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THE ANURAN MIDDLE EAR : DEVELOPMENTAL HETEROCHRONIES AND ADULT MORPHOLOGY DIVERSIFICATION

by

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SUMMARY

Many anuran related species with similar modes of life and similar mating calls display the occurrence of extreme differences in their middle ear morphology (for example, middle ear present in *Microhyla ornata* but absent in *M. heymonsi*; stapes present in *Bombina orientalis* but absent in *B. bombina*; the tympanic membrane is thin and transparent in *Bufo melanostictus,* but covered by thich unmodified skin in *B. bufo*). As loss or reduction of this system has a profound negative effect on hearing sensitivity, these patterns of middle ear diversification cannot be explained in terms of functional adaptivity. The formation of the anuran middle ear is greatly delayed and retarded, it begins at relatively late premetamorphic stages and progresses through postmetamorphic development. Because of the delayed and retarded morphogeny this system is subjected to paedomorphic underdevelopment through : progenesis (*M. heymonsi*), neoteny (*Bombina* species), and post-displacement (*B. bufo*). The late onset of the middle ear development is predetermined ; because of the peculiar anuran pattern of its morphogeny, it can be formed only at or after the end of metamorphosis. The author thinks that the occurrence of non-tympanic routes for airborne hearing decreases the pressure of functional requirements and allows developmental heterochronies to be the main factor in the diversification of the anuran middle ear.

Key words : Anura, middle ear, morphogeny, heterochrony, paedomorphosis.

INTRODUCTION

The anuran middle ear usually includes the middle-ear cavity, tympanic membrane, and plectrum, or stapes (Fig. 1). The plectrum consists of a medial cartilaginous pars interna plectri, ossified pars media plectri, and cartilaginous pars externa plectri. The latter distal element articulates with the tympanic membrane, which is stretched over the cartilaginous, ring-shaped tympanic annulus. A cartilaginous processus ascendens plectri extends from the pars externa plectri to the crista parotica.

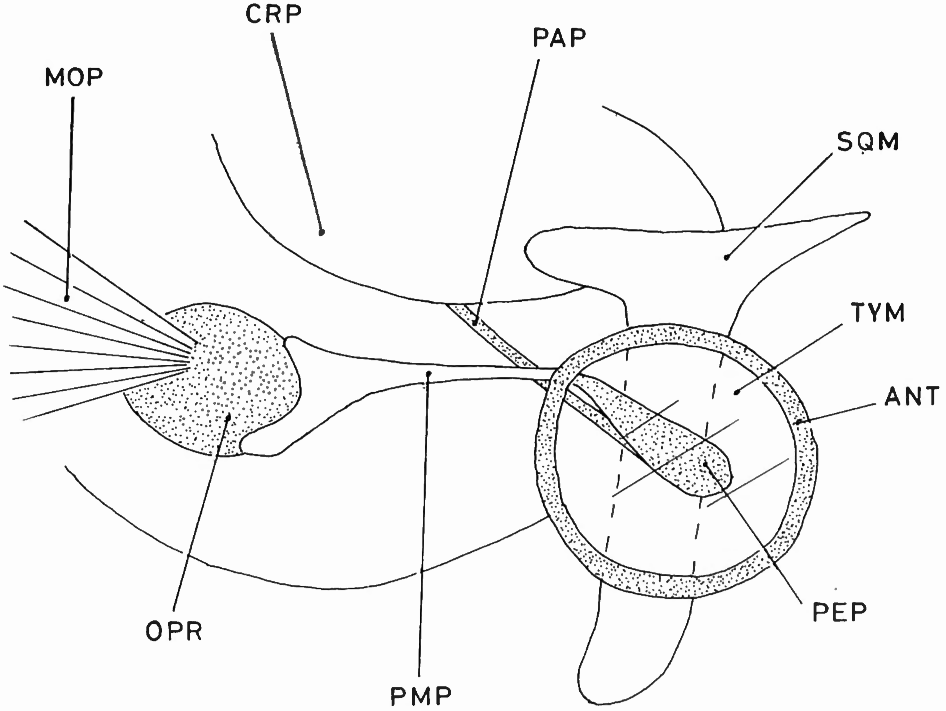

Fig. 1. — Scheme of the generalized anuran middle ear pattern.
ANT - annulus tympanicus ; CRP - crista parotica ; MOP - musculus opercularis ; OPR - operculum ; PAP - processus ascendens plectri ; PEP - pars externa plectri ; PMP - pars media plectri ; SQM - squamosum ; TYM - membrana tympani.

