Neuroanatomy influences speciation rates among anurans

(amphibian papilla/auditory neurobiology/macroevolution/mate recognition)

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ABSTRACT   Evolutionary divergence among animal courtship signals is an important component of the speciation process. In anurans, the preferential response of females to the mating call of conspecific males often maintains reproductive isolation among populations. Much of the information in the call is initially processed in the inner ear, and there is considerable variation in the structure of this organ among lineages of frogs. This variation is responsible for differences in the frequency range to which frog species are sensitive and thus influences the frequency range over which mating calls can effectively diverge. Data suggest that this influence of neuroanatomy on mating call divergence is partly responsible for the different rates of speciation among lineages of frogs.

The process of animal speciation and the factors that influence the number of species within a lineage are two major areas of interest in modern evolutionary biology (1–6). Many studies investigating differential rates of speciation among lineages have examined the influence of ecological and environmental factors on differential rates of species extinction and survival among lineages (7–9). However, reproductive isolation among populations [one of the major defining characteristics of a species (e.g., ref. 3)] might result from populational differences in courtship signals (10–12). Therefore, factors that have influenced the evolutionary divergence of courtship signals should also influence the rate at which new species have arisen. In this report, I argue that (*i*) there is variation in the structure of the inner ear among different lineages of anurans; (*ii*) due to this structural variation, there are differences among lineages in the range of frequencies (the "frequency window") that can be detected; (*iii*) this variation in the frequency window of the inner ear should result in differences among lineages in the frequency range over which mating calls can effectively diverge; and (*iv*) since mating call divergence is an important component of the speciation process, differences in the number of species in each lineage should be influenced by structural variation of the inner ear. Data available from studies of the neuroanatomy, neurophysiology, behavior, and phylogeny of anurans support this hypothesis.

In anurans it is well known that the mating call is an important behavioral isolating mechanism. There are >2700 species of anurans (13), and no two species have been found to have the same call (14). Calls can differ in frequency (15–18) and/or temporal (19–21) properties. It has been documented extensively that female frogs are attracted preferentially to the mating calls of conspecific males and that this female preference can prohibit or reduce reproductive interactions among species (14, 22). Evidence suggests that during the speciation process in frogs, the evolution of differences in calls usually occurs before the evolution of any postmating isolating mechanisms (e.g., genetic incompatibility) (14). Thus, mating call divergence can be a sufficient component of the speciation process. However, the range of call variation available for evolutionary experimentation is not unlimited. The auditory system of the female frog must be able to detect the frequencies contained in the call in order for the call to be an effective mate attractant. This is true whether the information that discriminates species is coded in frequency or temporal properties of the call: temporal cues can only be processed if they contain frequencies that fall within the frequency window of the inner ear. Therefore, the frequency response characteristics of the auditory system limit the degree to which frequencies in the calls can diverge effectively.

Initial neural processing of calls takes place in the inner ear. Anurans possess two inner ear organs that are especially sensitive to airborne sounds: an amphibian papilla (AP) and a basilar papilla (BP). The AP is innervated by one or two populations of fibers from the VIIIth cranial nerve. One population is sensitive to low-frequency sounds, and the other, if it occurs, responds to middle-frequency sounds. Fibers innervating the BP are sensitive to higher frequencies. In all species thus far examined, either one or two of these windows of frequency sensitivity match the frequency spectrum of the conspecific advertisement call. Only the bullfrog (*Rana catesbeiana*) is known to produce a call with substantial energy in the low range of frequency sensitivity of the inner ear (22). This probably is due to the large size of the frog; larger frogs produce calls with lower frequencies (23, 24). For all other species examined, call detection is limited to the middle and/or high windows of frequency sensitivity of the inner ear (22).

