229$) on average (Jones and Parijs, 1993). This difference is expected if 45-kHz bats were larger (see later discussion), since repetition rate (which is linked
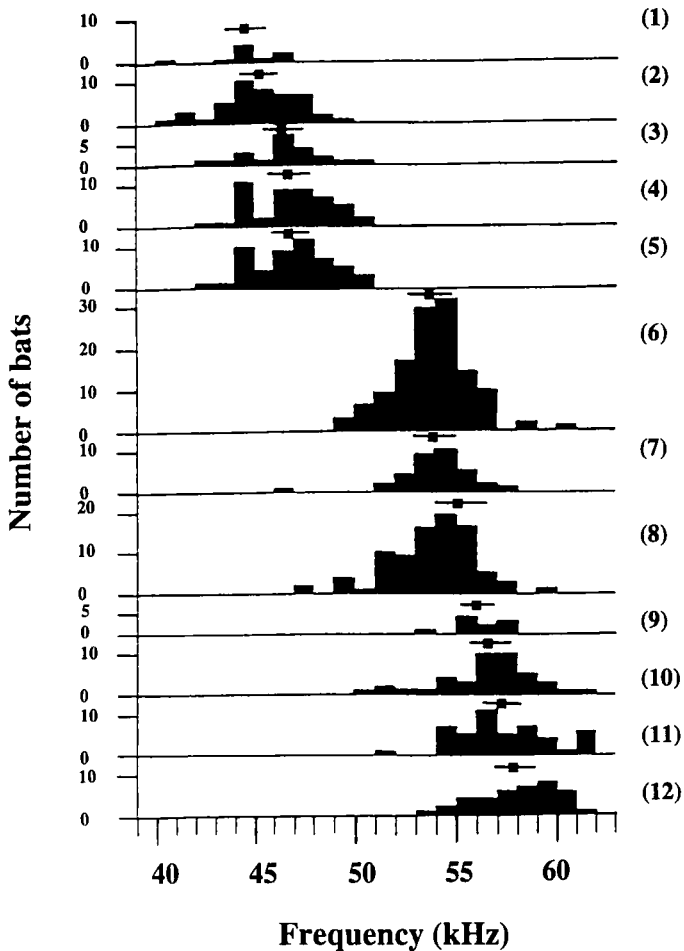
FIG. 2. Maternity colonies of pipistrelle bats contain only one phonic type of bats. Data are taken from bats exiting 12 maternity colonies, and represent frequency distributions of the frequencies of most energy of cells emitted by individual bats. Colonies are arranged in order of increasing mean call frequency for the sample. Means (solid squares) ± *SD*s of calls from each roost are illustrated. Redrawn from Jones and Parijs (1993) with permission from The Royal Society.

with wingbeat frequency 1:1 in the search phase for bats that hunt by aerial hawking) scales negatively with body mass in a cross-species comparison (Jones, 1994).

In Europe, 45-kHz bats have been recorded in France, The Netherlands (Jones and Parijs, 1993), Germany (Kalko and Schnitzler, 1993), Luxembourg (C. Harbusch, unpublished data) and in Poland, Slovakia, and The
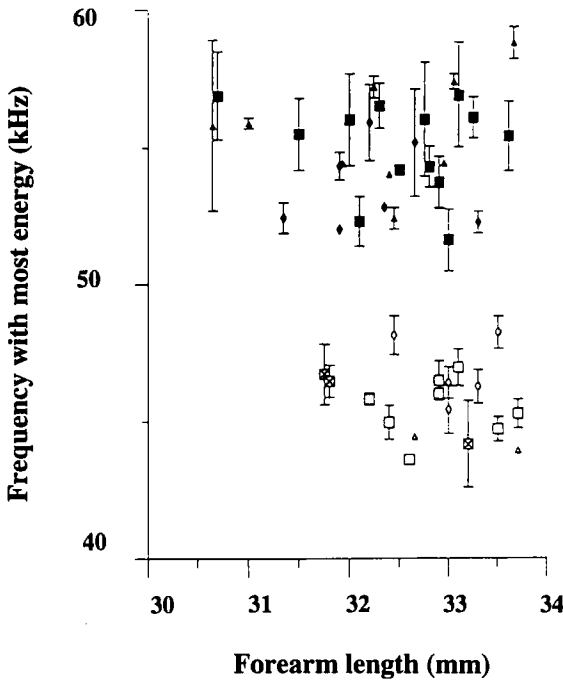
FIG. 3. Pipistrelle bats maintain phonic type when released in the same habitat. The relation between call frequency and forearm length is shown for 48 pregnant or lactating female pipistrelles from seven roosts. All bats were released in open habitats, away from clutter. Solid symbols are 55-kHz pipistrelles, open symbols are 45-kHz bats, and each symbol represents membership of a different maternity colony. Means ± SDs of five calls are illustrated. Redrawn from Jones and Parijs (1993) with permission from The Royal Society.

Czech Republic (J. Rydell and P. A. Racey, unpublished data; Fig. 4). However, most Scandinavian (Jones and Parijs, 1993; Denmark: Miller and Degn, 1981; Norway: T. Stormark, unpublished data) and Mediterranean (e.g., Greece: Weid and Helversen, 1987; Portugal and Spain: T. Guillen, A. Rainho, and N. Vaughan, unpublished data) records are of 55-kHz pipistrelles. Forty-five-kHz bats occur in south Denmark (H. Baagøe, unpublished data), and this phonic type has recently been discovered in northern Germany (H. J. G. A. Limpens, unpublished data) and northeastern Poland (A. Rachwald, unpublished data). Both phonic types are widespread and sympatric in Britain (Jones and Parijs, 1993), Northern Ireland (J. M. Russ, unpublished data) and Switzerland (Zingg, 1990). Despite extensive searching in the middle latitudes of Europe (e.g., The Netherlands: Kapteyn, 1993), 55-kHz pipistrelles appear to be absent over much of this area.
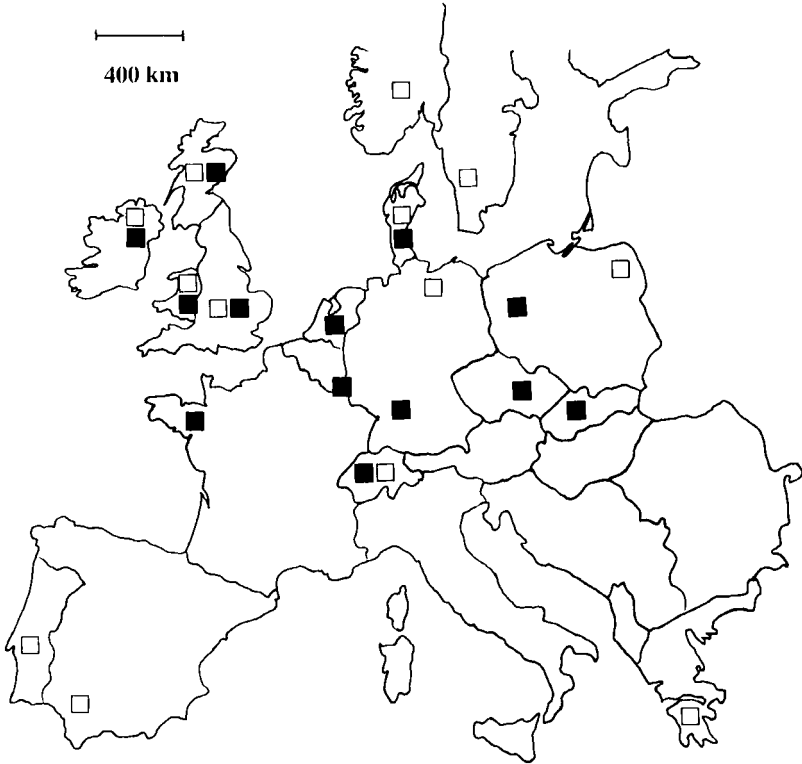
FIG. 4. The distribution of phonic types of pipistrelle bat in Europe. Open squares represent 55-kHz pipistrelles; solid squares represent 45-kHz bats. The source of data is given in the text. Note how 55-kHz bats may be absent from much of central Europe, though they are widespread in Scandinavia, Britain, and the Mediterranean. The map does not show the relative abundance of the two phonic types in different parts of their range (see text for details).

