

Has the evolution of complexity in the amphibian papilla influenced anuran speciation rates?

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Abstract

For anurans, increasing complexity of the inner ear has been correlated with speciation rates. The evolution of a complex amphibian papilla (AP) is thought to have facilitated speciation by extending the range of frequencies over which mating calls may diverge. Although this example has been proposed to represent a key innovation, the mechanism by which the AP is thought to promote speciation makes the questionable assumption that anurans generally use the AP for detection of their mating calls. This study uses mating calls from 852 species to test this assumption. Surprisingly, the calls of most species are not detected by the AP but by a second organ, the basilar papilla (BP). This refutes the role of AP complexity in facilitating call divergence and hence, speciation. Future research into the evolution of acoustically mediated reproductive isolation should focus instead on the BP as it may play a more critical role in anuran speciation.

Introduction

Exceptionally diverse taxa are thought to possess some trait or suite of traits which have promoted their rapid speciation. Such traits, termed key innovations, have often been hypothesized as mechanisms generating exceptional diversification (Miller, 1949; Simpson, 1953; de Queiroz, 2002). However, most key innovation arguments are based on the plausibility of the trait's promoting speciation rather than tests of these hypotheses (Cracraft, 1990; de Queiroz, 2002). A given trait may be hypothesized as a key innovation if there is (1) reason to suspect a causal connection between the trait and the success of the taxon and (2) a positive correlation between trait presence and speciation rate (Vermeij, 1988). Structures involved in intraspecific communication are often proposed as key innovations as they tend to be highly developed in species-rich taxa (West-Eberhard, 1983). As an example, traits influencing vocalization and learning ability in passerine birds have been proposed as key innovations facilitating the rapid

speciation of this group (Raikow, 1986; Fitzpatrick, 1988; Vermeij, 1988, but see Baptista & Trail, 1992).

Ryan (1986b) made a similar argument that the evolution of the morphologically complex amphibian papilla (AP), an organ in the anuran inner ear, has facilitated speciation by increasing the range of frequencies over which male mating calls may diverge and still be perceived by females. Intraspecific divergence in mating signals, whether generated by sexual selection, drift, pleiotropic effects, or other evolutionary processes, when correlated with divergence in female preference can lead to reproductive isolation and speciation (Lande, 1981; Thornhill & Alcock, 1983; West-Eberhard, 1983, 1984; Kaneshiro & Boake, 1987; Iwasa & Pomiankowski, 1995; Payne & Krakauer, 1997; Ptacek, 2000). Reproductive isolation among anuran populations is often maintained by females' preferences for the calls of conspecific males (Blair, 1964; Gerhardt, 1994; Giacoma & Castellano, 2001). This suggests that selection has likely favoured the evolution of a specialized neural auditory mechanism for extracting time and frequency information from such calls (Hall, 1994). The potential for reproductive isolation via differences in male calls and female preferences has been demonstrated for anurans among sympatric (Littlejohn, 1965; Fouquette, 1975; Loftus-Hills & Littlejohn, 1992) and parapatric (Hoskin *et al.*, 2005) sister species as well as

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among allopatric populations of the same species (Ryan & Wilczynski, 1991; Wilczynski *et al.*, 1992; Hoskin *et al.*, 2005). These studies support the plausibility of the evolution of a complex AP facilitating anuran speciation via the mechanism proposed by Ryan (1986b).

Morphological complexity of the amphibian papilla

Comparisons of the AP of 80 species from 13 anuran families (Lewis, 1978, 1981a,b, 1984; Lewis *et al.*, 1992) revealed that primitive anurans have just one patch of sensory cells in the AP whereas all other anurans have two, the second of which varies in length. Lewis (1984) subsequently classified each anuran examined as having one of the following morphological classes of the AP: (A) one-patch papilla; (B) two-patch papilla with the posterior patch ending at the tectorial curtain; (C) two-patch papilla with the posterior end extending beyond the tectorial curtain with no reversal in curvature; (D) two-patch papilla with the posterior patch reversing its curvature posteriomedial to the tectorial curtain and extending in a caudal direction (Fig. 1). The anuran AP is tonotopically organized, meaning that nerve fibres at the rostral end of this organ sense lower frequencies whereas those at the caudal end sense higher frequencies (Lewis *et al.*, 1982; Lewis & Leverenz, 1983; Simmons *et al.*, 1994; Smotherman & Narins, 1999). Thus as the AP increased in complexity (i.e. from class A to class D) this organ's range of frequency sensitivity also increased (Lewis, 1977, 1981b).

