Vocal morphology of the *Physalaemus pustulosus* species group (Leptodactylidae): morphological response to sexual selection for complex calls

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Most male frogs in the genus *Physalaemus* produce a whine-like advertisement call. Male *P. pustulosus*, however, add chucks to the call. This enhances the attractiveness of the call to females, and has evolved under the influence of sexual selection despite the increased predation risk from the frog-eating bat (*Trachops cirrhosus*). This complex call is unusual, if not unique, among anurans because the two call components overlap in time. Here we investigate the morphological changes responsible for the production of complex calls.

   The *Physalaemus pustulosus* species group consists of four species. *Physalaemus pustulosus* and *P. petersi* are sister species, and recently it has been shown that *P. petersi* produces chucks. *Physalaemus coloradorum* and *P. pustulatus* are sister species and neither is known to produce chucks. Two laryngeal characters vary within the species group. *Physalaemus pustulosus* has a large fibrous mass (FM1), whose vibration is responsible for production of the chuck. This mass is much smaller in the other three species. In *P. pustulosus* and *P. petersi* the FM1 is anchored dorsally, deep within the bronchial process, the attachment is more extensive in *P. pustulosus*. Neither *P. pustulatus* nor *P. coloradorum* have such a dorsal attachment associated with their FM1. This character is responsible for allowing the FM1 to vibrate independently of the vocal cords, that is, for the production of the complex call. Thus the morphological changes responsible for the evolution of this unusual behavioural innovation, the complex call, are gradual, and almost trival, in nature. This study also shows that the primitive condition of the larynx of the *P. pustulosus* and *P. petersi* ancestor, was predisposed to the production of complex calls.

   We also document ontogenetic and sexually dimorphic patterns in larynx structure.

KEY WORDS:—Advertisement calls – larynx – *Physalaemus pustulosus* – sexual selection – vocal morphology.

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INTRODUCTION

Traits that enhance the ability of males to attract mates, even if they decrease the survivorship of the bearer, can increase in frequency due to sexual selection (Darwin, 1871). The anuran advertisement call is such a trait (Ryan, 1988, 1990). Although this call functions in species recognition, and thus is important in ethological isolation among species, a number of studies have demonstrated that variation within the species' call can result in variation in the males' attractiveness to females (Ryan, 1980, 1983b, 1985; Gerhardt, 1982, 1988; Sullivan, 1982; Wells, 1988; Wells & Schwartz, 1984; Robertson, 1986); thus the evolution of these calls appears to be influenced by sexual selection (Littlejohn, 1981; Ryan, 1988).

A dramatic demonstration that sexual selection results in the evolution of call variants, even with the consequence of decreased survivorship, is provided by studies of the frog *Physalaemus pustulosus*. Males can produce calls with two separate and distinct components, a whine and a chuck. We refer to the two-component call as a complex call. Complex calls always are introduced by a whine and can be followed by 1–6 chucks (Fig. 1; Rand & Ryan, 1981). The whine is necessary and sufficient for species recognition, but the chuck does not elicit a response from either sex if not introduced by the whine (Ryan, 1983a). Males calling in isolation tend to produce only whines (simple calls), but add chucks during vocal interactions. When given a choice, females prefer calls with chucks (Rand & Ryan, 1981), and due to their preference for chucks with lower frequencies, females are more likely to mate with larger males (Ryan, 1980, 1983b, 1985). Thus the chuck has evolved in response to sexual selection (Ryan, 1985). The frog-eating bat, *Trachops cirrhosus*, eats frogs and uses the call for localization cues (Tuttle & Ryan, 1981). Like females the bats also are attracted preferentially to calls with chucks (Ryan, Tuttle & Rand, 1982). The ability of males to vary the number of chucks in the call seems to have evolved as a compromise in response to sexual selection by females and natural selection by bats. Although we have some understanding of the selection forces responsible for the evolution of complex calls, we have little knowledge of the evolutionary changes in morphology necessary for this behavioural response to selection. Such an investigation is the purpose of this study.

The *Physalaemus pustulosus* species group is a well-corroborated monophyletic unit consisting of four species, grouped as two species pairs (Cannatella &
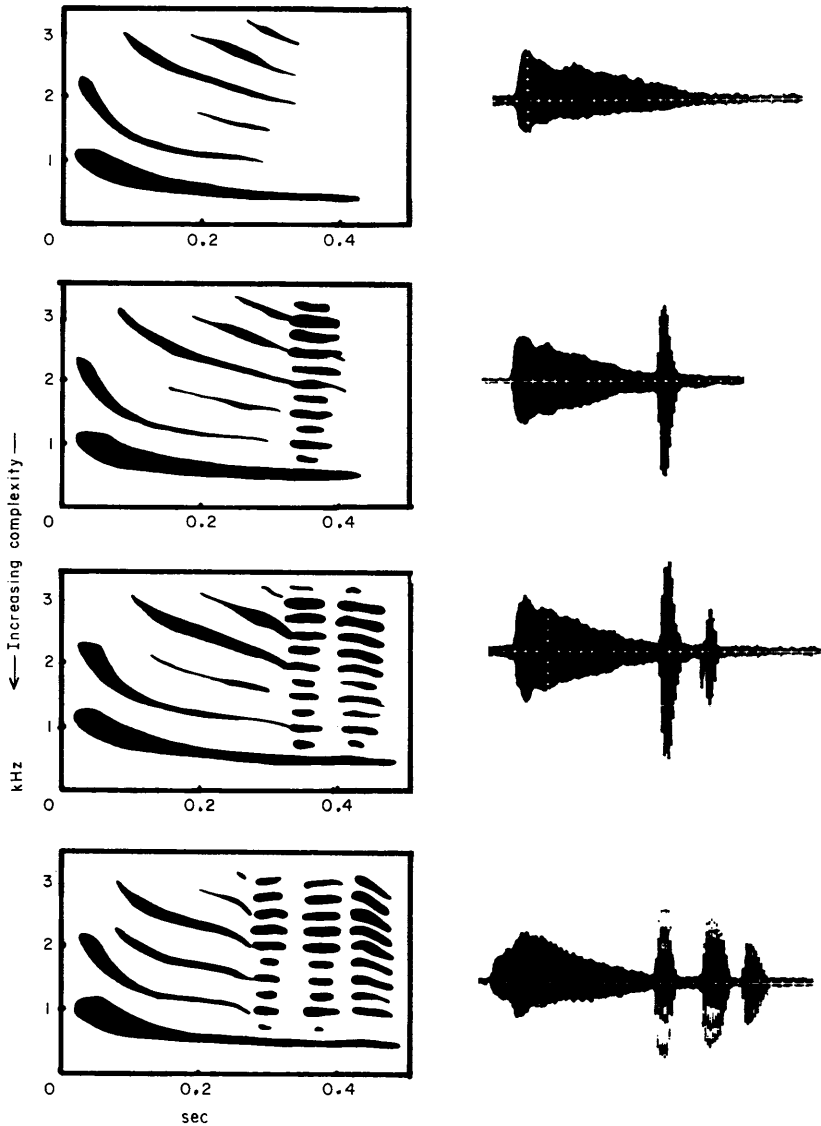
Figure 1. Advertisement call complexity series of *Physalaemus pustulosus*. Sonograms are on the right and oscillograms on the left. The top pair represents a whine only, the bottom pair represents a whine with three chucks.