The overall impression is that the 55-kHz phonic type is more abundant in northern and southern Europe, with the 45-kHz phonic type being found mainly in middle latitudes. Thus, 55-kHz bats may be pushed to the edge of the range of pipistrelles, on either side of a core distribution of 45-kHz bats.

B. SOCIAL CALLS

On foraging grounds, pipistrelles often emit "social calls." These are relatively low frequency calls (frequency of most energy typically between

16 and 23 kHz), and are more complex in structure and longer in duration (approximately 35 ms) than echolocation signals. Social calls are very similar in structure to the songflight calls illustrated in Fig. 5, but are repeated at a lower rate (Barlow and Jones, in press, b). Social calls probably travel further than echolocation calls, because the relatively low frequencies used in social calls suffer less from excess atmospheric attenuation (Lawrence and Simmons, 1982), and they are thus well designed for relatively long range communication. Social calls were believed to serve an agonistic function, as they were often associated with chasing behavior (Miller and Degn, 1981; Racey and Swift, 1985). Moreover, most chases (Racey and Swift, 1985) and social calls (Barlow and Jones, in press, c) occur at low insect densities, so social calls may be associated with defense of feeding sites.

The social calls of the two phonic types of pipistrelle differ substantially (Barlow and Jones, in press, a,b). Social calls of the 45-kHz bats tend to be slightly lower in frequency than those of 55-kHz bats, and usually consist of four, rather than three components (Barlow and Jones, in press, b). The function of the calls as agonistic signals was confirmed in playback experiments: bats of the 45-kHz phonic type were less active (fewer bat passes were recorded) during playbacks of 45-kHz social calls than during control (tape noise) playbacks, and 55-kHz bats showed a similar response
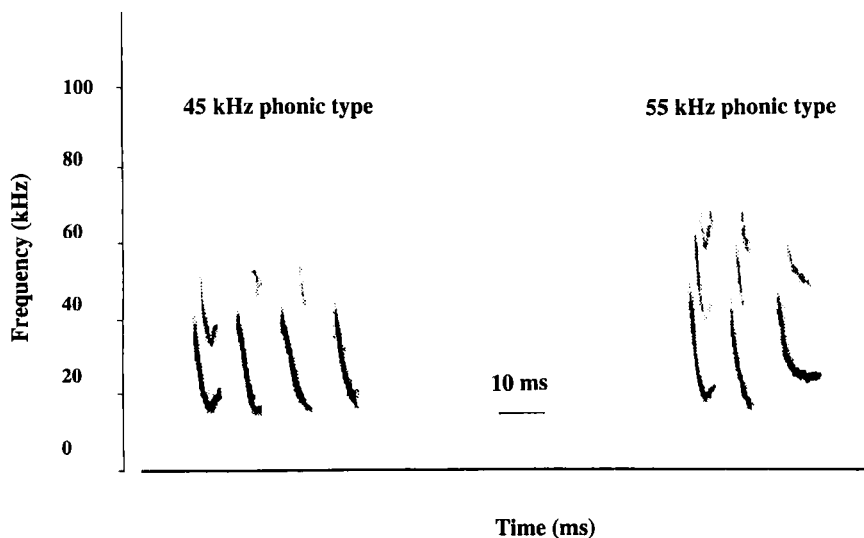

FIG. 5. Sonagrams of typical songflight calls of the 45-kHz and 55-kHz phonic types of pipistrelle.

to playbacks of calls from their own phonic type. A reduction in activity suggests an aversive response to playbacks because it implies that bats left the vicinity of the speaker. Interestingly, neither phonic type responded to playback of calls from the other phonic type, suggesting that social calls do not function in interspecific communication (Barlow and Jones, in press, c).

C. MALE SONGFLIGHT AND REPRODUCTIVE ISOLATION

The mating strategy of pipistrelles can be described as resource defense polygyny. Males occupy roosts in autumn, and call repeatedly during an advertisement flight (songflight) close to the roost, presumably to attract females. Mating groups of females with single males can be found in bat boxes during the autumn (Gerell and Lundberg, 1985; Lundberg and Gerell, 1986; Gerell-Lundberg and Gerell, 1994). The songflight calls (Fig. 5) are similar in structure to the social calls described earlier (Barlow and Jones, in press, b), but are repeated at a higher rate, often over several hours in the night, and over several weeks in the mating season. As with social calls, bats of the 45-kHz phonic type tend to emit songflight calls with fewer components and of relatively low frequency compared to calls of 55-kHz bats. Because the songflight calls of the two phonic types differ significantly in structure (all individuals can be assigned to the correct phonic type from their calls in multivariate analysis; Barlow and Jones, in press, b), female pipistrelles may use the songflight calls as cues for species recognition during the mating period.

The composition of mating groups of pipistrelles in bat boxes has been analyzed in relation to phonic type by Park et al. (1996). For 16 ringed males and 34 females, all of 27 mating groups investigated contained females of the same phonic type as the male. Where genetic data are available, females associate with males of the same genetic clade (discussed later) (E. M. Barratt, G. Jones, P. A. Racey, T. M. Burland, R. K. Wayne, M. W. Bruford, and R. Deaville, unpublished data). Assortative associations occurred even in areas where the phonic types were sympatric, and hence reproductive isolation between phonic types occurs.

D. MORPHOLOGICAL DIFFERENCES

Although the two phonic types of pipistrelle appear very similar superficially, subtle differences in appearance are obvious when the bats are examined closely. The 45-kHz bats have fur that is darker brown in color than that of 55-kHz bats. They also usually have a black face mask, while the eyes of 55-kHz bats are surrounded by flesh. It must be emphasized

that the two phonic types are very variable in fur color and in appearance, so characters such as these may be of little use in field identification.

The 45-kHz pipistrelles tended to be larger in the study of Jones and Parijs (1993): 45-kHz pipistrelles had a longer average forearm length (32.37 ± 0.59 (*SD*) mm, $N = 18$) than 55-kHz bats (31.91 ± 0.82 mm, $N = 30$) and a lower wing aspect ratio (45-kHz bats, 5.46 ± 0.42 (*SD*), $N = 18$; 55-kHz bats, 5.82 ± 0.72, $N = 30$). The results from this relatively small sample must be treated with caution, however. The difference in aspect ratio was not confirmed in a larger sample of bats (K. E. Barlow, unpublished data), and no significant differences were found between phonic types in univariate standard measurements of wing morphology (Norberg and Rayner, 1987) or in forearm length. Moreover, the two phonic types could not be separated by a multivariate analysis of wing-shape characters (Jones and Parijs, 1993). Thus, an interesting question arises: if the two phonic types are so similar in flight morphology, how do they partition resources? Presumably resource partitioning does occur, as both phonic types fail to respond to agonistic calls of the other phonic type at feeding sites and a response would be expected if interspecific competition was strong (Barlow and Jones, in press, c). Resource partitioning by diet occurs in sympatric populations of *Myotis lucifugus* and *M. volans* in Canada, despite the two species having similar morphology and echolocation calls (Saunders and Barclay, 1992). Hence resource partitioning between the phonic types of pipistrelle is expected given the radical differences in echolocation, even considering their similar flight morphologies.

Morphometric analysis of skulls revealed significant differences between the phonic types. In a multivariate analysis of 7 skull parameters from 57 bats, 79% were classified correctly, with cross-validation included in the model. The length of the mandibular tooth row between the upper canine and M^3 was the most important parameter separating the two phonic types (K. E. Barlow and G. Jones, unpublished data).

Morphological differences in the baculum (penis bone, or os penis) exist among several cryptic bat species (Lanza, 1960; Baagøe, 1973). If a difference in baculum shape results in a change in penile morphology, reproductive isolation between cryptic species may occur (Patterson and Thaeler, 1982). The bacula of the two phonic types of pipistrelle are very similar. Bacula have a paired basal flange, an elongate shaft, and a bifid tip (see Hill and Harrison, 1987). The bacula do not show the obvious differences seen between cryptic species in the genera *Plecotus* (Lanza, 1960) or *Myotis* (Baagøe, 1973). Slight differences do occur in the angle between basal flanges and the shaft between cryptic species, however. The bacula of the 45-kHz bats have flanges that rise gradually from the base, while the bacula of 55-kHz bats show a more acute angle between flange and shaft when

viewed in profile (K. E. Barlow and G. Jones, unpublished data). Overall, skull and bacular morphometrics emphasize the morphological similarity between the two cryptic pipistrelle species.