Lewis (25–27) examined the AP of nearly 80 species of anurans and showed that there are dramatic differences in the structure of this organ among species. This variation is of two sorts. Primitive anurans possess only one patch of sensory epithelium in the AP. All other anurans have two patches of sensory epithelium, but there is also variation in the occurrence and the degree of the extension of the caudal-most section of the papilla. Salamanders (order Urodela) possess only a single patch of sensory epithelium in the AP (25–27). This order is a sister group to the order Anura; thus, the cladistic method of outgroup comparison (28) suggests that the single patch of sensory epithelium is the primitive state of the anuran AP. Lewis (27) divided the known variation in the structure of the AP into four classes or character states: (A) one-patch papilla; (B) two-patch papilla with the posterior patch ending abruptly at the tectorial curtain; (C) two-patch papilla with the posterior patch extending beyond the tectorial curtain, without reversing its curvature; (D) two-patch papilla with the posterior patch reversing its curvature just posteromedial to the tectorial curtain and extending in a caudal direction (Fig. 1).

Variation in the structure of the AP has consequences for hearing abilities. Lewis *et al.* (29) showed that the AP of the

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Abbreviations: AP, amphibian papilla; BP, basilar papilla.
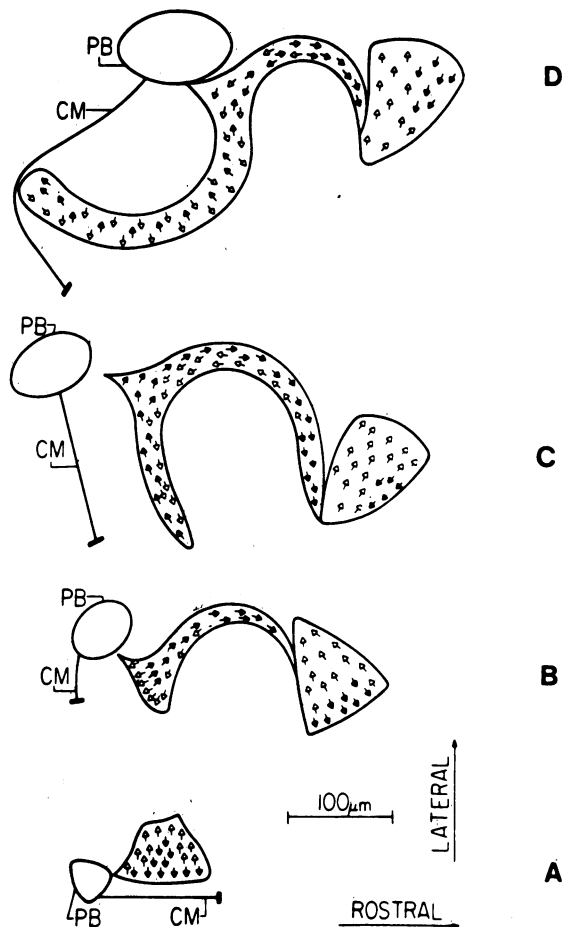*Present address: Department of Zoology, University of Texas, Austin, TX 78712.

FIG. 1. Sketches of the AP of four anurans representing four character states. (A) Ascaphus truei (Ascaphidae); (B) Bombina orientalis (Discoglossidae); (C) Scaphiopus couchi (Pelobatidae); (D) Kassina senegalensis (Hyperoliidae). PB, transected papillar branchlet of the VIIIth nerve; CM, contact membrane separating the papillar chamber from the amphibian periotic canal [reproduced with permission from Lewis (26)].