The information in a male anuran's call is initially processed in the inner ear of a female (Ryan, 1986a,b)

where sensitivity to airborne sound extends over the frequency range of about 100–4000 Hz, depending on species and body size (Hetherington, 1992). The inner ear contains two organs primarily sensitive to airborne sounds: the AP and the basilar papilla (BP). The AP is sensitive from about 100 to 1250 Hz in most advanced amphibians (Smotherman & Narins, 2000) whereas the BP is sensitive to higher frequencies (Smotherman & Narins, 2000). The frequencies to which one, or both of these organs are most sensitive tend to match the dominant (most emphasized) frequencies in male mating calls (Capranica, 1965; Loftus-Hills, 1973; Walkowiak *et al.*, 1981; Ryan, 1986a,b; Zakon & Wilczynski, 1988; Lewis & Narins, 1999; Gerhardt & Huber, 2002). This relationship between call frequency and auditory sensitivity is presumed to hold for all anurans and has been termed the 'matched spectral filter hypothesis' (Frishkopf *et al.*, 1968). The range of usable mating frequencies is therefore limited by both the ranges of male vocalization ability and female auditory sensitivity.

Apparent support for sensory complexity as key innovation

Ryan (1986b) showed that as the complexity of the AP increased among lineages (i.e. from morphological class A to D), the number of species per lineage also increased (Fig. 1). This difference in species numbers among lineages does not appear to be the result of differential extinction rates as this trend remains robust with the inclusion of fossil taxa. Instead, this trend appears to indicate an increase in speciation rate throughout anuran history correlated with the evolution of a complex AP (Ryan, 1986b). Unfortunately the anuran phylogeny is not known in sufficient detail to test the statistical association between AP complexity and speciation rates. However, even if a significant correlation was found, this would not necessarily demonstrate that the evolution of AP complexity indeed provided the mechanism by which speciation rates have increased. In order for the increased sensitivity range of a complex AP to influence anuran speciation rates in the way Ryan (1986b) proposed, species must use the AP to detect their mating calls rather than the BP. As Ryan's (1986b) study is often cited as an example by which a sexually selected key trait might influence speciation rates, the current study was undertaken to assess the validity of this assumption.

To test the hypothesis that species with a complex AP tend to call in the range of sensitivity of this organ, I examined patterns in mating calls of 852 anuran species representing the four morphological classes of the AP (Figs 1 and 2). If AP complexity has indeed provided the mechanism for increasing speciation rates, most species with complex AP should call within the range of sensitivity of this organ and not within the range of sensitivity of the BP. Furthermore, if the increased range of sensitivity of a complex AP has facilitated the evolution of




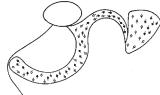
Morphological class Posterior → anterior	Genera		Species
	Extinct	Extant	Extant
 A	3	2	6
 B	8	4	21
 C	12	21	168
 D	44	327	4856

Fig. 1 Illustrations of the four morphological classes of the amphibian papilla (AP) (modified and reproduced with permission from Lewis, 1984) along with the estimated number of extant genera and species and extinct genera possessing each class of papilla. Numbers of extant species and genera are from (Frost, 2004). Extinct genera are from fossil data in Duellman & Trueb (1986). These numbers have been modified from those given in Ryan (1986b) to reflect current numbers of taxa. The frequencies to which AP hair cells are sensitive increase from anterior to posterior.