The generalized condition of the middle ear described here typifies most anurans, but many species display derived features down to the reduction or loss of the middle ear (SMIRNOV, 1984).

The main function of the anuran middle ear is the reception of high frequency air-borne sounds involved in mating calls (LOMBARD and STRAUGHAN, 1974). As acoustic communication plays an important role in the anuran life, one could suggest that selective forces would tend to improve the middle ear morphology to serve as an effective transducer of air-borne sounds. Sometimes this seems to be the case (SMIRNOV, 1984), but most patterns of the derived middle-ear morphology cannot be explained in terms of functional adaptivity. For example, two congeneric *Microhyla* species (Microhylidae) with similar modes of life and similar mating calls (the frequency constitution and wave form are essentially identical in both species — KURAMOTO, 1987) differ in their middle-ear morphology : *M. ornata* (DUMERIL and BIBRON, 1841) possesses a tympanic ear, whereas *M. heymonsi* VOGT, 1911 lacks it (Fig. 2A, B).

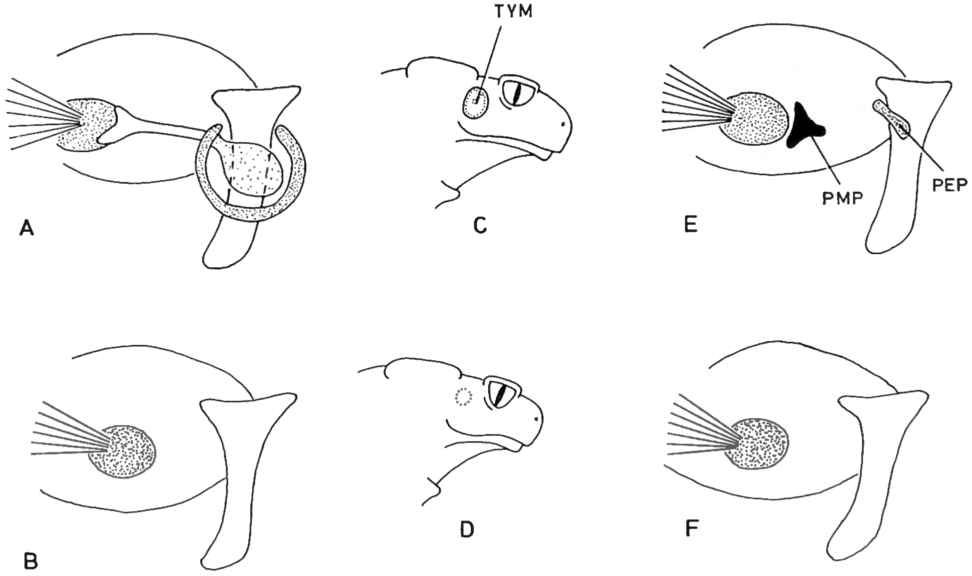

Fig. 2. — Diversification of the anuran middle ear patterns.
A - Microhyla ornata ; B - Microhyla heymonsi ; C - Bufo melanostictus ; D - Bufo bufo ; E - Bombina orientalis ; F - Bombina bombina ; PEP - pars externa plectri ; PMP - pars media plectri ; TYM - membrana tympani.

The occurrence of differences in the middle-ear morphology is displayed by many related congeneric anuran species with similar modes of life and similar mating calls : 1) *Bufo melanostictus* SCHNEIDER, 1799 (Bufonidae) has the middle ear with a large thin transparent tympanic membrane, whereas in *B. bufo* (LINNÉ, 1758) the tympanic membrane is covered by thick almost unmodified skin (Fig. 2C, D) ; 2) *Bombina orientalis* (BOULENGER, 1890) (Discoglossidae) displays the occurrence of bony stapes, whereas *B. bombina* (LINNÉ, 1761) lacks it (Fig. 2E, F).

As the loss or reduction of the middle ear has a profound negative effect on anuran air-borne hearing sensitivity, it is obvious that these patterns of diversification are absurd in terms of orthodox adaptivity related to selection on hearing sensitivity and cannot be explained on functional grounds.