E. BEHAVIORAL DIFFERENCES AND IMPLICATIONS FOR RESOURCE PARTITIONING

Can differences in echolocation pulses be used to predict differences in microhabitat use by the two phonic types? Bats that emit relatively short duration FM/CF signals are believed to process echoes that do not overlap with the outgoing pulse (Kalko and Schnitzler, 1993), and that return before the next search phase pulse is emitted (Roverud and Grinnell, 1985). For two calls of similar frequency structure but different duration, the shorter call may not mask an echo from a nearby target, while the longer one may. Since pulse duration does not differ between phonic types (approximately 5.3 ms; Jones and Parijs, 1993), a minimum detection distance (Kalko and Schnitzler, 1993) for insects of about 90 cm is expected for both phonic types. Kalko (1995) measured average distances for prey detection of 1.68 m for *P. pipistrellus* (presumed 45-kHz phonic type), and 1.63 m for *P.p. mediterraneus* (bats presumed to be of the 55-kHz phonic type). Pipistrelles often use calls of longer duration when searching for prey in open habitats (6–10 ms; Kalko, 1995), and this increased duration probably accounts for the discrepancy between measured detection distances and those predicted by the call durations provided by Jones and Parijs (1993).

The longer interpulse interval of the 45-kHz type may give it a greater maximum detection distance (Fenton, 1990), though most echoes from distant objects are probably attenuated severely at this distance (approximately 14 m). Although the 45-kHz phonic type may potentially have a greater overlap-free window (Kalko and Schnitzler, 1993), whether differences in interpulse interval have any implication for influencing habitat use by the phonic types is unlikely.

Assuming that the *P. pipistrellus mediterraneus* recorded by Kalko (1995) in Spain are bats of the 55-kHz phonic type of *P. pipistrellus,* then the 55-kHz bats have a lower flight speed than the 45-kHz pipistrelles. In a survey of habitat use by bats in southwestern England, Vaughan (1996) found 45-kHz bats over a wide range of habitats, while 55-kHz pipistrelles showed stronger selection for waterside habitats. The 55-kHz pipistrelle may form larger roosts than the 45-kHz phonic type in Britain. The maximum size of four maternity roosts of 45-kHz bats studied by Jones and Parijs (1993) was 160 bats, whereas four of seven roosts of 55-kHz pipistrelles contained more than 250 bats.

F. GENETIC DIFFERENCES

Phylogenetic relationships of the genus *Pipistrellus* have been determined using a 400-base-pair sequence of the cytochrome-*b* gene of mitochondrial DNA (mtDNA) amplified by the polymerase chain reaction (Barratt *et al.*, 1995). Mitochondrial DNA was used because it evolves rapidly at the sequence level, and cytochrome-*b* is a gene sequenced routinely in phylogenetic analyses, thus allowing comparisons to be made with other studies (Avise, 1994). A consensus of the 100 most parsimonious trees showed two distinct clades for the *Pipistrellus* species investigated, each clade differing by about 12% in cytochrome-*b* sequence (Fig. 6). Bats classified as *P. pipistrellus* appeared in both clades, implying that this species may have diphyletic origins. The phylogeny presented in Fig. 6 implies that the speciation event that separated the two clades of *P. pipistrellus* occurred a long time ago, and may have been followed by other speciation events within the same lineage. Importantly, the two clades of *P. pipistrellus* have recently been found to correspond completely with the two phonic types described by Jones and Parijs (1993) (E. M. Barratt, G. Jones, P. A. Racey, T. M. Burland, R. K. Wayne, R. Deaville, and M. W. Bruford, unpublished data). A 12% sequence divergence in mtDNA (Barratt *et al.*, 1995) confirms that the two phonic types differ substantially in genetic constitution, despite their morphological similarity. The magnitude of the sequence divergence between the phonic types of pipistrelle described by Barratt *et al.* (1995) is similar to that found among noncryptic species of *Phyllostomus* (Van Den Bussche and Baker, 1993), in other genera of phyllostomid bats (Van Den Bussche, Baker, Wichman, and Hamilton, 1993; Baker, Taddei, Hudgeons, and Van Den Bussche, 1994), and among species in the molossid genus *Nyctinomops* (Sudman, Barkley, and Hafner, 1994) (Fig. 7).

In summary, the two phonic types of pipistrelles are classic cryptic species. Despite superficial similarities in appearance, they differ radically in their echolocation calls and in mtDNA sequence. Differences are also apparent in skull morphology, social calls, habitat use, and roost size. Differences in male songflight calls may facilitate reproductive isolation of the cryptic species, and assortative associations between males and females are found in bat boxes during the mating season. The nomenclature of *Pipistrellus pipistrellus* is now being revised in light of these findings (G. Jones, in preparation).

III. ACOUSTIC RESOURCE PARTITIONING BY ECHOLOCATION

Why might differences in echolocation call frequency promote resource partitioning in echolocating bats? The target strength of insects is likely to
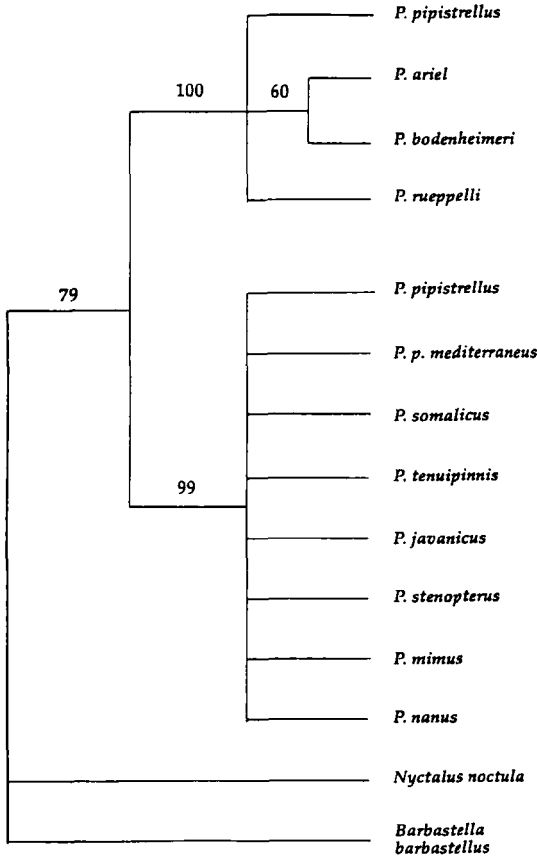
FIG. 6. Phylogeny of bats in the genus *Pipistrellus* based on parsimony analysis of cytochrome-*b* gene sequences of mitochondrial DNA. A consensus tree from 100 trees was generated by using heuristic search in PAUP (phylogenetic analysis using parsimony) version 3.0 (Swofford, 1989), with *Nyctalus noctula* and *Barbastella barbastellus* as outgroups. Numbers on the tree show bootstrap node confidence values from 100 replications. *P. p. mediterraneus* is almost certainly the 55-kHz phonic type of pipistrelle. Hence, *P. pipistrellus* in the lower clade is likely to be the 55-kHz phonic type, that in the upper clade the 45-kHz phonic type. From Barratt *et al.* (1995), and reproduced with permission from The Zoological Society of London.

depend largely on call frequency (Pye, 1993). For spheres, echo strength falls rapidly when wavelength (the inverse of frequency) exceeds target circumference (Fig. 8). Effectively this relation means that if insects reflect sound in a way similar to spheres, frequencies lower than (i.e., with wavelengths greater than) some measure of target circumference will return