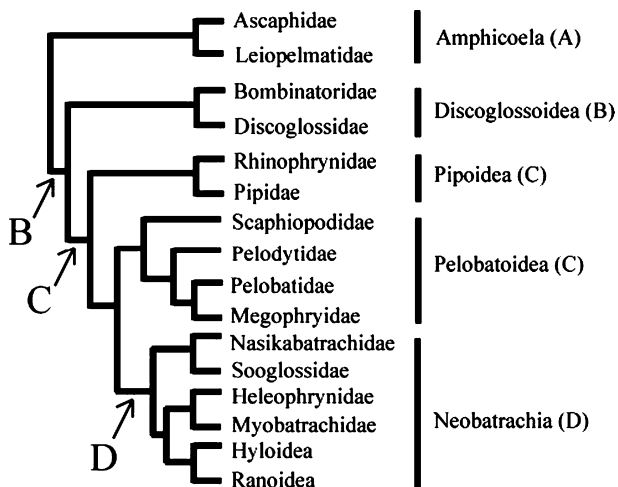


Fig. 2 Phylogeny of anuran familial relationships coded by amphibian papilla (AP) class. Relationships in the Amphicoela, Discoglossoidea, Pipoidae and Pelobatoidea follow Roelants & Bossuyt (2005). Placements of Nasikabatrachidae, Sooglossidae, Heleophrynidae, and Myobatrachidae and definitions of the clades Ranoidea and Hyloidea follow Biju & Bossuyt (2003). Families belonging to Ranoidea and Hyloidea have been lumped together as all possess class D APs. Character state designations and transitions follow Ryan (1986b) and Lewis (1984).

reproductive isolation among anuran species with a complex AP, the proportion of species using the AP for the detection of their mating calls should increase with each increase AP complexity (i.e. from class A to D, see Fig. 1). This is because each increase in AP complexity is thought to have increased the range of available frequencies for mating calls, and hence the potential for call variation leading to reproductive isolation.

Materials and methods

Three aspects of male mating calls were collected from the literature for 852 anuran species: (1) dominant (most emphasized) frequencies, (2) frequency ranges and (3) call complexity (i.e. calls were coded as simple or complex. Complex calls were coded as being either frequency modulated, or containing multiple pitches of different frequencies). For most species, dominant frequencies were reported directly in the literature whereas for some this information was extracted from power spectra (frequency vs. amplitude plots). Dominant call frequencies were included in these analyses as females most commonly respond and are thought to have evolved the greatest sensitivity to these frequencies (Frishkopf *et al.*, 1968; Ryan, 1986b; Ryan & Wilczynski, 1988). High and low frequencies were also included in the analysis to determine which of the auditory papillae were stimulated by the call. Body sizes of the calling individuals, or an average male body size for the species if

no size measurements were reported in the call literature, were also recorded.

Species were coded as either AP or BP depending on whether the frequencies contained in their calls fell into the range of sensitivity of the AP or BP respectively (Fig. 1). Both intra- and inter-generic comparisons have demonstrated that in species with longer, more complex APs the AP is also sensitive to a wider frequency range (Capranica & Moffat, 1975; Lewis, 1981b). Species with class A through C APs have been demonstrated to have AP sensitivity to <1000 Hz (class A to 600 Hz based on *Ascaphus truei*, Lewis, 1981a, class C to 800 Hz based on *Scaphiopus couchi*, Zakon & Wilczynski, 1988 and Capranica & Moffat, 1975). For class D species, frequency sensitivity of the AP may extend to 1600 Hz (Lewis & Narins, 1999). As AP sensitivity information is available for only a handful of species and not for all four AP classes, call frequencies were coded as AP or BP in two ways. First, class A, B and C species with call frequencies <1000 Hz were classed as AP, as the available information indicates the upper end to their AP sensitivity is <1000 Hz. For class D species the upper end of the AP range was set at 1600 Hz as this is the highest reported frequency detected by the AP for any class D species. Secondly, as a more conservative measure of AP use, due to the limitations of the available sensitivity data, all species (classes A–D) were considered to have AP range calls if their call frequencies fell at or below 1600 Hz. In cases where the dominant frequency was reported as a range of frequencies which overlapped the ranges of sensitivity of both papillae, the median of this range was recorded and the species was coded as APBP indicating the possible use of both the AP and BP in call detection and mate choice.