However, numerous attempts to find functional explanations for reduction or loss of the anuran middle ear continue to be made (see review by JASLOW *et al.*, 1988 for details). Concerning the background of general adaptationist's enthusiasms, surprisingly little attention was paid to PARKER's (1940) hypothesis that heterochronic shifts account for the loss or reduction of the anuran middle ear. Only recently this heterochronic explanation was rejected all over again by TRUEB and ALBERCH (1985) who proposed the loss of the anuran stapes to be a consequence of paedomorphic trend in miniature anuran species. While this hypothesis is rather intriguing, it is scarcely well founded without proper evidence supporting it. However, three points indicate the validity of the heterochronic explanation for reduction and loss of the anuran middle ear : 1) heterochronic shifts in the sexual or somatic development resulting in paedomorphosis are common events among anurans (TRUEB and ALBERCH, 1985 ; SMIRNOV, 1989), 2) paedomorphosis is accompanied by the reduction or loss of morphological features that develop later (TRUEB and ALBERCH, 1985), and 3) in anurans the formation of the middle ear is one of the latest ontogenetical events ; it begins at relatively late metamorphic stages and usually progresses through postmetamorphic development (VOROBYEVA and SMIRNOV, 1987 ; HETHERINGTON, 1987).

The aim of the current study is to provide evidence supporting the heterochronic explanation for diversification of the anuran middle ear. For this purpose the mechanism of the middle ear diversification was studied in three pairs of related congeneric anuran species displaying the similar modes of life and similar mating calls, but differing greatly in their middle-ear morphology : 1) *Microhyla ornata* - *M. heymonsi* ; 2) *Bombina orientalis* - *B. bombina* ; 3) *Bufo melanostictus* - *B. bufo.*

MATERIALS AND METHODS

The main idea of the current study is based on two mutually complementary working hypotheses : 1) it is paedomorphosis resulting from developmental heterochronies, that may account for the middle ear reduction, and 2) in species under study the middle ear formation is delayed and retarded, and that is why this system may be subjected to paedomorphic reduction. To prove them one needs information about the rate of the middle ear development as well as information about the rate of somatic development (evaluated here on grounds of anuran cranial ontogeny). For these purposes information about sequence and timing of the middle ear development as well as of cranial development was gathered from ontogenic series stained with Alizarin Red S. These series included newly metamorphosed individuals, juveniles of various ages, half-grown individuals, and adult specimens of the following taxa : *Microhyla ornata, M. heymonsi, M. pulchra* (HALLOWELL, 1860), and *Kaloula pulchra* GRAY, 1831 (Microhylidae), *Alytes obstetricans* (LAURENTI, 1768), *Discoglossus pictus* OTTH, 1837, *Bombina orientalis,* and *B. bombina* (Discoglossidae), *Bufo melanostictus* and *B. bufo* (Bufonidae). Additional information was obtained from histological sections of newly metamorphosed anurans — *Microhyla ornata* and *M. pulchra* (Microhylidae), *Discoglossus pictus* (Discoglossidae), and *Bufo bufo* (Bufonidae). These species were sectioned transversely at a thickness of 10 µm, and the sections Mallory stained. All information was derived from specimens used in earlier studies (SMIRNOV, 1986, 1989, 1991) and readers interested in details are referred to these papers.

RESULTS

Microhyla ornata - M. heymonsi

Microhyla species display the retarded rate of the middle-ear morphogeny as may be seen from the underdevelopment of this system in the postmetamorphic animals. For example, in newly metamorphosed *M. pulchra* only the pars interna plectri achieves its definitive state of histological differentiation and is chondrified, whereas all other middle ear elements are represented by mesenchymatous primordia. In somewhat older *M. ornata* froglets (several days after metamorphosis is complete) the middle ear morphogeny is more advanced and one can record the first signs of chondrification in pars media plectri and annulus tympanicus, but the pars externa plectri is still mesenchymatous and the anlage of the tympanic cavity is discontinuous from the Eustachian tube. On the whole, one may conclude that *Microhyla* species metamorphose when the middle ear development is far beyond the end.