0

1 –

2 –

3 – ◄— *Chiroderma trinitatum - C. doriae*
     ◄— *Dermanura (Dermanura) azteca - D. phaeotis & Chiroderma*
4 –   *trinitatum - C. villosum*
     ◄— *Dermanura azteca - D. tolteca*
     ◄— *Dermanura phaeotis - D. tolteca*

5 –

6 – ◄◄— *Nyctinomops aurispinosus - N. laticaudatus*
          Artibeus fuliginosus - A. lituratus

7 – ◄— *Phyllostomus latifolious - P. discolor*

8 – ◄— *Dermanura phaeotis - D. cinerea*

9 – ◄— *Phyllostomus hastatus - P. elongatus*
     ◄— *Chiroderma salvini - C. trinitatum*
10 – ◄— *Dermanura cinerea - D. tolteca (tolteca)*
     ◄— *Phyllostomus latifolious - P. hastatus*
     ◄— *Phyllostomus latifolious - P. elongatus*

11 – ◄— *Phyllostomus latifolious - P. stenops*

—► 12 –
Pipistrellus pipistrellus

13 – ◄— *Phyllostomus stenops - P. hastatus*
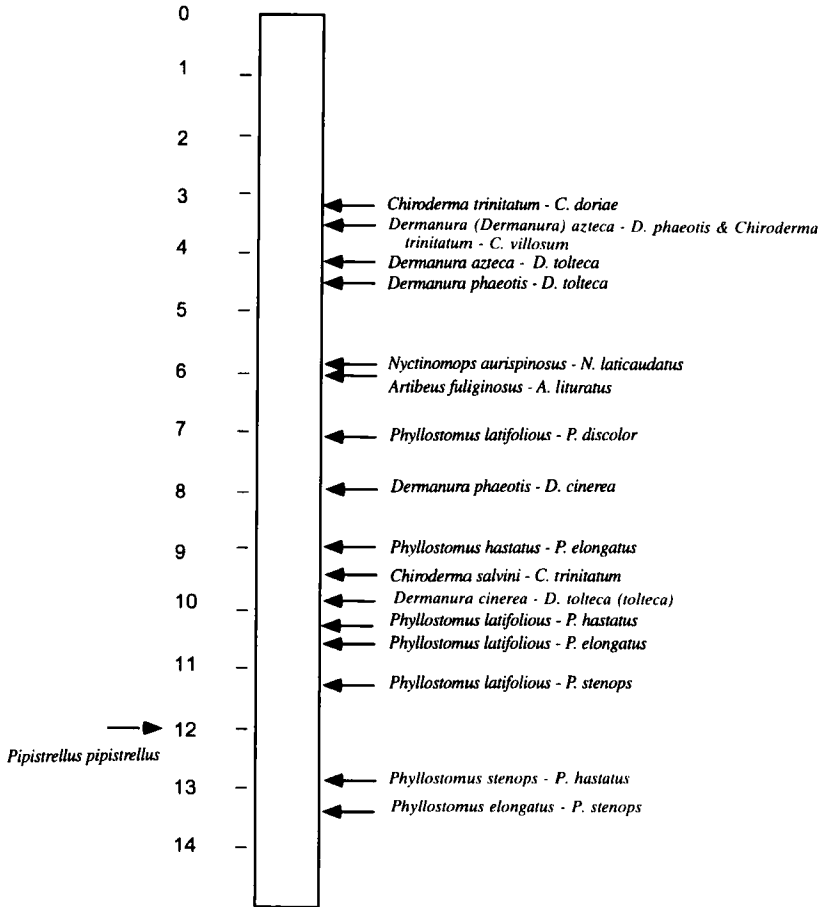   ◄— *Phyllostomus elongatus - P. stenops*

14 –

FIG. 7. Magnitude of sequence divergence (%) in cytochrome-b gene sequences (400–1140 base pairs) for a range of congeneric microchiropteran bats. Data from Van Den Bussche and Baker (1993), Van Den Bussche *et al.* (1993), Baker *et al.* (1994), Sudman *et al.* (1994), and Barratt *et al.* (1995).

little energy in echoes. When wavelength is considerably less than target circumference, then echo intensity remains relatively high and constant. In a simplified way, then, frequencies whose wavelength exceeds sphere circumference will reflect echoes poorly, and high frequencies give stronger echoes from smaller targets.

The frequency when echo intensity begins to decline can be predicted with accuracy for spheres, but do such predictions hold for more complex targets such as insects? The relation between target strength and frequency
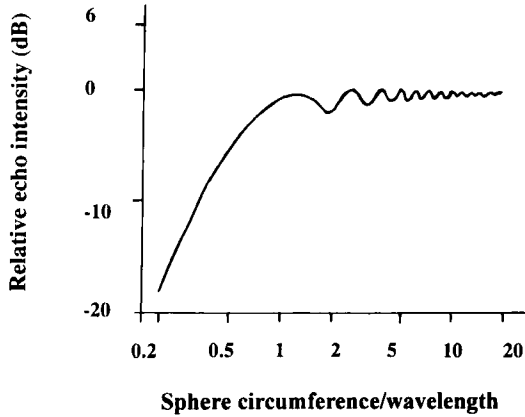
FIG. 8. The relation between echo intensity reflected from a sphere in relation to sound frequency. Where sphere circumference/wavelength is greater than 1, echo intensity is highest. When wavelength is longer than sphere circumference, echo intensity falls off rapidly as wavelength increases. The x-axis represents the scale in frequency for a sphere of 10.8 cm diameter. Adapted from Pye (1993).

has received little empirical study: Waters, Rydell, and Jones (1995) found no obvious relation between target strength and frequency for moths and small flies between 20 and 100 kHz, and argued that the target strength predictions for spheres may not hold for insects. Nevertheless, recent empirical studies on discs (A. P. Norman, unpublished data) and spheres (R. D. Houston, unpublished data) supported Pye's predicted relationship between target strength and call frequency. If, therefore, target strength from insects did depend on frequency in the way measured for spheres and discs, I predict that the 55-kHz pipistrelles would receive stronger echoes from smaller flying insects than would 45-kHz bats. I would then predict that 55-kHz pipistrelles should eat smaller prey than 45-kHz bats, if the 1.2-mm difference in wavelength between the two phonic types is biologically significant. This hypothesis is now being tested (K. E. Barlow, in preparation).

If the sphere model of target strength applies to insect targets, then the echolocation call frequency used by a bat should influence the bat's ability to detect prey of a given size. Call frequency could be thought of as the ecological equivalent of bill size in birds, with bats that use higher frequencies specializing in eating smaller prey. Hence, there may be strong selective pressure for divergence in call frequency between species of echolocating bats to minimize interspecific competition for insect prey. Intraspecific competition for different sizes of prey may indeed have been the driving force for speciation in pipistrelles.

Perhaps the most likely cases of acoustic resource partitioning in bats can be seen in rhinolophid and hipposiderid bats. These bats use CF calls that are either short (hipposiderids) or long (rhinolophids) in duration. The high duty cycles (proportion of time filled with sound) of these bats mean that echoes returning to the bats have potential to contain many glints (small deviations in amplitude or frequency of echoes) from insect wingbeats (Schnitzler, 1987). Bats that use long-duration CF calls, like the greater horseshoe bat *Rhinolophus ferrumequinum,* select prey in ways predicted by optimal foraging theory. Unprofitable prey, such as small Diptera and ichneumonids, were rejected except when more profitable prey such as moths were scarce (Jones, 1990). Prey size selection in relation to prey availability by *R. ferrumequinum* showed remarkable parallels with that seen in visually hunting insectivorous birds, such as swallows, *Hirundo rustica* (Turner, 1982), and spotted flycatchers, *Muscicapa striata* (Davies, 1977). Bats that use long CF calls may therefore obtain considerable information about prey characteristics in echoes. The echolocation calls of rhinolophoid bats are relatively simple in structure (Fig. 9) and are dominated by a relatively intense CF portion. Frequency differences in this CF portion are therefore likely to have the most impact on any acoustic resource partitioning in these bats.