In order to regard species, despite being part of a hierarchically structured phylogeny, as independent data (Felsenstein, 1985) I assumed that call dominant frequencies respond quickly to natural selection and thus are not phylogenetically autocorrelated. Evidence that bird song frequencies (analogous characters to anuran call frequencies) are free from phylogenetic inertia (Rheindt *et al.*, 2004) support this assumption, as does the occurrence of call frequency shifts among anuran populations under different environmental pressures (Nevo & Capranica, 1985; Gerhardt, 1994; Feng *et al.*, 2002; Lardner & bin Lakim, 2002).

Although the anuran phylogeny is not known in sufficient detail to apply a phylogenetic comparative method for all species, I did test the assumption of phylogenetic independence of call frequencies and snout–vent lengths on subsets of data from five molecular phylogenies (*Physalaemus pustulosus* species group: Cannatella *et al.*, 1998; genus *Limnodynastes*: Schauble *et al.*, 2000; genera *Crinia* and *Geocrinia*: Read *et al.*, 2001; Hyloidea: Darst & Cannatella, 2004 and the genus *Pseudacris*: Moriarty & Cannatella, 2004). A test for serial independence was performed for each phylogeny using

the program PHYLOGENETIC INDEPENDENCE (Abouheif, 1999), shuffling the original data 1000 times and randomly rotating each node 1000 times. The test for phylogenetic autocorrelation was two-tailed as call frequency and body size could be either positively or negatively correlated with phylogeny. In addition, more conservative analyses were performed using one, randomly chosen species per genus under the assumption that any phylogenetic effect on body size or call frequency would not be present above the genus level. Although the significance of statistical tests which use all species' calls and body sizes as independent data may be inflated by phylogenetic autocorrelation, the significance of analogous tests using a single species per genus should not be so affected.

Results

The test for serial independence indicated that call frequencies were not positively or negatively phylogenetically autocorrelated ($P > 0.025$) except for the *Phyllsalaemus pustulosus* ($P = 0.0113$) species group. Snout-vent lengths were also not autocorrelated except in the case of *Crinia* and *Geocrinia* ($P = 0.003$). For the remaining three phylogenies tested, which represent taxonomic scales from a single genus to all of Hyloidea, both call frequencies and snout-vent lengths were phylogenetically independent. These results suggest that the need to correct for phylogeny is limited to comparisons among closely related species (e.g. *P. pustulosus* species group) and not among distant taxa. As congeners were included in this study for 91 of the 153 sampled genera, all analyses were additionally performed using one, randomly selected species per genus in an effort to correct for the potential effect of phylogenetic inertia on body size and call frequency. This did not qualitatively alter the results of this study.

As none of the species in AP class A are thought to give mating calls they are not included in the call frequency results. Call frequency distributions for each of the remaining morphological classes (Fig. 3) show that the range of frequencies used in calls of species in classes B, C and D increases with AP complexity. However, fewer than 30% of species (31% if only one species per genus is considered, see Table 2) with the most complex AP (class D) have call dominant frequencies in the AP sensitivity range (Table 1). Class B contains the greatest percentage of species with call dominant frequencies in the AP range, regardless of whether the AP range is considered to extend to 1000 or 1600 Hz or whether one or multiple species per genus are considered. The proportion of species whose calls merely contain frequencies in the AP sensitivity range (dominant frequency may be sensed by either organ) is also highest for class B, where over 50% of sampled species' calls contain frequencies detected by the AP. This result holds if only one species is considered per genus.

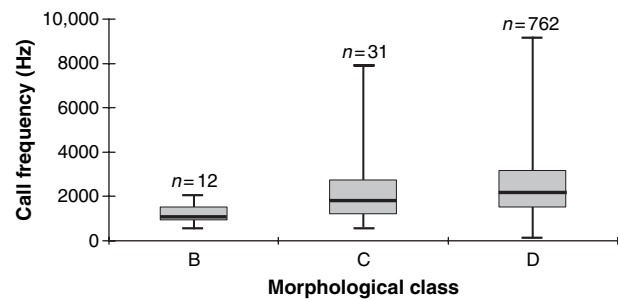


Fig. 3 Boxplots of call dominant frequencies for species in amphibian papilla (AP) classes B, C and D. As the AP increases in complexity (i.e. from class B to class D) the range of frequencies used in mating calls also increases. However, the number of species is not spread evenly among morphological classes. Sample sizes are given above boxplots.