Duellman, 1984). The vicariance of the species pairs resulted from a trans-Andean divergence, while the two species within each pair are thought to be derived from a cis-Andean divergence. *Physalaemus pustulosus* and *P. petersi* are found on the eastern side of the Andes, with *P. pustulosus* extending well into Central America; *P. coloradorum* and *P. pustulatus* are on the western side of the Andes (Cannatella & Duellman, 1984). Extensive behavioural experiments using playbacks of conspecific advertisement calls revealed that *P. petersi* in Peru also add chucks to their calls, albeit infrequently (Cocroft & McDiarmid, unpublished observations). There is no other information on the function of the

complex call in this species. In similar experiments with *P. coloradorum*, males never produced chucks (Ryan & Rand, unpublished observations). These experiments have yet to be conducted with *P. pustulatus*, but a survey of recorded calls does not reveal chucks.

The complex call of *P. pustulosus* and *P. petersi* is unusual for several reasons. There are no published reports indicating that any of the other 35 species in the genus produce calls with two distinct components (e.g. see Barrio, 1965; Cannatella & Duellman, 1984; Ryan, 1985). Furthermore, while other anurans add notes to their calls, we know of no other species that adds a note which is so distinctly different in structure from the introductory note, and in which there is a partitioning of function within the call for species recognition (whine) and sexual selection (chuck). The most unusual feature of the chuck is that it temporally overlaps the whine, suggesting two distinct sources of vibration for the call (Drewry, Heyer & Rand, 1982), analogous to the syrinx of oscine birds (Greenwalt, 1968) but previously unknown in anurans. Thus the evolution of the chuck represents a major innovation in anuran sound production.

Athough the chuck enhances the attractiveness of the call in *P. pustulosus*, it is not the only alternative for a more attractive call. The chuck has a broad frequency range (220–3300 Hz; Fig. 1) which encompasses those frequencies to which the two hearing organs in the frog's inner ear are most sensitive. In general, the amphibian papilla (AP) is more sensitive to frequencies below 1500 Hz, while the basilar papilla (BP) is more sensitive to frequencies above 1500 Hz (Zakon & Wilczynski, 1988). In *P. pustulosus*, the AP is most sensitive to 500 Hz, while the BP is maximally sensitive to 2100 Hz (Ryan *et al.*, 1990). Ryan & Rand (in press) showed that calls with synthetic chucks having only the lower portion or the higher portion of the normal frequency range, and thus primarily stimulating either only the AP or only the BP, are equally as attractive as the normal chuck, if they contained the same amount of energy, which simultaneously stimulates both inner ear organs.

The female's sensory system exerts equally strong selection favouring three alternatives for more attractive complex calls: a full chuck, or a chuck containing only the upper or only the lower frequencies (Ryan & Rand, in press). However, the evolution of behaviour can be constrained by morphology. The purpose of our investigation is to identify those evolutionary changes and possible historical constraints responsible for this particular response to sexual selection–evolution of the chuck. We propose to accomplish this by a careful analysis of the larynges and calls of the four species of the *Physalaemus pustulosus* species group. Using a phylogenetic approach, we hypothesize the ancestral state of the larynx previous to the evolution of the complex advertisement call, and identify those morphological changes that took place in the larynx in response to sexual selection.

Proposed mechanism of vocalization

The whine of *P. pustulosus* appears to be produced by vibration of the vocal cords; the tension of the cords is changed by the contraction of intrinsic laryngeal muscles that change larynx shape and thus cause the frequency modulation of the whine. The chuck is produced by vibration of a large fibrous mass that is located in the passageway created by the bronchial process of the cricoid ring.

The chuck begins during production of the whine. On a sonogram the whine appears to grade into the chuck with the fundamental frequency of the whine being the second harmonic of the chuck (Drewry et al., 1982; Fig. 1). Given the similarities of the calls and larynges of P. pustulosus and P. petersi, we assume similar mechanisms of vocalization for both species.