In the Krau Game Reserve, an area of primary rainforest and high biodiversity in Peninsular Malaysia, at least 12 rhinolophoid species coexist. The CFs of these species range between 40 kHz and 200 kHz, but the distribution of call frequencies is not random: frequencies lying close together are avoided, suggesting that resource partitioning may occur with respect to prey size as a mechanism for reducing interspecific competition for insect food (Heller and Helversen, 1989). Large bat species use lower frequencies than small species (Heller and Helversen, 1989; Barclay and Brigham, 1991; Jones, 1996), so separating the effects of body size and call frequency on prey size selection can be problematic because of the confounding effects of body size and call frequency. Three sympatric Indian hipposiderids use CFs filling the spectrum between 130 kHz and 170 kHz. There is, however, virtually no overlap in CF between species, and in one species, *Hipposideros speoris,* males emitted higher frequencies than females (Jones *et al.,* 1994; Fig. 9). Sexual differences in CF occur in several rhinolophoid bats (Jones, 1995), so acoustic resource partitioning even between the sexes is possible.

If acoustic resource partitioning by echolocation does occur, then I predict that acoustic character displacement will exist (see Grant, 1986, for character displacement in bill sizes of Darwin's finches). In the case of pipistrelles, for example, I predict that 55-kHz bats will use lower frequencies and 45-kHz bats will use higher frequencies in areas of allopatry than when in
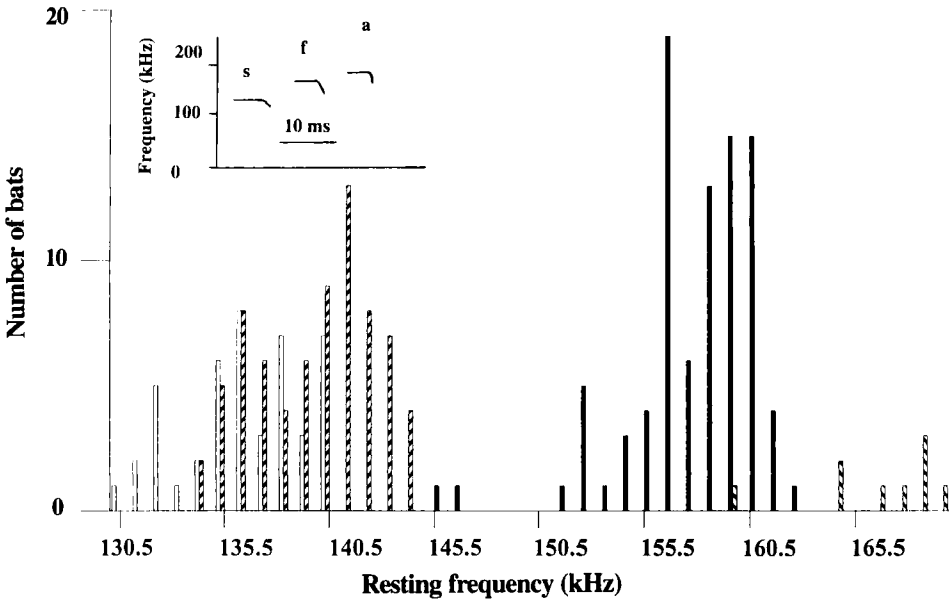
FIG. 9. Frequency distribution of resting frequencies of three sympatric species of hipposide-
rid bats in southern India. Open bars, *Hipposideros speoris* females; upwards cross-hatching,
H. speoris males; solid bars, *H. fulvus;* downwards cross-hatching, *H. ater.* The smaller species
use higher frequency calls, and overlap between species in call frequency is generally avoided.
The inset shows sonagrams of typical calls from the three species: s, *H. speoris;* f, *H. fulvus;*
and a, *H. ater.* Modified from Jones, Sripathi, Waters, and Marimuthu (1994), reproduced
with permission from The Academy of Sciences of the Czech Republic.

sympatry. In allopatry, the absence of interspecific competition may allow
bats of one phonic type to occupy some of the niche space normally occupied
by the other phonic type during sympatry.

IV.   CRYPTIC SPECIES OF ECHOLOCATING BATS

How widespread are cryptic bat species? Are cryptic species of bats
relatively more frequent than cryptic species in other animals? The second
question is difficult to answer, but nevertheless I predict that many bat
species remain undescribed because of the likely importance of echoloca-
tion in determining resource use, because selection for acoustic divergence
may be stronger than for differences in appearance, and because differences
in echolocation calls are difficult for us to study and appreciate.

Several other cryptic bat species appear to use different echolocation call frequencies. Pye (1972) found two groups of putative *Hipposideros commersoni* using CFs at 56 kHz and 66 kHz, respectively, inhabiting one cave in Kenya. He interpreted the difference as an intraspecific variation to prevent interference from band spreading due to beating wings when several bats were flying together. The high-frequency bats were significantly smaller, however, and I predict that the two echolocating types are indeed cryptic species. The cryptic species *Hipposideros caffer* and *H. ruber* are widespread in Africa, and are difficult to key to species on external characters (Fenton, 1986). Bats keyed as *H. caffer* used CFs between 128 and 153 kHz, while those keyed as *H. ruber* used 121–136 kHz (Jones, Morton, Hughes, and Budden, 1993). It seems likely that more bats currently believed to belong to a single species will be found to comprise several species from analysis of their echolocation calls, and acoustic resource partitioning in bats certainly merits close attention.

Cryptic species are known from several bat faunas. In Britain, for example, the long-eared bats, *Plecotus auritus* and *P. auriculus*, and the whiskered and Brandt's bats, *Myotis mystacinus* and *M. brandtii*, are considered as pairs of sibling species, with the new cryptic species discovered only in the past 40 years (Schober and Grimmberger, 1987). Hence, in the well-studied British bat fauna, 6 of 15 species (40%) form sibling species pairs. Baker (1984) discovered the cryptic species *Rhogeesa genowaysi* in Central America by karyotyping, and the bat was not distinct in morphology from other species in its genus. In continental Europe *Myotis myotis* and *Myotis blythii* are extremely similar in morphology, yet show clear genetic differences and trophic resource partitioning (Arlettaz, 1995, 1996; Arlettaz and Perrin, 1995). *Myotis myotis* forages mainly in meadows, orchards, and forests without undergrowth where it feeds on ground-dwelling prey, especially carabid beetles. *Myotis blythii* is associated more with grassland habitats, where it catches large numbers of bushcrickets (Arlettaz, 1995). Arlettaz (1995) believes that allopatric mechanisms, perhaps occurring after glaciations, were responsible for speciation in *M. myotis* and *M. blythii.*

A new, cryptic specis in the *M. mystacinus* group was discovered in Greece by analysis of mtDNA (Nemeth and Helversen, 1994). The genus *Myotis* also contains several cryptic species in North America; the long-eared species *M. evotis, M. keenii,* and *M. septentrionalis* overlap in distribution in some areas, and their field identification is problematic (Zyll de Jong and Nagorsen, 1994). In British Columbia, *Myotis lucifugus* and *M. yumanensis* coexist. These species are extremely similar in morphology, and some individuals appear intermediate in morphology between the two. Nevertheless, no evidence of hybridization was found from electrophoretic

studies, and resource partitioning by habitat use and diet was discovered (Herd and Fenton, 1983). Obstacle negotiation experiments confirmed that *M. yumanensis* was the more maneuverable species, partly as a consequence of its lower wing loading and wingspan (Aldridge, 1986). *Myotis nigricans* may be a composite of sibling species based on bacular morphology (LaVal, 1973). Cryptic species of bats are clearly widespread, perhaps especially in the genus *Myotis,* and they occur frequently even in well-documented faunas. What is not known at this stage is whether many species that resemble one another in morphology differ in their echolocation calls in the way that pipistrelles do.