Table 1 Numbers of sampled species in each amphibian papilla (AP) class with call frequencies detected by either the AP or basilar papilla (BP). Species coded as APBP are included in both AP and BP columns. For AP classes B and C, results are included for analyses where AP < 1000 Hz and AP < 1600 Hz (more conservative estimate). The proportion of species with AP contained frequencies differs significantly among morphological classes for both AP estimates (conservative or not). However, when only dominant frequencies are considered, proportions differ only when using the more conservative AP estimate.

AP class	Dominant frequency*		Contained frequencies†		Mute species	Species sampled/extant
	AP	BP	AP	BP		
A	0	0	0	0	5	5/6 (83%)
B‡	5 (42%)	7	9 (58%)	7	0	12/21 (57%)
B§	10 (83%)	2	12 (100%)	5		
C‡	6 (19%)	25	6 (19%)	28	0	31/168 (18%)
C§	15 (48%)	16	15 (48%)	17		
D	221 (27%)	542	297 (30%)	589	41	804/4856 (17%)

*Organ(s) which is/are stimulated by the dominant frequency of the mating call.

†Organ(s) which is/are stimulated by any frequency contained in the call.

‡AP < 1000 Hz, BP > 1000 Hz.

§AP < 1600 Hz, BP > 1600 Hz (conservative estimate).

The proportion of species utilizing the AP for detection of their mating call varies among morphological classes for the conservative AP classification (when all classes were assumed to be sensitive up to 1600 Hz), regardless of whether dominant call frequencies ($\chi^2_2 = 21.32$, $P < 0.0001$) or all frequencies contained in the call ($\chi^2_2 = 12.31$, $P = 0.0021$) are considered. However, if classes B and C are assumed to have APs sensitive to only 1000 Hz, there is no difference among classes in terms of the proportion of species with AP-range call dominant frequencies ($\chi^2_2 =$

Table 2 Numbers of sampled species in each amphibian papilla (AP) class with call frequencies detected by either the AP or basilar papilla (BP) when one species is randomly selected per genus to account for potential phylogenetic inertia in call frequency. The proportion of species with AP-contained frequencies does not differ significantly among morphological classes for either AP estimate (conservative or not). However, when only dominant frequencies are considered, proportions differ significantly when using the more conservative AP estimate.

AP class	Dominant frequency*		Contained frequencies†	
	AP	BP	AP	BP
A	0	0	0	0
B‡	1 (33%)	2	2 (66%)	2
B§	3 (100%)	0	3 (100%)	1
C‡	2 (22%)	7	2 (18%)	9
C§	6 (66%)	4	6 (66%)	5
D	40 (31%)	89	53 (41%)	100

*Organ(s) which is/are stimulated by the dominant frequency of the mating call.

†Organ(s) which is/are stimulated by any frequency contained in the call.

‡AP < 1000 Hz, BP > 1000 Hz.

§AP < 1600 Hz, BP > 1600 Hz (conservative estimate).

2.330, $P = 0.3119$). The per cent of species whose calls contain any AP-range frequencies does vary among classes ($\chi^2 = 7.560$, $P = 0.0228$). If only one species per genus is considered (Table 2), AP use differs by class only when the conservative AP range estimate is used and dominant call frequencies are considered ($\chi^2 = 9.27$, $P = 0.0097$). However, none of these differences support the hypothesis that species with more complex APs call more often within the AP sensitivity range as AP-range calls are more common in class B than in classes C or D, which have more complex AP.

In morphological class D (Fig. 4), seven of 16 families sampled do not contain species calling in the AP range. Additionally, in all but one class D family (Rhacophoridae) fewer than 50% of sampled species call in the AP sensitivity range. Within some class D families the distribution of AP- or BP-use differed greatly among subfamilies and genera. As an example, *Bufo* and *Schismaderma* were the only bufonid genera with species calling in the AP range. Marked differences in AP use were also found between subfamilies of Myobatrachidae and Hyperoliidae.