Table 1. Character states of the AP

Character state	Family	Extinct genera	Extant Genera	Extant Species	Species per character state
A	Ascaphidae	2	1	1	
A	Leiopelmatidae	1	1	3	4
B	Discoglossidae	5	4	9	9
C	Pipidae	5	4	17	
C	Pelobatidae	3	8	49	66
D	Leptodactylidae	2	41	635	
D	Dendrobatidae	0	3	60	
D	Hylidae	3	33	560	
D	Bufonidae	1	19	277	2489
D	Ranidae	2	45	586	
D	Hyperoliidae	0	14	54	
D	Rhacophoridae	0	14	96	
D	Microhylidae	0	58	221	

Character states of the AP are from Lewis (25–27). The combination of anuran families and character states is determined from Fig. 3. The number of species and genera in each family is from Dowling and Duellman (ref. 13; see legend to Fig. 1).

bullfrog (a character state D papilla) is tonotopically organized—that is, nerve fibers traced to the caudal end of the AP are responsive to higher frequencies (analogous to the middle-frequency peak), and those traced to the rostral end are responsive to lower frequencies. Fibers traced to intermediate areas of the papilla are responsive to intermediate frequencies. Lewis (25–27) suggested that due to the tonotopic organization of the AP, a caudal extension of the papilla corresponds to an extension of the higher frequency end of the range of sensitivity of the AP. This hypothesis is supported by neurophysiological data. Scaphiopus couchi (Pelobatidae) lacks a population of VIIIth nerve fibers sensitive to middle-frequency sounds (30). The lack of middle-frequency-sensitive fibers seems to be due to the lack of the caudal extension of the AP in this species (Table 1); the neural substrate needed to perceive these frequencies is simply not available to frogs in this lineage (25–27). Thus, mating call discrimination in this species is restricted to the frequency window of the BP. Therefore, variation in papilla structure should strongly influence the amount of potential variation in the mating calls of these frogs (Fig. 2). Neurophysiological recordings from Ascaphus truei (Ascaphidae), Bufo debilis and Bufo americanus (Bufonidae), Hyla versicolor (Hylidae), and Kassina senegalensis and Kassina maculatus (Hyperoliidae) also support this relationship between structure and function (R. R. Capranica, personal communication).

The AP seems to have evolved in a conservative manner. Character state A is the least complex, and because it is shared with the sister group—order Urodela—it is thought to be the primitive state. The complexity of the AP increases from presumed primitive to advanced lineages of anurans (Fig. 3). Therefore, different lineages exhibit different character states of the AP. If call divergence is an important component of the speciation process and if auditory morphology and physiology influenced the degree to which calls diverged, there should be some relationship between the character state of the AP and the number of species in a lineage. This can be tested by comparing the total number of species among lineages with different character states of the AP (Table 1). As the complexity of the AP increases among lineages (i.e., evolves from state A to state D), so does the number of species. The same trend holds if the number of species per family is compared (although this is a less appropriate test of the hypothesis): families with character state D always have more species than those with character state C; C has more than B; and B has more than A (Table 1). There are no exceptions to this trend. Of course, it is possible that the concordance of character states of the AP and species number within lineages is spurious.

The number of species in a lineage is a function of the speciation rate and of species extinctions. The above results would be confounded if extinction rates among lineages showed the opposite trend exhibited by species numbers. The fossil record does not provide much data on extinctions for anuran species. However, there are data for extinction of genera, and these data give some indication as to the prevalence of extinction among lineages. Although there is a trend in the number of extinct genera among lineages, this trend is in the same direction as that of species number (Table 1). Therefore, the differences in the number of species among lineages does not result from differential extinction rates. Since the more speciose lineages have radiated more recently, and thus have had less time for speciation to take place, this suggests that the differences in species number must be due to differences in rates of speciation among lineages.

The evolution of the anuran AP exhibits an evolutionary trend of increased complexity. An interpretation of most accepted anuran phylogenies combined with the distribution of character states suggests that there are no known evolutionary reversals in the trend of increasing complexity of the AP. Therefore, the hypothesized influence of the neuroanatomy of the inner ear on the number of species within a lineage could be confounded by other characters that exhibit similar