Drewry et al. (1982) examined the larynges of two other species of Physalaemus. Physalaemus gracilis lacks a large fibrous mass and its call is similar to the whine of P. pustulosus. Physalaemus olfersi possesses a large fibrous mass similar in proportions to that of P. pustulosus. However, this mass cannot vibrate independently of the vocal cords, and the call of P. olfersi is similar to the chuck of P. pustulosus, except much longer in duration. In P. pustulosus the large fibrous mass does not vibrate at the beginning of the call; the independent vibration of the vocal cords results in the whine. Chucks are added to the call as this large fibrous mass vibrates. It is the ability of the frog to control the vibration of this large fibrous mass, independent of the vibration of the vocal cords, that is thought to result in the variable complexity of the P. pustulosus call.

MATERIAL AND METHODS

Laryngeal morphology

Our terminology follows Trewavas (1933) and Drewry et al. (1982). Dissection of muscles was aided by use of Weigert's Lugol solution. Each hyolaryngeal apparatus was removed in toto after partial detachment and deflection of the pectoral girdle, and then dissected and examined ex situ. Illustrations were drawn from photomicrographs. Specimens are listed in the Appendix.

Advertisement call analysis

Representative calls from each species of the species group were analysed. The tape recording of the vocalizations of each species were obtained from: P. pustulosus (30.0 mm snout to vent length (SVL)), MJR; P. petersi (30.4 mm SVL), R, Cocroft; P. pustulatus (24.1–28.3 mm SVL), KU; P. coloradorum (size is not available, but average species size is 20.4 mm SVL (Cannatella & Duellman, 1984)), R. McDiarmid. Power spectra and oscillograms were analysed with a DATA 6000 digital waveform analyser. Sonograms were analysed with a Uniscan II real-time spectrum analyser.

RESULTS

Laryngeal morphology

Physalaemus pustulosus. The intrinsic musculature of the larynx of P. pustulosus is similar to that described by Trewavas (1933) for Eupemphix nana (= Physalaemus nanus). Given the locality of the specimen dissected by Trewavas, it is more likely to be P. signiferus (Cannatella, personal communication). The vocal cords contain three accretions or concentrations of fibrous materials (Fig. 2). In keeping with Drewry et al. (1982) the two larger concentrations are referred to as fibrous masses, and the third is referred to as a transverse thickening. The smaller fibrous mass is the FM2 (Fig. 2). This mass is sausage-shaped, and is
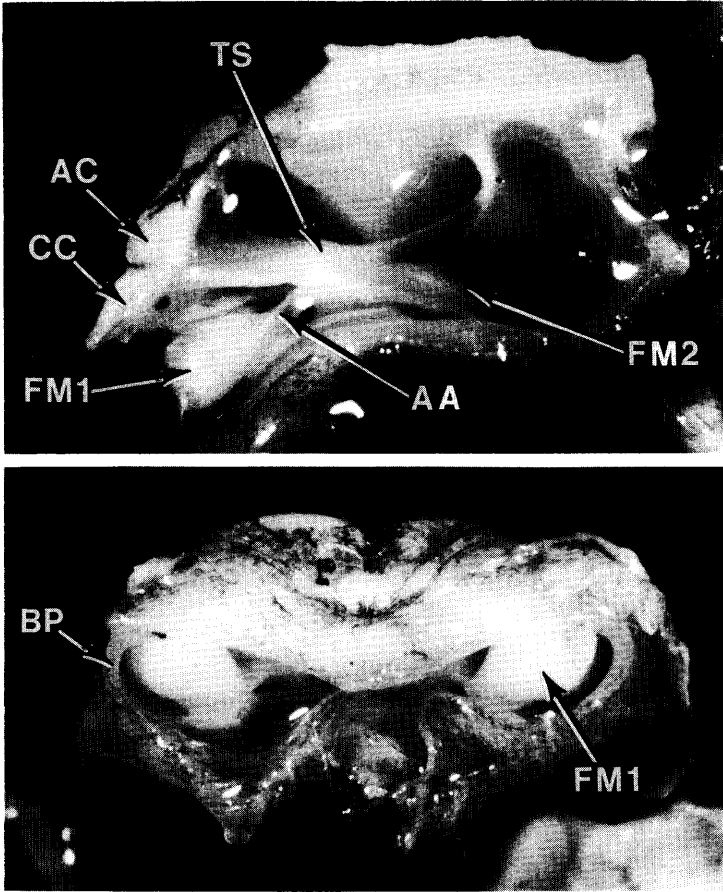
Figure 2. Photographs of a larynx of *Physalaemus pustulosus*. A, Left side of sagittal section of larynx. AA anterior attachment of the FM1 at transverse ridge; AC arytenoid cartilage; CC cricoid cartilage; FM1 primary fibrous mass; FM2 smaller fibrous mass; TS transverse thickening on vocal cords. B, Posterior view of entire larynx. BP bronchial process of crichoid arch.

anteriorly directed at the base of the point of attachment of the primary fibrous mass (FM1) and the vocal cords. It is laterally displaced so that it is adjacent to the inner surface of the medio-distal part of the wall of the arytenoid cartilage. Given its placement, it seems probable that this mass does not vibrate with the vocal cords.