The regressions of log (dominant frequency) on log (snout-vent length) for all AP classes together ($F_{1,700} = 619.54$, $P < 0.001$), as well as for classes C ($F_{1,27} = 27.54$, $P < 0.001$) and D ($F_{1,659} = 588.96$, $P < 0.001$) individually, were highly significant (Fig. 5). The effect of body size on frequency was not significant ($F_{1,10} = 0.433$, $P > 0.1$) for morphological class B, likely due to small sample size. The slope of

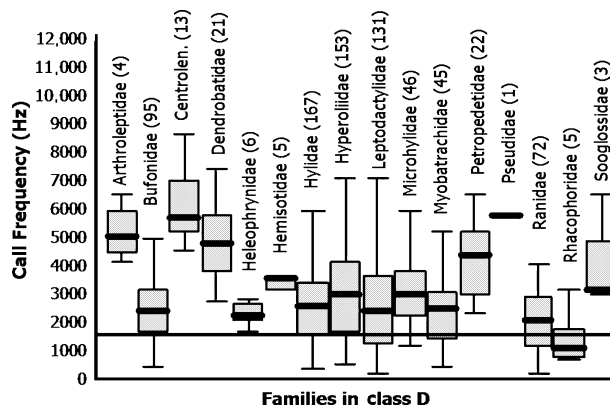


Fig. 4 Boxplots of call dominant frequency for 16 sampled families possessing class D amphibian papillae (AP). The horizontal bar at 1400 Hz separates frequencies sensed by the AP (below the bar) and those sensed by the basilar papilla (above the bar). Families are listed above their respective boxplots, followed by the sample sizes in parentheses.

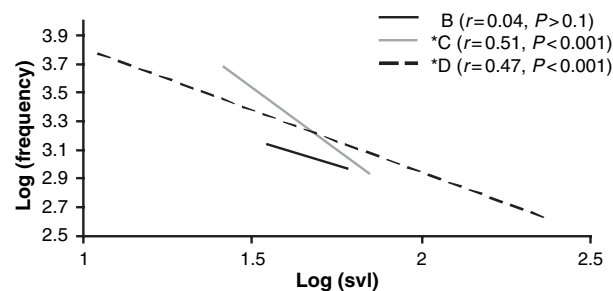


Fig. 5 Linear regressions of log (call frequency) on log (body size) for all sampled species in each amphibian papilla class. The regressions for classes C and D were significant. However, the regression for B was not, likely due to small sample size. Regressions for classes C and D had significantly different slopes and intercepts as indicated by the asterisk.

the frequency-body size regression differed (Sokal & Rohlf, 1981, pairwise test of slopes p. 507, $P = 0.045$) between classes C and D. An ANOVA performed on the residuals from the linear regressions for morphological classes B, C and D showed no differences among population means ($F_{2,701} = 0.2241$, $P > 0.1$) indicating that after the effect of body size was removed, dominant frequency means did not differ among morphological classes. The regression of log (dominant frequency) on log (snout-vent length) for all classes together ($F_{1,123} = 148.703$, $P < 0.001$) and for class D alone ($F_{1,112} = 142.090$, $P < 0.001$) remained significant when only one species per genus was used. However, the significance of the regressions for classes B and C could not be tested as too few genera were available to be sampled.

Discussion

As Ryan (1986b) predicted, the range of dominant frequencies used in anuran mating calls increased as AP became more derived. However, this increase has been mostly to the upper end of the range, outside the sensitivity of the AP. This calls into question the purported causal link between AP complexity and increasing speciation rates. If the evolution of AP complexity played a role in promoting speciation we would expect species with a complex AP to call at frequencies which stimulate the AP rather than the BP. This is not the case for the majority of species with complex AP, whether analyses using single or multiple species in each genus are considered. Although the extended range of sensitivity of a complex AP is undoubtedly important for mate recognition in the species whose calls directly exploit this range, it does not appear to be important for the majority of species which have class B, C, or D APs and call in the BP range of sensitivity.