On the other hand, *Microhyla* species constitute a group of small-sized frogs which display some features of cranial underdevelopment (reduced sphenethmoids and quadrato-jugals, reduced or lost palatines) if compared with larger generalized related species (for example, *Kaloula pulchra*). These bones (the sphenethmoids, quadrato-jugals, and palatines) are among the last cranial elements to appear in the sequence of anuran skull ossification (TRUEB, 1985), and their failure to develop as well as small body size characteristic for *Microhyla* species indicate the possibility of the paedomorphic origin of the *Microhyla* genus.

Paedomorphosis, which can be defined as the occurrence of ancestral juvenile morphology in a descendent adult (GOULD, 1977), can occur in three principle ways : progenesis, neoteny, and post-displacement (MCNAMARA, 1986). Progenesis results from acceleration in rate of sexual development. As precocious sexual maturation produces the overall truncation of the somatic growth and development, progenesis is accompanied by small body size, reduced somatic morphology, and loss of features that develop later (ALBERCH and ALBERCH, 1981 ; TRUEB and ALBERCH, 1985). As *Microhyla* species meet all these requirements, one may conclude that they are progenetic animals. Earlier it was shown that the middle ear development is retarded in *Microhyla* species and is one of the latest ontogenetical events. Then one could suggest that if *Microhyla* species are progenetic, their middle ear would also be subjected to paedomorphic underdevelopment and, moreover, the smallest species would display the more pronounced morphological reduction of this system. This seems to be the case : if compared with the middle ear morphology of the generalized larger related *Kaloula pulchra,* the adult middle ear of *Microhyla* species displays some features of underdevelopment and resembles the immature condition in *K. pulchra* (VOROBYEVA and SMIRNOV, 1987) and, moreover, in the smallest *Microhyla* species — *M. heymonsi* — the middle ear is completely lost.

Bombina orientalis - B. bombina

The middle ear may be lost another paedomorphic way-by neoteny. Neoteny occurs by retardation in the rate of somatic development (GOULD, 1977; MCNAMARA, 1986) and is accompanied by reduced morphological development as only juvenile morphology is achieved to the onset of sexual maturity which causes the cessation of somatic development.

Bombina, aquatic anurans, have a highly derived morphology. In comparison with generalized anurans, the main derived features of *Bombina* are as follows : retention of the lateral line system in adult animals (FRITZSCH *et al.,* 1987), juvenilized teeth structure (CLEMEN and GREVEN, 1980), absence or reduction of some cranial ossifications (for example, palatines), and reduced middle ear. These features are larval or juvenile for generalized anurans.

If compared with generalized discoglossids (*Alytes obstetricans* and *Discoglossus pictus*), *Bombina orientalis* displays the retarded rate of development of different systems (the hyoid apparatus, cranial ossifications, and middle ear).

For example, in *B. orientalis* the first appearence of the os parahyoideum (bone attached to the ventral surface of the corpus of the hyoid apparatus) is recorded in 2-years-old specimens (mean SVL = 33,3 mm), whereas in *A. obstetricans* and *D. pictus* this bone appears as an ossification in even recently metamorphosed animals (mean SVL = 18,0 and 19,0 mm respectively).

The similar trend to retarded developments is displayed by cranial ossifications in *B. orientalis* : whereas in *A. obstetricans* adult cranial state is attained prior to sexual maturation (SMIRNOV, 1991), in *B. orientalis* cranial development is greatly prolonged in time and sexual maturation is achieved before osteogenesis of the cranium is complete (SMIRNOV, 1989).

In *A. obstetricans* the middle ear elements complete histological differentiation within a short time after the end of metamorphosis and in recently metamorphosed juveniles (mean SVL = 18 mm) the pars media plectri is ossified and the annulus tympanicus and pars externa plectri are chondrified. In *D. pictus* the ossification of the pars media plectri and chondrification of the annulus tympanicus and pars externa plectri begin even before the metamorphosis is complete, as was exemplified by the tadpole of 45 GOSNER stage, and in recently metamorphosed animals (mean SVL = 19 mm) the process of histological differentiation of the middle ear elements is already completed. However, in *B. orientalis* the first appearence of the bony pars media plectri is recorded only in a 2-years-old animal (SVL = 33,9 mm).