The transverse thickening of the vocal cords is apparent as a transverse ridge on the dorsal surface of the vocal cords, approximately one-third of the distance anterior to the bronchial process of the cricoid cartilage. This linear thickening appears to be composed of the combination of the attachments of the two fibrous masses to the vocal cords. Just anterior to the thickened ridge, the outer lateral edge of the vocal cord material, which is attached to the inner wall of the arytenoid cartilage, begins to rotate dorsally from an essentially horizontal to an oblique orientation, forming a thin, upwardly-sloping sheet in the anterior two-thirds of the dorsal chamber of the arytenoid cavity. This sheet is continuous, and there are no apparent openings through it to the lower cavity formed by the cricoid cartilages.
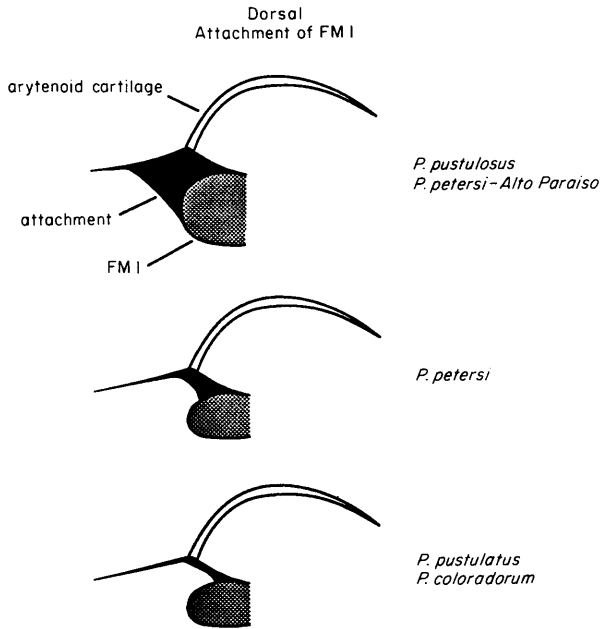
Figure 3. Scheme showing dorsal ligamentous connection of the FM1 to the bronchial process of the cricoid arch (*P. pustulosus* and *P. petersi*) or to both the bronchial process and the arytenoid cartilage (*P. pustulatus* and *P. coloradorum*) oriented as in top photo in Fig. 2.

The primary fibrous mass, the FM1, is located in a passageway formed by the bronchial process of the cricoid ring to which the lungs attach (Figs 2, 3). The mass is large relative to the diameter of the passageway. When viewed from a posterior perspective, it occludes one-half to three-fourths of the diameter of the bronchial process, and thus most of the passageway by which air enters the larynx (Figs 2, 3).

The FM1 has two attachments; one, narrow but thick, attaches the mass anteroventrally to the floor of the hyoarytenoid membrane and then proceeds dorsally where it invests in the vocal cords, its terminus apparently involved in the transverse thickened ridge described above. The other connection is dorsal. This attachment proceeds dorso-posteriorly and attaches well inside the dorsal inner wall of the bronchial process; it then extends anteriorly and continuously where it invests heavily in the vocal cords at the transverse local thickening (Fig. 2). The dorsal attachments of the FM1 preclude passage of air through the bronchial processes by any route except medio-ventrally. This, coupled with the continuous nature of the anterior vocal cord sheets indicates that air can only pass from the ventral chamber to the dorsal chamber of the larynx through the space between the free medial margins of the vocal cords ('FE' in fig. 6A of Drewry *et al.* (1982)).

The smallest size at which males breed in nature is 24 mm SVL (Ryan, 1985). Three juvenile males were examined (13.4 mm, 16.8 mm and 18.7 mm SVL). In each specimen the testes were readily identifiable. There were no discernible differences between FM1 of the largest juvenile and that of mature *P. pustulosus* males that we examined. However, an ontogenetic trend toward lengthening of FM1 is suggested when the two smaller males are examined. At 13.4 mm SVL,

this fibrous mass appears as a vertically oriented thickening as opposed to the large, rounded protuberant mass in adults when viewed posteriorly, that is, through the bronchial process. However, the postero-dorsal attachments of the fibrous mass extend into the bronchial process as in adults. Thus, ontogenetically, the FM1 must elongate posteriorly with increasing size as the male matures, eventually partially occluding the bronchial process as it does in the adult. This is evidenced by the 16.8 mm SVL male in which the FM1 is intermediate in size between the 13.4 mm SVL and 18.7 mm SVL males.

Physalaemus petersi. The intrinsic musculature, the FM2, and the local thickening of the vocal cords are as in *P. pustulosus* (Fig. 2). The FM1 is relatively smaller in *P. petersi* than in *P. pustulosus*, occluding only one-fifth to one-fourth of the opening formed by the bronchial process in *P. petersi* (Fig. 3) in all specimens examined except those from the Alto Paraiso area of Brazil (see below). The connections of the mass are similar in these two species, but with some difference in the dorsal connection. In *P. petersi* the dorsal connection also proceeds posteriorly, such that the mass is anchored in the bronchial process, but the connection inserts more anteriorly and thus less extensively within the process than it does in *P. pustulosus* (Fig. 3).

R. Crombie (personal communication) reported that *P. petersi* in the Alto Paraiso area of Brazil produce complex calls frequently, which is more similar to *P. pustulosus* than to the *P. petersi* studied in Peru (R. Cocroft & R. McDiarmid, unpublished observations). These calls have not been recorded. These individuals are larger than the *P. petersi* in Ecuador and Peru. The Alto Paraiso *P. petersi* also tend to differ from these conspecifics in two laryngeal characters. FM1 tends to be larger and tends to be anchored more deeply in the bronchial process. In both these characters *P. petersi* from the Brazilian population and *P. pustulosus* tend to be similar.

Although *P. petersi* from the Alto Paraiso area in Brazil exhibit all five of the diagnostic characters for this species reported by Cannatella & Duellman (1984), it is possible they might represent another species (D. Cannatella, personal communication). Given this uncertainty, we restrict our discussion to the *P. petersi* from Ecuador and Peru noting that an intense taxonomic and behavioural study of (cf.) *P. petersi* from Alto Paraiso in Brazil might offer intriguing insights into the evolution of complex calls.