As the vast majority of species exhibit the most complex AP state, and the majority of those call only within the range of sensitivity of the BP, it appears that the evolution of the BP rather than the AP may have played an important role in the detection of mating signals over anuran history. This refutes the idea proposed in Ryan's (1986b) often-cited study that the evolution of a complex AP has acted as a key innovation facilitating anuran speciation. The increase in call frequency range documented in this study and predicted in Ryan's (1986b) study may be simply a byproduct of the increase in species numbers from AP class A to D as more speciose groups may contain species that are also more diverse.

Alternative links between AP complexity and speciation

It is worthwhile to consider that the evolution of a complex AP could have favoured call diversification and hence, speciation without being directly linked to mating call frequencies. For example, the evolution of a complex AP may have released BP tuning from natural selection if the AP replaced the BP as the primary auditory mechanism for sensing predators, competitors, heterospecifics, or prey. This would leave the BP free to function primarily in mate recognition and we would then expect

tighter coevolution between BP tuning and call frequency in species with a complex AP. Unfortunately, auditory tuning curves have only been published for a few species with other than class D AP (see Gerhardt & Schwartz, 2001) making it impossible to test whether the correlation between BP tuning and call frequency is tighter in lineages with more complex APs.

Alternatively, a complex AP may have facilitated anuran speciation by enabling more sophisticated auditory processing and thus permitting the evolution of more complex calls. Anuran mating call complexity can take many forms, including frequency and amplitude modulations, variations in temporal structure, and the incorporation of multiple note types within a single call. As the BP allows better resolution of temporal and amplitude modulations than the AP (Zakon & Wilczynski, 1988) it is unlikely that the evolution of a complex AP led to the evolution of these types of call complexity. The AP is, however, thought to allow pitch discrimination whereas the BP is not (Lewis, 1984; Ryan, 1988) so that frequency modulations cannot be decoded by the BP. Perhaps the evolution of a complex AP facilitated anuran speciation by enabling pitch discrimination and hence the ability to recognize multiple frequency components in complex mating calls?

The per cent of species sampled in this study with complex calls (i.e. frequency modulated calls or calls containing components of differing frequencies) increased with AP complexity (Table 3), as did the numbers of species with multiple-component and frequency modulated calls individually. However, of the 242 sampled species with complex calls, only 83 (34%) have calls containing frequencies in the AP sensitivity range. Thus, although a larger percentage of species in class D than in other classes have complex calls, the majority of these calls are detected by the BP and not the AP, a surprising result given the BP's inability to discriminate pitch. Although these results do not necessarily refute the hypothesis that a complex AP facilitated speciation by allowing for the evolution of more complex mating calls, neither do they strongly support it.

In the light of this study, it seems likely that selection acting outside the context of mate recognition, such as finding food, detecting predators, avoiding heterospecifics, or male/male competition (Boughman, 2002) may have favoured a complex AP. AP nerve fibres have lower

Table 3 Numbers of sampled species in each amphibian papilla (AP) class with complex calls.

AP class	Complex calls*	AP-range complex calls†	Multiple-component calls	Frequency modulation	Sampling sampled/extant
A	0	0	0	0	5/6 (83%)
B	0	0	0	0	12/21 (57%)
C	4 (13%)	0	2 (6%)	2 (6%)	31/168 (18%)
D	238 (32%)	83 (11%)	118 (16%)	135 (18%)	750/4856 (15%)

*All species with complex calls regardless of AP- or BP-range.

†Species with complex calls within the range of sensitivity of the AP (<1600 Hz).

minimum thresholds than BP fibres, meaning the AP is more sensitive and can detect frequencies at lower amplitudes (volumes) than the BP (Smotherman & Narins, 2000). The evolution of AP complexity and its resulting extension of low- to mid-range frequency sensitivity may simply have allowed anurans to perceive acoustically more of their environment (Ryan, 1986b), aiding in predator and prey detection.

Body size as a constraint

A number of factors may influence the effect a key innovation, such as a complex AP, has on diversification. For example, competitive or predator–prey interactions might affect the extent to which a trait promotes speciation (de Queiroz, 2002). Furthermore, some traits may only be able to promote diversification when found in combination with others or in certain physical or environmental contexts. Anuran calls and acoustic perception may evolve as side effects of other traits under natural selection or differ among populations by chance. If so, this could have influenced the extent to which AP complexity had the potential to affect speciation rates. Call frequencies are constrained by morphology as the structure of the larynx and mass of the vocal cords are important determinants of anuran call frequency, pulse rate and harmonic structure. Vocal cord mass increases with body size causing a decrease in call frequency (Blair, 1972) as seen in the highly significant effect of body size on dominant frequency for the species in this study (Fig. 5).