V. ACOUSTIC SIGNALS AND CRYPTIC SPECIES IN NONECHOLOCATING ANIMALS

Swarms of cryptic species are known in several insect groups that produce near-field, substrate-borne signals. Although the physical nature of these songs differs from far-field acoustic signals, their songs are difficult for humans to detect, and a discussion of such species seems relevant in a consideration of cryptic species. Henry (1994) has argued that species that use such signals are conducive to speciation via acoustic recognition and assortative mating according to song type. Cryptic species of planthoppers (order Hemiptera) and lacewings (order Neuroptera) are described (review in Henry, 1994). At least five "song morphs" of the lacewing species *Chrysoperla carnea* exist in North America, with a further five in Europe. The morphs are virtually identical in morphology (Henry, 1985a; 1994), yet each has a distinctive song and responds strongly only to the song of its own morph (Wells and Henry, 1992). Henry (1985b) argued that the simple genetic transmission of song type could be modified easily by mutations, and sexual selection for elaborate male songs by females could drive speciation. It is not clear whether speciation in these lacewings occurs sympatrically or whether it is facilitated by geographical isolation (Henry, 1985a,b).

There are numerous examples of cryptic insect species that use far-field acoustic signals. Advances in bioacoustics led to a proliferation in the numbers of species of singing orthopterans that were described. About a quarter of North America's ensiferan Orthoptera remained undescribed until their songs were studied in detail (Walker, 1964). Further examples of cryptic species of insects that use acoustic signals are reviewed by Ewing (1989) and Bailey (1991).

Cryptic species are widespread in anuran taxa where males use advertisement calls. For example, the leopard frog *Rana pipiens* was found to be a complex of four species after studying variation in its vocalizations (Little-

john and Oldham, 1968). Many Australian frogs are best separated by vocal differences (review in Littlejohn and Watson, 1985). The frog *Leptodactylus mystaceus* was believed to be a single species from morphological investigations, but analysis of advertisement calls suggests that at least two sibling species are present (Heyer, García-Lopez, and Cardoso, 1996). Species number in the anuran genus *Xenopus* has trebled in the last 20 years (review in Kobel, Loumont, and Tinsley, 1996). Many cryptic species exist in the genus, some of which are allopolyploid species. Differences in male advertisement calls exist among many of the cryptic species (Vigny, 1979), and may be useful clues in understanding the taxonomy and phylogeny of *Xenopus*. First, the gross structure of advertisement calls is often similar in apparently related species with different ploidy levels. Second, some taxa currently described as subspecies have different advertisement calls and show genetic differences that suggest they may be better considered as cryptic species (Kobel *et al.*, 1996). Most *Xenopus* species are found in cloudy water conditions, where they locate prey by using a lateral line system (Elepfandt, 1996), and where acoustic advertisement would be favored over visual communication for mate attraction. This acoustic diversity, rather than variability in morphology or color, contributes to the relatively high number of cryptic species in this genus. Allopolyploidy makes *Xenopus* unusual among vertebrates, and it is possible that new species arise by hybridization when previously allopatric species reestablish contact. This could occur, for example, when a rise in water level allows species previously separated by mountain ranges to establish sympatry (J. Measey, personal communication).

Among European birds, sibling species are found in the warbler genera *Phylloscopus* and *Acrocephalus,* in the titmice and treecreepers described in the Introduction, and in nightingales in the genus *Luscinia.* Mayr (1963) believed that sibling species were relatively uncommon among birds, constituting about 5% of the class. He ascribed the relative paucity of avian sibling species to the importance of vision in the recognition of secondary sexual characters in bird behavior. Mayr believed that sibling species were more widespread in taxa where chemical senses were more highly developed than vision. He made little reference to the possible importance of acoustic differences between sibling species.

It is notable that sibling species are frequent in taxa of marine invertebrates. Knowlton (1993) argues that the number of marine species may increase by an order of magnitude if sibling species are considered, and that our current lack of knowledge about sibling species in the sea arises from our reliance on auditory and visual senses, whereas chemical recognition is widespread in marine species.

VI. Speciation in Cryptic Species That Use Acoustic Signals

In this section, I review possible speciation mechanisms that may account for the diversity of cryptic species that use acoustic signals. I discuss mechanisms that may apply to echolocating bats, to animals other than bats that use acoustic signals for mate attraction, and to all species. In any discussion of speciation in *Pipistrellus pipistrellus*, it is, of course, important to realize that the speciation event separating the two clades was probably ancient, and that differences observed today in, for example, acoustic behavior, may not have been associated with the speciation event.

A. Allopatric Speciation

Allopatric speciation is widely believed to be the most likely mechanism of speciation in most animal taxa (Mayr, 1963, 1977). Several recent studies of cryptic species favor allopatric explanations of speciation (sticklebacks, Schluter and McPhail, 1992; mouse-eared bats, Arlettaz, 1995). Allopatric speciation involves geographical isolation of a population from other populations of the parental species, and acquisition of characters that promote or ensure reproductive isolation once sympatry is reestablished.

A plausible scenario for pipistrelle speciation may be as follows. Imagine that bats in the parent population echolocate at 45 kHz. A small population becomes isolated by, for example, mountain barriers. Perhaps a glaciation event pushed this isolated population into a refuge that ensured its isolation. Bats in the isolated population changed echolocation call frequency to 55 kHz, social call structure altered, but morphological conformity with the parent population was maintained. Perhaps echolocation call frequency changed to exploit a new insect resource encountered by the isolates. As conditions became warmer, barriers between the populations broke down; however, the changes that occurred during isolation caused the previous parent and isolated populations to remain reproductively isolated. Both nascent species then spread over a wide geographic range and avoided competition because they used different call frequencies. This scenario may be testable if the date of divergence of the species could be ascertained by application of a molecular clock. Conditions at the time of divergence could be explored, to determine if geographic separation of populations would have been facilitated by climatic conditions at that time.

B. Sympatric Speciation by Disruptive Selection

As described earlier, differences in call frequencies used in echolocation may allow resource partitioning in bats. Call frequency can therefore be

viewed as a "resource acquisition character" that may be favored to diverge if a new niche becomes available, hence allowing some individuals to exploit the new niche and minimize competition with other animals. Models involving disruptive selection on resource acquisition characters have been described as "competitive speciation" models (e.g., Maynard Smith 1966; Rosenzweig, 1978; Pimm, 1979; Wilson and Turelli, 1986; Wilson, 1989). Disruptive selection favoring extreme resource acquisition characters (e.g., beak size, echolocation call frequency) may promote sympatric speciation if the distribution of resources is not normal (Seger, 1985). What may initially begin as a polymorphism in resource use (Skúlason and Smith, 1995) may result in sympatric speciation if reduced gene flow occurs between sympatric morphs (Bush, 1994).

In pipistrelles, it is easy to understand how a shift in call frequency by some bats may reduce competition with conspecifics if a new niche becomes available. However, one difficulty sometimes raised about competitive speciation models is how the bimodality in the resource acquisition character is maintained. If random breeding occurs, recombination will result in intermediate forms remaining in the population (Felsenstein, 1981). This difficulty has been circumvented by several authors. Pimm (1979) and Wilson and Turelli (1986) modeled scenarios where new niches were invaded by poorly adapted heterozygotes. The heterozygote is later eliminated by homozygotes for the new character, with the result that unfit heterozygotes are replaced. Disruptive selection may result in character divergence if assortative mating occurs between the divergent phenotypes. The evolution of character divergence by disruptive selection and assortative mating of divergent phenotypes might be unlikely (Felsenstein, 1981). A more plausible scenario is that disruptive selection favors divergence in mating traits (such as timing of breeding), and resource acquisition characters are pleiotropic with the mating trait (Rice and Hostert, 1993). If new morphs that arise live in novel habitats away from the parental population, assortative mating among morphs may be promoted via habitat segregation (e.g., Johnson, Hoppensteadt, Smith, and Bush, 1996). Competitive speciation models may be realistic scenarios for the evolution of echolocation call divergence in pipistrelle bats if some of the above assumptions are met.

C.   SYMPATRIC SPECIATION BY SEXUAL SELECTION

Advertisement calls cannot be viewed as resource acquisition characters, and so are unlikely to evolve by competitive speciation. Natural selection imposes tight constraints on the design of echolocation signals in bats: bats that forage in similar habitats show convergence in the design of

echolocation signals, even though the species may be phylogenetically distant (Norberg and Rayner, 1987; Neuweiler, 1989; Fenton, 1990).