Physalaemus pustulatus. The intrinsic musculature, the FM2, and the transverse thickening of the vocal cords are as in *P. pustulosus* and *P. petersi* (Fig. 2). The relative size of the mass is as in *P. petersi;* that is, much smaller than in *P. pustulosus* (Fig. 3). The dorsal connection of FM1 differs from both *P. pustulosus* and *P. petersi* in that the involvement with the cricoid is reduced because the dorsal connection does not proceed posteriorly into the bronchial process; rather, it attaches to both the ventral margin of the arytenoid and dorsal margin of the cricoid where the two come together (Fig. 3).

Physalaemus coloradorum. The intrinsic musculature, the FM2, and the transverse thickening of the vocal cords are as in *P. pustulosus*, *P. petersi*, and *P. pustulatus* (Fig. 2). The relative size of the mass is as in *P. petersi* and *P. pustulatus*, and the attachments of the FM1 are as in *P. pustulatus* (Fig. 3).

Females of the Physalaemus pustulosus *species group*. All females examined are similar to one another in laryngeal morphology and are different from their male counterparts. Extrinsic musculature of larynges of females is greatly reduced in

mass in comparison to males, and females lack both fibrous masses (FM1 and FM2) and the local thickening of the vocal cords.

Advertisement call structure

Physalaemus pustulosus. The call structure of this species has been described in detail (Ryan, 1985). A representative call is shown in Fig. 4A. The fundamental frequency of the whine is modulated from 950 Hz to 465 Hz over a period of 269 ms. The dominant frequency of the entire whine is 833 Hz, indicating that most of the energy is concentrated in the fundamental frequency of the whine near the beginning of the call. Both the sonogram and the power spectrum indicate significant energy in the second harmonic. The chuck is much shorter, in this case only 37 ms. The frequency spectrum of the chuck is harmonically structured with little energy present in the fundamental of 275 Hz. The dominant frequency in the chuck of this call is the eighth harmonic, 2200 Hz.

Physalaemus petersi. The advertisement call of *P. petersi* (Fig. 4B) shares many similarities with that of *P. pustulosus.* In the representative call, the whine is 87 ms in duration and is modulated from 1160 Hz to 280 Hz. The drop in frequency is not smooth as in *P. pustulosus,* but is much more rapid at the call's onset. The dominant frequency of this component is 546 Hz. The chuck is much longer than in *P. pustulosus,* 64 ms. The chuck's dominant frequency is 2340 Hz, the fourteenth harmonic of a fundamental frequency of 167 Hz. There is little energy in the first several harmonics.

Physalaemus pustulatus. This species also has a call (Fig. 4C) similar to the whine of *P. pustulosus.* Frequency modulation is more complicated. Six harmonics are evident. The fundamental frequency of the call begins at 840 Hz, increases to 1023 Hz and then decreases throughout the remainder of the call to 440 Hz. The dominant frequency is 947 Hz, and the duration is 280 ms.

Physalaemus coloradorum. The call of this species (Fig. 4D) is also whine-like. The call is harmonically structured, but only a few harmonics are present; in this character the call is more similar to that of *P. pustulosus.* The call begins at a frequency of about 1200 Hz and sweeps to 610 Hz. The dominant frequency is 1060 Hz. The call's short duration of 169 ms makes it more similar to the call of *P. petersi* than to either *P. pustulatus* or *P. pustulosus.*

DISCUSSION

Sexual dimorphism

Darwin (1871) first conceived of the theory of sexual selection as an attempt to explain the evolution of secondary sexual traits. Production of the advertisement call is the most obvious secondary sexual trait of male anurans. Sexual dimorphism in the underlying morphology responsible for calls has not been investigated thoroughly, and for the most part these sexual differences in larynx structure appear to be quantitative. For example, Trewavas (1933) reported that the larynges of males are usually larger and the intrinsic musculature associated with the larynx more massive than in female conspecifics. Kelley (1986) reported that, associated with the larynx in *Xenopus laevis,* adult males
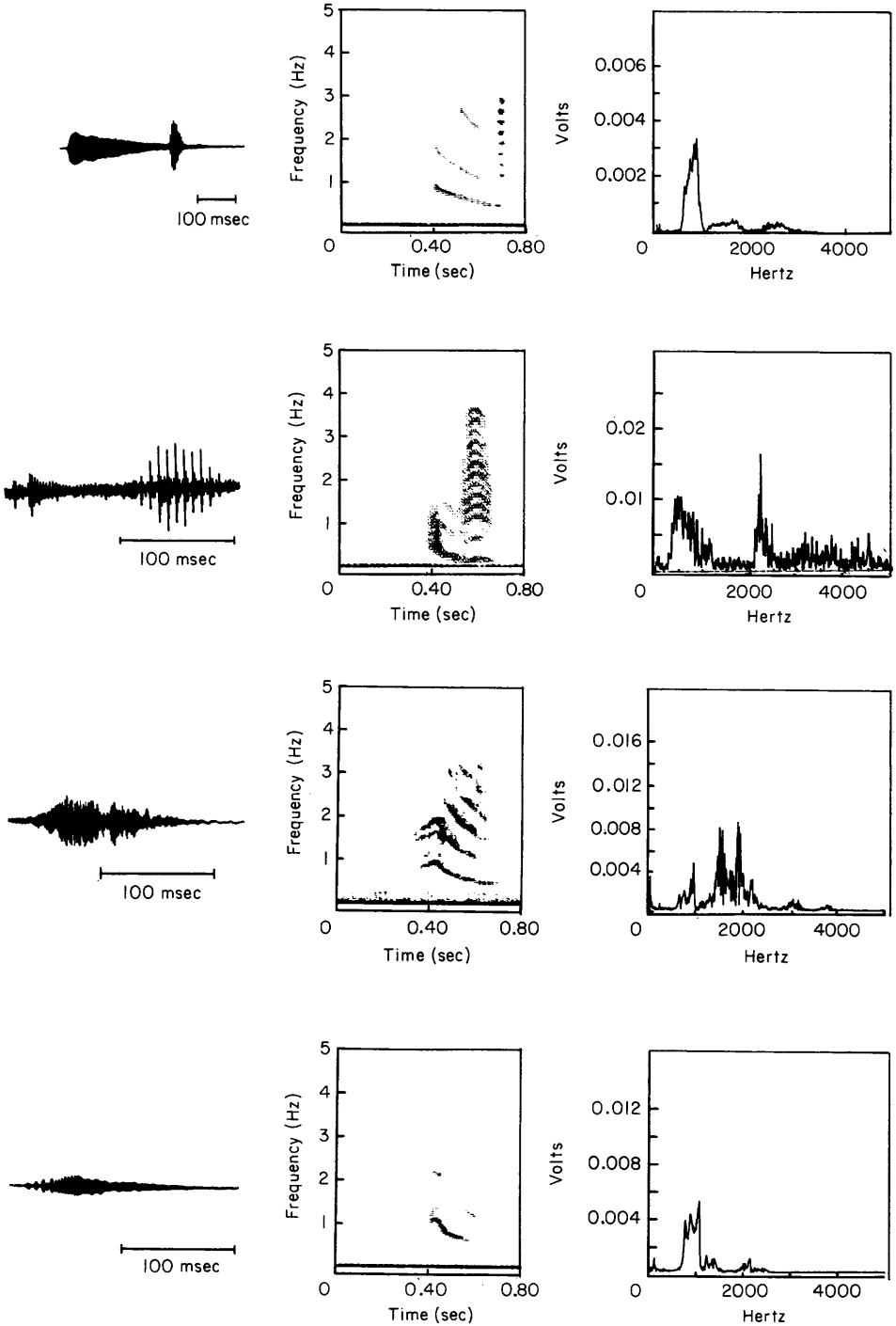
Figure 4. Advertisement calls of members of the *Physalaemus pustulosus* species group. A, *P. pustulosus*. B, *P. petersi*. C, *P. pustulatus*. D, *P. coloradorum*. From left to right, amplitude versus time (oscillogram), frequency versus time (sonogram), and energy versus frequency (power spectrum) are represented.