Evolution: Ryan

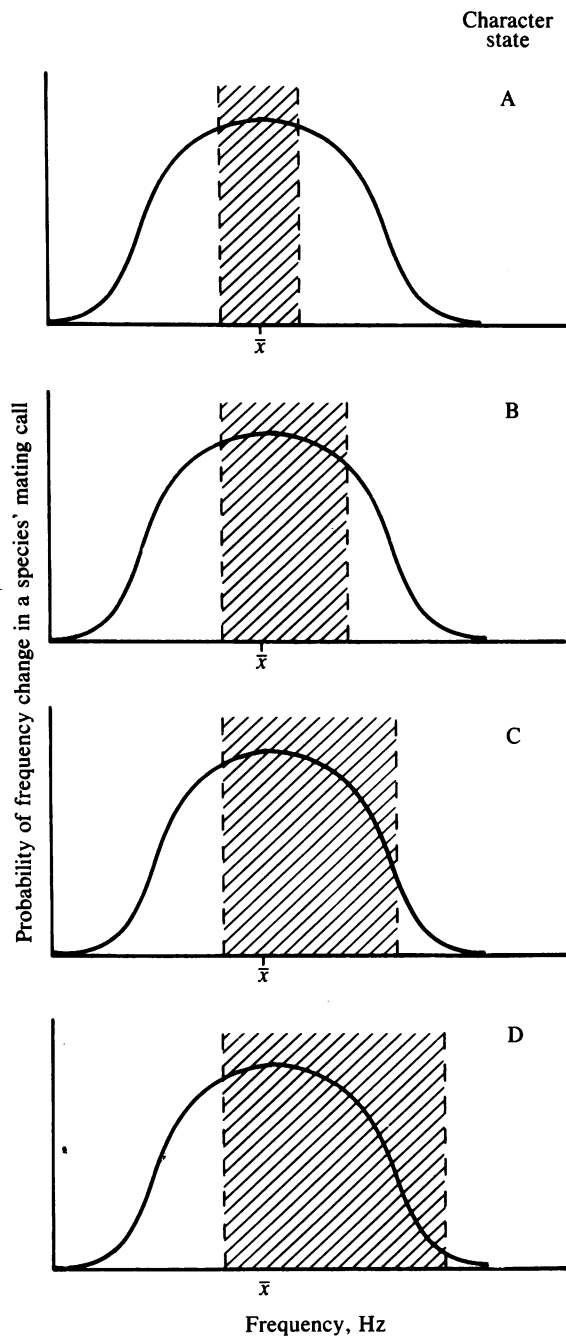*Proc. Natl. Acad. Sci. USA 83 (1986)* 1381

FIG. 2. A graphical representation of the hypothesized mechanism by which variation in the AP might restrict the opportunity for evolutionary divergence of mating calls. For each character state of the AP, the hatched rectangle represents the range of frequencies to which the AP is potentially sensitive and the curve is a density function representing the probability of a mutation changing the frequency of the mating call (represented by $\bar{x}$) to other frequencies represented on the horizontal axis. (The hypothesis does not assume a Gaussian probability density function.) From state A to D the frequency range of the AP encompasses higher frequencies and thus also encompasses a larger portion of the probability density function.
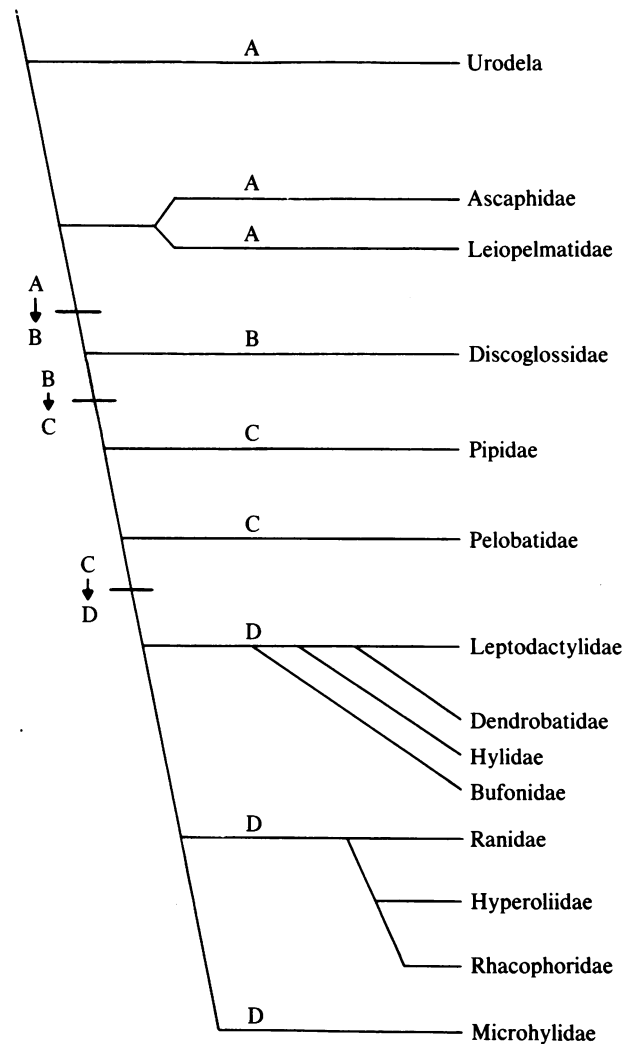