Although natural selection will shape the design of acoustic signals in other animals, there is likely to be more scope for signal flexibility than in bats, where echolocation call design is likely to be tightly shaped for orientation and especially for the detection of insect prey. Songflight and social calls of bats should show less stereotypy than echolocation calls, and this has indeed been shown by Fenton (1994), who argued that echolocation calls were less likely to be used as dishonest signals than were social calls.

The ways in which habitat influences the design of relatively low frequency acoustic signals have been studied for temperate (Marten and Marler, 1977) and tropical (Marten, Quine, and Marler, 1977) habitats. How habitat features influence the design of bird song was described by Morton (1975). Although features such as vegetation structure are likely to select for the best frequencies to, say, maximize the range of sound transmission, there will still be scope for flexibility in signal design within a particular frequency window. For example, repetition rate of pulses, pulse duration, and components that describe elaboration of bird song may be little affected by habitat structure; the constraints may be less tight than those shaping the design of bat echolocation pulses. Consequently I predict that scope for variation in the vocalizations of birds, anurans, and insects should be greater than that in bat echolocation. Bat echolocation calls will be shaped strongly by natural selection, making the signals designed optimally for the detection of, for example, different types of insects. The songs of insects, anurans, and birds will probably be shaped more by sexual selection, with female choice for particular signal designs being a potentially strong force shaping divergence in acoustic signals of these groups. Consequently the potential role of sexual selection in the evolution of animals that use acoustic signals in mate advertisement deserves scrutiny.

Can divergence in advertisement calls occur within a population, and hence promote speciation? Several models have been proposed to show how sympatric speciation may occur as the consequence of female choice for elaborate male traits. Most models involve Fisherian runaway selection, with correlated selection between the degrees of male trait elaboration and female response (e.g., Lande, 1981; West-Eberhard, 1983). Lande and Kirkpatrick (1988) proposed a mechanism of sympatric speciation that could occur if female choice of mates was based on ecologically important characters and if more than one ecological niche was available for the species. Lande and Kirkpatrick's model relied on the trait that was being sexually selected also being important for niche differentiation by natural selection, and considered body size as being a trait that would be important both in female choice and in niche differentiation. Wu (1985) developed

a model of sympatric speciation by runaway sexual selection, and concluded that "one would expect to find *occasionally* in natural populations the existence of two or more reproductive units. These units may have diverged very little in their morphological and ecological characters" (p. 79).

Divergence in female preference and male sexual ornaments has been modeled under non-Fisherian conditions by Schluter and Price (1993), when male traits are viewed as handicaps. Although many models of speciation by sexual selection usually involve disruptive natural selection as being important, a recent model (Turner and Burrows, 1995) claimed that sympatric speciation may occur in the absence of disruptive natural selection.

If sexual selection has been important in speciation, then secondary sexual traits should show the most remarkable differences in taxonomic groups containing many species (Andersson, 1994). However, female preferences are predicted to vary less than male traits, and the most plausible examples of sexual selection driving speciation are when closely related species show divergent mate preferences but little post mating isolation (Schluter and Price, 1993). Some authors have argued that acoustic signals will diverge *after* populations that underwent allopatric speciation meet again in sympatry. Signal divergence may be the consequence of reinforcement (the unlikely situation where gene flow between populations still occurs), or more probably reproductive character displacement (no gene flow) when, rather than promoting speciation, acoustic divergence occurs rapidly after speciation (Butlin, 1987). Divergence between song types before reproductive isolation may require assortative mating between females that prefer a particular song type, and males who sing it. Currently, however, there is no convincing evidence for a genetic coupling between signal production and reception in animals. It remains possible that coevolution of characters affecting signal production and reception has occurred, rather than signal and reception characters having a common genetical basis (Butlin and Ritchie, 1989; Boake, 1991). It remains difficult to reject the view that female preferences evolved after speciation.

For species in which sexual selection by female choice on vocalizations is important (insects, anurans, birds), song probably evolves in a less conservative fashion than morphology, and may be subject to faster evolutionary change. Small mutations in song structure may impose less of a cost to the bearer than would small changes in appearance. The groups of passerine birds containing the greatest number of species are those with the most advanced capacity for sound production (Raikow, 1986), suggesting that female choice for song may have contributed to speciation in these taxa. Mistakes during vocal learning may also have contributed to species diversity in passerines, though its importance has been disputed (Fitzpatrick, 1988; Vermeij, 1988; Baptista and Trail, 1992). In a similar way, those taxa

of birds containing the greatest number of species also contain the highest proportion of sexually dichromatic species, a trend expected if sexual selection by female choice increased speciation rate (Barraclough, Harvey, and Nee, 1995). The taxa of frogs that contain large numbers of species appear to be those that detect the widest range of frequencies (Ryan, 1986). Rapid change may occur in song when small populations become isolated, and divergence may occur rapidly in sympatric populations (Lande, 1981; Wu, 1985). Natural selection may constrain an animal's morphological design within tight limits, but sexual selection may elaborate song rapidly by runaway selection. Plumage or coloration may also be constrained by natural selection, especially if an animal's main predators hunt visually. Divergence in song would then occur faster than divergence in appearance, and hence the large genetic differences seen in some cryptic species are not surprising if appearance and morphology are constrained tightly by natural selection.

A potential speciation mechanism that may apply to pipistrelles links sexual selection by females with resource acquisition in males. Females choose male characters that affect resource exploitation, and if the resource acquisition characters are inherited, sexual selection may drive speciation (see also the model of Lande and Kirkpatrick, 1988, described earlier). The mechanism may not be unique to bats, and the way in which female choice may drive resource acquisition in bats is slightly more complicated than in other animals. In bats, one feature of resource acquisition, echolocation call frequency, may be correlated with the character driven by female choice, songflight call frequency. In other animals, female choice may influence the evolution of the resource acquisition character directly (Lande and Kirkpatrick, 1988), and female choice for echolocation call frequency may have this effect in bats.

Hence, female choice for songflight call frequency might drive divergence in echolocation call frequency. The entire population of bats may not be selected to shift to lower echolocation call frequencies because, although some bats would benefit from moving into a new foraging niche (exploitation of larger prey associated with lower call frequencies), others would benefit from reduced interspecific competition by remaining with a higher call frequency and being better able to exploit smaller prey.

D. EVALUATION OF THE SPECIATION MODELS

Allopatric speciation is widely accepted, and should not be dismissed in pipistrelles. However, one problem with allopatric hypotheses concerns finding a reason why call frequency should diverge in allopatry, with the species later becoming largely sympatric. Competitive events in sympatry

may be a more plausible driving force for the divergence in call frequency. Although the adaptive advantages of bimodality in call frequency are easy to visualize, how bimodality would be maintained in an interbreeding population is problematic. There is at present no evidence for assortative mating according to call frequency within phonic types of pipistrelle (Park *et al.,* 1996). Thus, disruptive selection models may involve mechanisms other than assortative mating to maintain character divergence.

Sympatric speciation by sexual selection in pipistrelles has little empirical support. Within phonic types of pipistrelle there is no correlation between songflight call frequency and echolocation call frequency (Barlow and Jones, in press, b), and such a relation might be expected in sexual selection models that involve genetic correlations between echolocation call and songflight call frequencies. Speciation by sexual selection would be restricted to bat species that choose mates on the basis of acoustic cues from songflights: pipistrelles are some of the few bats for which songflight displays are known. Pipistrelle bats may be animals where mate choice depends on resource choice (or vice versa), and such species are prime candidates for possible sympatric speciation (Bush, 1994). An alternative explanation for the differences in call frequency and songflight call frequency between phonic types is that disruptive natural selection favors divergence in echolocation call frequency, and songflight frequencies change as a correlated response. This speciation mechanism could occur without geographical isolation, but would not involve sexual selection by female choice. A further alternative is that divergence in echolocation and songflight call frequencies evolved independently, with divergence in echolocation calls being favored by disruptive natural selection, and songflight calls evolving largely under the influence of sexual selection. Thus, although speciation by sexual selection seems unlikely in pipistrelles, it remains possible for animal species that use acoustic signals for mate attraction, such as birds, anurans, and orthopterans. As in the competitive speciation models, intermediate song morphs must be selected against if extreme song types are to be favored. Genetic coupling of signal type and reception may promote song divergence, but, as described earlier, there is no evidence for coupling to date. It is also important to realise that sexual selection may promote rapid divergence in advertisement calls in small geographically isolated populations. In conclusion, it is likely that sexual selection facilitates acoustic divergence in cryptic taxa *after* divergence, but there is no convincing evidence to date that it promotes divergence *before* populations are isolated.