possess eight times as many muscle fibres as females, and that they also have more motor neurons with larger soma and dendritic trees.

To our knowledge, our study is the first to report the existence of qualitative differences in laryngeal structure between the sexes. Females in the *P. pustulosus* species group not only have smaller larynges and intrinsic muscles, but they also lack structures found in the male larynx, specifically the FM1, the FM2, and the transverse local thickening on the vocal cords. These results are not surprising; sexual dimorphism in behaviour should be reflected by dimorphism at either the morphological or physiological level.

Ontogeny

We are especially concerned with the ontogeny of the two laryngeal characters that vary among species (see below): the posterior insertion of the dorsal attachment of the FM1, and mass of the FM1. In the smallest male *P. pustulosus* examined the dorsal attachment is already anchored deeply in the bronchial process, as in the adult. The FM1 at this size, however, is quite small and does not enter the bronchial process. During development the attachment thickens posteriorly, growing into the bronchial process. It is interesting that in the 16 mm male, the FM1 is still relatively small compared to that of the 18 mm SVL male. Eighteen mm SVL is the minimum size at which males raised in the laboratory are first able to produce viable sperm (Davidson & Hough, 1969), although in the field males are not present at the breeding site until they are 24 mm SVL (Ryan, 1983b, 1985). Given female preference for calls with low frequency chucks, it is expected that the FM1 might reach its maximum size at the time of sexual maturity. The time at which the development of sexual dimorphism in these traits begins is not known. The larynx of *Xenopus laevis* is sexually monomorphic at metamorphosis (Kelley, 1986).

Mechanics of complex call production

Drewry *et al.* (1982) suggested that the presence of the FM1, and its ability to vibrate independently of the vocal cords, allow production of the complex call of *P. pustulosus* with its two temporally-overlapping components. Drewry *et al.* (1982) further suggested that independent production of the chuck is achieved by changes in the shape of the larynx that inhibit vibration of the FM1. They did not state how this is achieved, but there appear to be only two likely possibilities. Either larynx shape is changed such that the FM1 is drawn out of the flow of air through the passageway formed by the bronchial process of the cricoid ring, or changes in the shape of the larynx tighten the FM1 such that it no longer vibrates. In *P. pustulosus* and to a lesser extent in *P. petersi*, the process of the dorsal attachment initially proceeds posteriorly and is firmly anchored in the bronchial process. It seems to us that change in larynx shape cannot remove the FM1 from the air flow. The alternative, that a change in larynx shape increases tension on the FM1 and prohibits vibration, seems more plausible. This might be especially so when the FM1 is anchored firmly to both the bronchial process and the vocal cords and arytenoid wall. Thus contraction of muscles causing anteroventral rotation of the arytenoid cartilages on the cricoid wall would result in a stretching of the FM1. Our main point is that movement of the
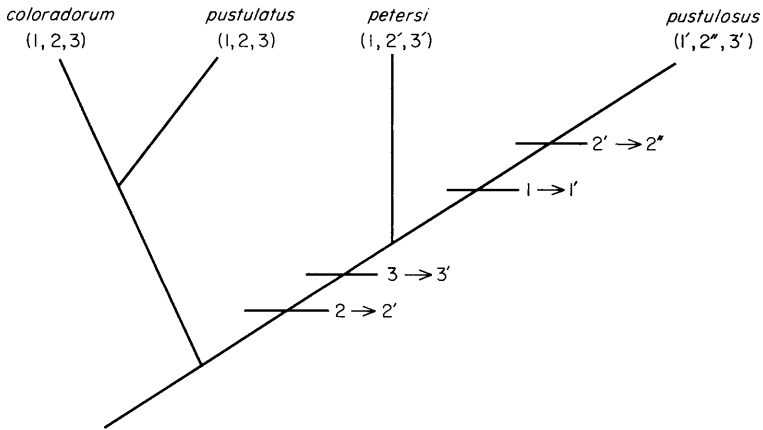
Figure 5. Cladogram representing phylogenetic hypotheses for the species relationships of the *Physalaemus pustulosus* species group and character state distributions for call and larnygeal morphology characters. 1- size of FM1 (1-small, 1'large); 2-connection of FM1 to larynx (2-arytenoid and bronchial process connection, 2'-only shallow bronchial process connection, 2"-only deep bronchial process connection; see Fig. 3); 3-call complexity (3-simple, 3'complex).