Basilar papilla frequency perception also varies with body size (Narins & Capranica, 1976; Nevo & Capranica, 1985; Zakon & Wilczynski, 1988; Lewis *et al.*, 1992; Hetherington, 1994) suggesting that constraints imposed on the evolution of body size have the potential to secondarily constrain both call frequency and perception (Gerhardt, 1994). Thus, we might expect allopatric populations evolving to different body sizes due to different prey distributions, climates, predators, or other ecological factors of the local environment to also evolve divergent call and perception characteristics. As an example, variation in the call frequency of *Acris crepitans* has been attributed to a pleiotropic consequence of an increase in body size due to selection for desiccation resistance in the more arid western part of its range (Nevo & Capranica, 1985; Gerhardt, 1994, but see Ryan & Wilczynski, 1991). Thus, natural selection acting on body size may have constrained the potential of the increased range of sensitivity of the AP to influence speciation rates. Selection to maintain a small body size would likely preclude many species from calling within the range of sensitivity of even the most complex AP.

The role of the basilar papilla

The frequencies of the majority of anuran mating calls fall within the range of sensitivity of the BP rather than

the AP. What advantage might calling in the BP range confer over that of the AP? The BP is always tuned to a narrow range of frequencies >1000 Hz (Zakon & Wilczynski, 1988). However, the specific frequencies to which this organ is tuned can vary with sex, body size and geographical location within species (Narins & Capranica, 1976; Nevo & Capranica, 1985; Zakon & Wilczynski, 1988; Lewis *et al.*, 1992; Hetherington, 1994). In addition, although AP tuning is restricted to somewhere between the ranges of 100–600 Hz and 100–1600 Hz for all anurans, BP tuning can extend to 4000 Hz in small frogs (Hetherington, 1992). The dominant frequency of a male's call may or may not fall within the range of sensitivity of the AP. But, for the vast majority of species, the most sensitive frequency of the BP is at or close to the dominant frequency of the male's mating call (Capranica, 1965; Loftus-Hills, 1973; Walkowiak *et al.*, 1981; Zakon & Wilczynski, 1988) highlighting the importance of this organ for mate recognition.

For many species, the BP, and not the AP appears to play a dominant role in providing a matched filter between female sensitivity and male calls as predicted by the sensory drive hypothesis (Endler, 1992; Lewis & Narins, 1999; Gerhardt & Huber, 2002). Both intra- and interspecific comparisons have shown that BP tuning is inversely related to body size (Loftus-Hills, 1973; Walkowiak *et al.*, 1981; Wilczynski *et al.*, 1984) so that both call frequency and the frequency to which the BP is most finely tuned increase with decreasing body size. Such correlated changes in call frequency and BP sensitivity could allow filter matching to persist through changes in body size in diverging populations and thus simplify the evolution of premating isolation through call divergence for species communicating via the BP when compared with the AP.

Conclusion

Although Ryan's (1986b) study is frequently cited as an example of a key innovation and of the evolution of complexity begetting species diversity, call frequency data do not support the hypothesis that the evolution of a complex AP has influenced speciation rates among anuran lineages. Although the AP's changing morphology may have increased the frequency range, resolution and/or sensitivity of this organ, there is little evidence linking this trait to the dramatic differences in speciation rates exhibited among anuran lineages. The frequencies of most mating calls fall within the range of sensitivity of the BP rather than the AP, suggesting that future research into the evolution of acoustically-mediated reproductive isolation and differential speciation rates should focus on the role of body size in maintaining a matched filter between the sensitivity of the BP and the dominant frequency of species-specific mating calls. The evolution of complexity in the AP has more likely been

shaped by selection for adaptation to different environments rather than for increasing the width of the auditory niche available for inter-sexual communication.

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