VII.   CRYPTIC SPECIES, GENETIC DIVERGENCE, AND HIDDEN BIODIVERSITY

Many cryptic species do in fact show small morphological differences, and such subtle differences may influence niche use (e.g., Marchetti, Price,

and Richman, 1995). It would be of interest to determine whether cryptic species that are morphologically indistinguishable (e.g., many *Chrysoperla* lacewings) show any differences in habitat use (especially if habitat divergence promotes assortative mating between morphs), and whether the relative genetic divergence in such species is smaller than that found in cryptic species that show subtle morphological differences (e.g., *Phylloscopus* warblers). Genetic markers allow the relative evolutionary rates of morphological conservatism and acoustic divergence to be quantified in ways that have hitherto been impossible, and should shed more light on the evolutionary reasons why cryptic species proliferate in certain taxa.

Recent advances in molecular biology may allow insights into speciation in cryptic species, and whether cryptic species evolve at similar rates to species that look different in appearance to our eyes. Sequence divergence in mtDNA could be compared between cryptic species and other closely related species that differ in appearance to infer whether cryptic species are more similar genetically, and hence at an earlier stage in their phylogenetic history. If genetic divergence can serve as a molecular clock, then dates for speciation events may be inferred, as may the role of geographical events in driving speciation. As already mentioned, the cytochrome-*b* data for pipistrelles show a large sequence divergence between phonic types, of a magnitude typical of species differences in taxa that are morphologically more obviously different (Fig. 7). Avise and Zink (1988) studied genetic differences within four pairs of avian sibling species where the merits of specific status were unclear. For three pairs (rails *Rallus elegans* and *R. longirostris;* titmice *Parus bicolor bicolor* and *P.b. artricristatus,* and grackles *Quiscalus major* and *Q. mexicana*) results were equivocal, with mtDNA distances typical of extremely closely related species, but overlapping with maximum values reported for some conspecific pairs. However, distance for the dowitchers *Limnodromus scolopaceus* and *L. griseus* was large, and close to maximum values reported for avian congeners (Avise and Zink, 1988).

Genetic differences between cryptic species of *Phylloscopus* warblers have been calculated by Helbig, Seibold, Martens, and Wink (1995). There appear to be parallels with pipistrelle bats in that study. The western *Phylloscopus b. bonelli* and eastern *P. b. orientalis* Bonelli's warblers were conventionally classified as subspecies. The warblers are allopatric, and, although very similar in morphology and apparently in plumage, have very different songs. Genetic differences between the warblers were large, with an 8.3–8.6 sequence divergence in a sequence of cytochrome-*b* of mtDNA. Thus, the warblers were genetically more different than were the shoveler (*Anas clypeata*) and teal (*A. crecca*) ducks, that are obviously different in appearance to humans. Clearly, the two subspecies of Bonelli's warbler merit specific status because of vocal differences (and species-specific re-

sponses to song playbacks; Helb, Bergmann, and Martens, 1982) and be-
cause of the large genetic differences. It is important to emphasize that the
small morphological differences in cryptic taxa such as *Phylloscopus* may
have ecological importance. *Phylloscopus b. orientalis* has longer and more
pointed wings, for example, perhaps as an adaptation to a longer migration
distance (Helbig *et al.*, 1995). Subtle ecological differences occur in *Phyllos-
copus* warblers as a consequence of small morphological differences (Rich-
man and Price, 1992; Marchetti *et al.*, 1995). Species with longer wings
hover less and migrate further, while species with more pointed wings
migrate further and are more arboreal (Marchetti *et al.*, 1995). Before the
revolution in molecular genetics, Mayr (1977) pointed out correctly that
sibling species are not necessarily species that have formed in the recent
past. As the previous discussion outlines, cryptic species can be as distinct
genetically as other species, and may often be cryptic only to human vision.

The average rate of sequence divergence in the entire mtDNA genome
is about 2% per million years in birds and mammals (Wilson *et al.*, 1985).
If the cytochrome-*b* mutation rate approximates that of the entire mtDNA
genome, then the two Bonelli's warblers may have separated from a com-
mon ancestor over 4 million years ago (Helbig *et al.*, 1995). The divergence
of the cryptic pipistrelle species may have occurred at a similar time. Even
if these extrapolations are incorrect, the point still remains that cryptic
species can be as genetically distinct as are more morphologically divergent
congeners (see Henry, 1985a, for a discussion of possible exceptions). There
is potential for considerable hidden genetic diversity in cryptic species, and
some of that may be unrealized as species become extinct through habitat
destruction (Wilson, 1992). At least four new species of *Phylloscopus* were
described in 1992–1993 (Alström, Olsson, and Colston, 1992; Olsson, Al-
ström, and Colston, 1993). The chiffchaff, *P. collybita*, is probably at least
three cryptic species in Europe, each with a distinct male song (Helbig,
Martens, Seibold, Hening, Schottler and Wink, 1996). Our emphasis on
morphological rather than behavioral characters, and our sensory biases
in determining the extent of biodiversity may mean that many cryptic
species remain undiscovered, or may even evade discovery before extinc-
tion. Given the potentially large genetic divergence between some cryptic
species, the amount of genetic diversity as yet undescribed on earth may
be substantially greater than generally assumed.

VIII.   SUMMARY

Animal taxonomy has often relied on separating species by their appear-
ance or by morphological characters. Sometimes behavioral features such

as differences in vocalizations have been overlooked as indicators of species differences. Moreover, humans are limited in sensory abilities. Although we primarily use vision for sensing the world, even our visual capacities are restricted when compared with those of other animals. Such restrictions are important in our appreciation of biodiversity. Our appreciation of diversity in nonvisual signals in animals is limited. Even if animals resemble one another in appearance, they may differ in, for example, acoustic or olfactory signals, and such differences may be important isolating mechanisms between animal species that appear similar to our eyes. Moreover, even our visual perception of the world may fail to detect differences in, for example, animal coloration that may distinguish different species through the eyes of the animals concerned. Hence, sensory limitations in humans probably limit our description of species diversity to species that we can most easily distinguish.

Species that appear similar to us are termed cryptic species, yet many cryptic species use quite different acoustic signals, and the diversity of such animals has only begun to be appreciated since advances in bioacoustical analysis have made their study possible. In many animals, factors such as predation pressure from visual predators may select for conservation in appearance during speciation, while acoustic signals may be free to diversify more rapidly. The taxa of frogs and birds containing the largest number of species are sometimes those with the most complex auditory morphology or song structure. In singing insects, anurans, and birds, sexual selection may in theory drive acoustic divergence rapidly. Speciation by such divergence should involve assortative mating within morphs, however, and whether sympatric speciation can result from female choice remains controversial. It is more likely that female choice would accelerate acoustic divergence in small, isolated populations.

Cryptic species are widespread in bats, and their diversity may be underestimated because of their use of signals above the frequencies of human hearing. Acoustic signals may be important mechanisms for resource partitioning in echolocating bats because, at least in theory, call frequency will determine echo strength from prey of different sizes. Bat acoustic signals are probably more severely constrained in their flexibility by natural selection than are the songs of insects, anurans, and birds, but sympatric speciation may still be possible if the frequency of calls used in mate attraction is coupled to echolocation call frequency, or if differences in echolocation call frequencies are favored by disruptive selection. Assortative mating within morphs may again be a prerequisite for sympatric speciation. Evidence from echolocation calls, social calls, mating associations, morphology, and genetics shows that Britain's commonest bat, the pipistrelle, is actually two cryptic species, and many more cryptic bat species probably await

discovery. Techniques from molecular genetics show great promise in revealing the hidden genetic diversity shown by cryptic species. Studies on warblers and pipistrelle bats suggest that cryptic species are often not recently formed species, and that visual appearance may have been conserved during their evolution, while acoustic divergence has been substantial.

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