FM1 out of the air flow seems a very unlikely mechanism for inhibition of the FM1 vibration. We suggest that the postero-dorsal connection is important in generation of the complex call because it somehow prohibits vibration of the FM1 in *P. pustulosus*.

Another consideration in the ability to produce the complex call is the integration of the mass into the vocal cords. Drewry *et al.* (1982) stated that *P. olfersi* produces a chuck-like call because the FM1 is extensively connected to the vocal cords and thus cannot vibrate independently. Since only *P. pustulosus* and *P. petersi* produce a two-component call, we assume with Drewry *et al.* (1982) that the FM1 of the other two species always vibrates with the vocal cords. However, in *P. pustulosus* the anterior ligament of the FM1 invests heavily in the vocal cords at the transverse thickening of the cords. This attachment is more exaggerated in *P. pustulosus* than in the other three species. It should be noted that in *P. olfersi*, the FM1 is of similar size to the FM1 in *P. pustulosus*. We do not dispute the claim by Drewry *et al.* (1982) for the role of the vocal cord attachment in the *P. pustulosus-P. olfersi* comparison; we only indicate that it is not a plausible explanation for the lack of two components in *P. pustulatus* and *P. coloradorum*.

All four species in the group possess the transverse thickening of the vocal cords approximately at the same site and of the same dimensions. As Drewry *et al.* (1982) suggested, this mass clearly loads the vocal cords and, in part, explains the very low frequencies produced by such small frogs. The FM2, which also is similar among all species of the group, is adjacent to the arytenoid wall. It seems to us that the FM2 can not vibrate with the vocal cords, and we question its significance as a mass-loading device.

We would revise the mechanism proposed by Drewry *et al.* (1982) for production of the complex call in the following ways: (1) changes in larynx shape can not prohibit vibration on the FM1 by moving the mass out of the air flow; (2) the posterior extension of the dorsal attachment of FM1 is implicated, and

changes in larynx shape might tighten the FM1 thus prohibiting vibration; (3) the anterior attachment of the FM1 to the vocal cords does not explain the lack of independent vibration of FM1 in *P. pustulatus* and *P. coloradorum*; if anything, the anterior attachment of FM1 of *P. pustulosus* is more heavily invested in the vocal cords than is that of the other three species. Given that *P. pustulosus* and *P. petersi* both can produce chucks, and differ in the size of FM1, we are not able to speculate as to the role of the size of this mass in the production of chuck. It is somewhat surprising that the fundamental frequency of the chuck in *P. petersi* is lower than that of *P. pustulosus*, even given its smaller size. The size of FM1 is not important in allowing independent production of the chuck. This is consistent with the fact that *P. olfersi* has an FM1 the same size as that of *P. pustulosus* but does not produce a two-component call.

The vocal mechanics of the calls of *P. pustulosus* and of *P. petersi* are clearly complex and investigations of vocal morphology can only offer suggestions. These suggestions should serve as hypotheses to be tested with realistic physical models or ablation experiments. Both would be technically difficult and perhaps not tractable.

Evolution of the complex call

Drewry *et al.* (1982) argue convincingly for the morphology responsible for the complex call of *P. pustulosus*, and, by extension, of *P. petersi*. In comparison with two other congenerics, *P. gracilis* and *P. olfersi*, it appeared that *P. pustulosus* had evolved a suite of complex morphological structures responsible for the complex call. Using the phylogeny of the *P. pustulosus* species group hypothesized by Cannatella & Duellman (1984; Fig. 5), we propose the laryngeal morphology of the hypothetical ancestor of *P. pustulosus-P. petersi*, and hypothesize the specific changes in morphology involved in the evolution of the complex call.

The distribution of call and laryngeal characters, which were not used in the analysis of other morphological characters by Cannatella & Duellman (1984), are consistent with the cladogram derived from their study (Fig. 5). *Physalaemus pustulosus* is the most derived species of the group with respect to those characters analysed (Fig. 5). Two changes in laryngeal morphology are apparent as we proceed from the root to *P. pustulosus*: increase in size of the FM1 (Character 1, state 1′), and movement of its dorsal attachment posteriorly into the bronchial process (Character 2, state 2″). The phylogeny of the genus *Physalaemus* is not understood in enough detail to warrant meaningful outgroup comparisons. Thus the suggestion that the small FM1 is primitive for the *P. pustulosus* species group does not imply it is primitive for the genus.