The occurrence of retardation in the development of different systems (the hyoid apparatus, cranial ossifications, and middle ear) argues for the coordinated overall retardation of the *Bombina*'s somatic development rather than independent retardations in the morphogeny of these systems. This conclusion is supported by the retarded rate of the hemoglobin change in metamorphosing *Bombina* (CARDELLINI and SALA, 1979) and by the occurrence of the highest DNA level (genome size) among anuran species, indicating the low rate of the *Bombina*'s development (OLMO *et al.,* 1982).

Then from the foregoing data it can be gathered that : *a*) *Bombina* displays the retarded rate of somatic development if compared with the generalized relatives, *b*) *Bombina* displays the occurrence of paedomorphic features in the morphology of different systems. These two points indicate that in the *Bombina* genus retention of the paedomorphic morphology was attained through the retardation of the somatic development, in other words, through neoteny.

Retardation of the middle ear morphogeny accompanying the overall retardation of the somatic development leads to the severe reduction of the middle ear in *B. orientalis* : only chondrified pars interna plectri and bony pars media plectri, appearing earlier in the anuran ontogeny, achieve their definitive state of histological differentiation, while the annulus tympanicus and pars externa plectri normally appearing later are lacking in *B. orientalis* (1).

If compared with *B. orientalis,* the development of *B. bombina* proceeds slower (UTESHEV and VASILIEV, 1986). Then one could suggest that more retarded somatic development would result in more pronounced morphological reduction in *B. bombina.* This seems to be the case. *B. bombina* displays much more juvenilized morphology if compared with *B. orientalis* (SMIRNOV, 1989) and lacks any signs of the pars media plectri.

Bufo melanostictus - Bufo bufo

In the previous two examples heterochronies occurring in sexual (*M. heymonsi*) or in overall somatic development (*Bombina* species) result in the diversification of the middle ear morphology. However, the middle ear itself may display developmental heterochronies which produce differences in the adult middle ear morphology.

Two *Bufo* species under study greatly differ in the rate of their middle ear morphogeny. Thus, in a recently metamorphosed *B. bufo* toadlet (SVL = 11 mm) all components of the middle ear are still mesenchymatous except for the pars interna plectri which is chondrified. In a juvenile specimen (SVL = 18,5 mm) the tympanic membrane is absent, the pars media plectri still unossified, and the annulus tympanicus and the pars externa plectri mesenchymatous. In even larger specimens (SVL = 40-45 mm) the annulus tympanicus is not yet fully chondrified, and the pars media plectri is ossified only partionally. Only in sexually matured animals did the middle ear complete its development, but the tympanic membrane remains covered with thick skin. In contrast, in *B. melanostictus* the middle ear morphogeny is greatly accelerated if compared with *B. bufo.* Even in a juvenile specimen (SVL = 17 mm) (2) the histological differentiation of the middle ear components is complete, and a thin tympanic membrane is already formed (VOROBYEVA and SMIRNOV, 1987).

(1) The morphogenetic basis for their development retains and in some old animals the rudimentary cartilaginous pars externa plectri may appear (Fig. 2 E).

(2) We can use size as an indirect indicator of age as both species metamorphose at similar sizes and are related by their adult size and age of sexual maturation.

The formation of the tympanic membrane is the last event in anuran middle ear morphogeny (SMIRNOV, 1986). On the other hand, a tympanic membrane development is a rather complicated process itself : it proceeds through several histological changes of integument overlying the annulus tympanicus, and ends in the transformation of this integument into a thin tympanic membrane (HELFF, 1928). Then one may conclude that the occurrence of a thick skin covering the tympanic membrane in *B. bufo,* indicates the failure to complete the histological tympanic membrane changes. Moreover, it indicates the truncated middle ear development in *B. bufo.*

Then if compared with *B. melanostictus,* two features are characteristic for the middle ear in *B. bufo* : 1) its truncated development and 2) delayed onset of its morphogeny. These two points indicate the post-displacement (the third principal way of paedomorphosis occurring by a change in timing of the onset of development of certain structures — MCNAMARA, 1986) to account for the derived middle ear condition exemplified by *B. bufo.*