FIG. 3. The phylogeny of frog families follows Dowling and Duellman (13) with one exception: I do not combine the families Ascaphidae and Leiopelmatidae as they do, since the only justification for combining these families is based on shared primitive characters. The occurrence of evolutionary changes of the character states is deduced from the familial distribution of the character states of AP among the nearly 80 species examined by Lewis (25–27). From this figure, a character state can be assigned to any species examined unequivocally with the following exceptions: two discoglossids have an intermediate B–C character state and one pelobatid has an intermediate B–D character state. The evolutionary significance, if any, of these intermediate states is not known.

trends and that might also contribute to rates of speciation. There does not appear to be any such characters. Trueb (31) identifies evolutionary trends in several osteological characters: neurocranium, vertebral column, pectoral girdle, pelvic girdle, appendages. It is not readily apparent how the different states of these characters would influence the speciation process. Two important characteristics of anuran mating systems (32), the length of the breeding season and the amplectic period, do not exhibit any clear evolutionary trends

(33), and a review of anuran reproductive patterns (e.g., internal or external fertilization, direct development, parental care, site of egg deposition) also does not exhibit any evolutionary trends within the order Anura (34).

I suggest that the neuroanatomy of the inner ear has had a direct influence on the variation in numbers of species among lineages as opposed to the alternative hypothesis that differences in neuroanatomy are merely correlated with other factors that influenced species number. Since the evolution of the anuran inner ear exhibits a clear evolutionary trend, it is possible, although speculative, that the inner ear has responded to directional selection. Lewis (27) suggested that this trend reflects the evolution of a traveling wave structure, perhaps functionally analogous to the mammalian cochlea. He discusses potential selective advantages for coding frequency with this type of structure relative to a resonating structure. Another potential selective advantage is that the more complex AP and its greater range of frequency sensitivity might allow the individual to perceive acoustically more

of its environment, including potential predators and prey. The suggestion that observed evolutionary changes in AP structure represent adaptive modifications has no bearing on the relationship between neuroanatomy and speciation that I propose (see also refs. 8, 11, and 12). The cause and effect relationship I propose could (only) be reversed if one proposed a species-selection argument.

There are a number of other deterministic and stochastic factors that undoubtedly influenced differences in species numbers among lineages, and I am not suggesting that variations in the neuroanatomy of the inner ear determined species number. However, I do suggest that since divergence of courtship signals is an integral component of the speciation process, if the neural substrate restricts or constrains an aspect of signal divergence it should influence the rate of speciation. Further, I suggest that this possible interaction between structure, function, and major patterns of evolution be considered in other organisms in which courtship is restricted primarily to a single sensory modality.

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