The complex call and the correlated vocal morphology of *P. pustulosus* and *P. petersi* are presumed to be derived since these two characters are not present together in the other members of the genus, in females, or, as far as is known, in any other frogs. The morphological change necessary for the evolution of this unique behavioural innovation—the complex call—appears to be simple and of a gradual nature: the anchoring of FM1 inside the bronchial process. This character—anchoring of the FM1—has three character states. We argue that the most likely polarization of the character states is as follows: $2 \rightarrow 2' \rightarrow 2''$. We suggest that initially there was a change in articulation ($2 \rightarrow 2'$), then a change in thickness of the connection ($2' \rightarrow 2''$). It also could be argued that this transition

actually should be considered as two characters. One character is the position of the articulation (2 vs. 2' and 2''), and the other character is the thickness of the articulation (2, 2' vs. 2''). For our purposes, the precise character state scenario is unimportant. Of significance is the suggestion that the change from the primitive condition of the FM1 attachment seen in *P. pustulatus* and *P. coloradorum* (2) to the dorsal attachment inside the bronchial wall (2' and 2'') in *P. petersi* and *P. pustulosus* was necessary for the evolution of the chuck.

The above interpretation of the morphological changes necessary for evolution of the complex call suggests that this unique behavioural innovation was the outcome of simple and gradual morphological evolution. Initially (Drewry *et al.*, 1982) it appeared that *P. pustulosus* (and now, *P. petersi*) had evolved a suite of complex morphological characters permitting production of the complex call, an advertisement call that is unique relative to other congenerics and, perhaps, to all other anurans. Our analysis shows that all necessary structures were present in the hypothetical ancestor, and that what appears to be only one relatively simple morphological change took place in the evolution of the complex call. The morphological substrate available for sexual selection to act upon—the primitive larynx—predisposed the ancestor of *P. pustulosus* and *P. petersi* towards evolution of its complex call. Interesting questions that remain unresolved are how does the size of FM1 affect the call, and what selection forces, if any, would favour increased size of the FM1. It seems that a better understanding of the phylogenetic position and vocal behaviour of cf. *P. petersi* in Brazil might be enlightening.

Ryan & Rand (in press) showed that the sensory system of female *P. pustulosus* would exert equally strong selection favouring at least three alternatives for the evolution of more attractive complex calls: the full chuck, and chucks containing either only the upper or only the lower frequencies. This study shows that historical constraints biased males towards the evolution of the full chuck. Because the production of the low and high frequencies in the chuck are mechanically coupled, alternatives besides the full chuck might be morphologically possible, but would require evolutionary changes (e.g. low or high frequency filters) in addition to those needed for the evolution of the full chuck, and there would be no selective advantage to doing so, since these alternatives are no more attractive to females than the full chuck.

In *P. pustulosus*, studies of the female's sensory system and behavioural responses to call variants have elucidated the potential selection forces favouring call variants (Ryan & Rand, in press). This study has illustrated how the call responded to these selection forces, and why one alternative call variant was more likely to evolve. These studies should illustrate the utility, if not the necessity, of an interdisciplinary approach to understanding a multi-dimensional problem such as sexual selection.

SUMMARY

The advertisement call of the frog *Physalaemus pustulosus* has two components. The first, the whine, is necessary and sufficient for species recognition; the second, the chuck, enhances the attractiveness of the call to females, and appears to have evolved under the influence of sexual selection. Recent data show that *P. petersi* also produces a complex call. We examined laryngeal morphology and

call structure of the four species of the *Physalaemus pustulosus* species group, and suggested pathways for the evolution of the complex call. We also showed for the first time qualitative differences in laryngeal structures between sexes, and we examined an ontogenetic pattern of development of the FM1 responsible for production of the chuck.

We have amended the original theory of vocal mechanics in *P. pustulosus* proposed by Drewry *et al.* (1982) by speculating that changes in the shape of the larynx tighten the FM1, which occludes the passageway formed by the bronchial process of the cricoid ring. This change in shape prevents the mass from vibrating during the whine portion of the call. Our suggestion is supported by the fact that this character is also present in the only other *Physalaemus* reported to produce a complex call: *P. petersi*. The evolutionary changes responsible for the production of a call with both a whine and a chuck appear to be quite simple: a change in the anchoring of the mass that occludes the bronchial process within that process, is the only change that appears to have taken place since the hypothetical ancester of *P. pustulosus* and *P. petersi* that is correlated with production of the chuck. Thus the ancestor appears to have possessed a laryngeal structure predisposed for the action of sexual selection to result in a complex call. We emphasize that the suggestions of the factors responsible for the chuck be treated as hypotheses that should be tested further by models, that the function of the relatively large FM1 in *P. pustulosus* is not clear, and that the enigmatic population of *P. petersi* in the Alto Paraiso area of Brazil might offer intriguing insights into the evolution of complex calls.

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APPENDIX

A list of specimens examined: CAS, California Academy of Sciences, KU, Museum of Natural History, University of Kansas; USNM, U.S. National Museum of Natural History; MVZ, Museum of Vertebrate Zoology , University of California, Berkeley. All measurements are of snout to vent length. *Physalaemus pustulosus:* KU 80379 male–28.3 mm (all measures are of snout-vent length); KU 58913 male–24.0 mm; KU 58914 male–25.4 mm; KU 65643 male–23.6 mm; CAS 156157 female–30.2 mm; CAS 143319 male–26.1 mm; three Fl, laboratory-raised males, CAS 161402–18.6 mm. CAS 161401–16.8 mm, CAS 161400–13.4 mm. *Physalaemus petersi*: KU 149388 male–23.2 mm; KU 148389 male–23.5 mm; KU 122552 female–31.4 mm, USNM 247429 male–27.1 mm; USNM (field) 048404 male–29.0 mm.

Physalaemus pustulatus: KU 162371 male–28.3 mm; MVZ 123115 male–24.9 mm; MVZ 123111 female–23.8 mm.

Physalaemus coloradorum: KU 52642 female; KU 178272 male–20.0 mm; KU 117783 male–24.5 mm.