However, it should be said in all fairness that the middle ear underdevelopment in *B. bufo* might be attained through another heterochronic mechanism — through changes in the time of inductive tissue interaction. Earlier the experiments of HELLF (1928) have indicated quite conclusively that in anurans the annulus tympanicus constitutes the immediate influence responsible for the skin transformation which results in the formation of the tympanic membrane. As the annulus tympanicus and skin constitute a system of inductive tissue interactions, the period of development during which they are physiologically active is an important point. If the appearence of the annulus tympanicus is delayed, it may appear when the skin has already lost its ability to transform into the typical tympanic membrane. This seems to be the case in *B. bufo.* Late appearing of the chondrified annulus tympanicus is accompanied by only imperfect modification of skin covering the tympanic membrane. As a result, in *B. bufo* the tympanic membrane is covered by thick almost unmodified skin, whereas in *B. melanostictus* displaying the accelerated development of the annulus tympanicus, the tympanic membrane is thin and transparent.

As one may conclude, the exact mechanism of the middle ear underdevelopment (through post-displacement or through changes in the time of inductive tissue interaction) in *B. bufo* is obscure, but whatever it is, it is obvious that the derived middle ear state in *B. bufo* is a consequence of heterochronic shifts in its middle ear morphogeny.

DISCUSSION

All these examples indicate that diversification seen in the anuran middle-ear morphology is often accompanied by the reduction of the middle ear as a transducer of air-borne sounds. This diversification is not a result of selection for middle-ear morphology but is a consequence of developmental heterochronies as well as of the late onset of the middle ear morphogeny. Then there may be a question : is there any possibility for acceleration of the onset of middle-ear development ? The answer is no. There are strict developmental constraints precluding this acceleration. The anuran middle ear develops from two sets of elements : one set including the pars interna plectri and pars media plectri appears near the otic capsule, and another one including the annulus tympanicus and pars externa plectri develops near the rostral end of the larval palato-quadrate cartilage (according to GAUPP (1893) the annulus tympanicus forms as an outgrowth of this cartilage). The middle ear may be formed only when these two sets of elements meet each other. However, this event is related to the posterior drift of the palato-quadrate cartilage — the event that determines a change in the jaw mechanism from larval state to the adult one (Fig. 3). This change means the end of metamorphosis, so the middle ear may be formed only at the end of metamorphosis, and the late appearence of the anuran middle ear is predetermined.

But the middle ear is not the only structure that develops late. Many anuran features appear after the end of metamorphosis, but in spite of their delayed and retarded development they do occur, whereas the middle ear is absent. The second question is why the anuran middle ear may be omitted from the ontogeny and why it is greatly subjected to underdevelopment ? It seems that the occurrence of other channels for sound conduction that can participate in perception of acoustic reproductive information decreases the pressure of functional requirements and allows the developmental heterochronies to be the main factor in generating diversification in the anuran middle ear morphology. The main candidate for such an alternative sound conducting channel is an opercular system.

The operculum is an oval or round cartilage that fits into the oval window and provides an insertion for the opercular muscle — a muscle that originates from the suprascapula of the pectoral girdle (Fig. 1). The main function of the amphibian opercular system is the providing of seismic sensitivity to ground vibrations (KINGSBURY and REED, 1909 ; HETHERINGTON, 1985), but there are certain data suggesting the possible functioning of the opercular system in airborne hearing (LOMBARD and STRAUGHAN, 1974). Another possible extratympanal pathway for airborne sounds was recently proposed by NARINS *et al.* (1988). This route includes the lateral body wall, lungs and endolymphatic sacs. The exact mechanism of the extratympanal airborne hearing is obscure, yet whatever it is, it is obvious that frogs can use non-tympanic routes of transmission of airborne sounds. As WILCZYNSKI *et al.* (1987) has shown for *Rana pipiens* SCHREBER 1782, frogs may be equally sensitive to sounds below 1000 Hz through either the tympanic ear or through an alternative route.

As both presumed non-tympanic routes appear before the end of metamorphosis, the middle ear morphogeny occurs when they are already formed and functional. As these alternative routes of hearing can provide the function of the middle ear (airborne hearing), the tympanic middle ear may be subjected to loss or reduction as a by-product of a selection for small body size or early sexual maturation (in progenetic species), a selection for retaining larval features in adult morphology (as seems to be the case in aquatic neotenic *Bombina* which retain the lateral line system), or as a by-product of a selection for other yet unknown adaptive features.

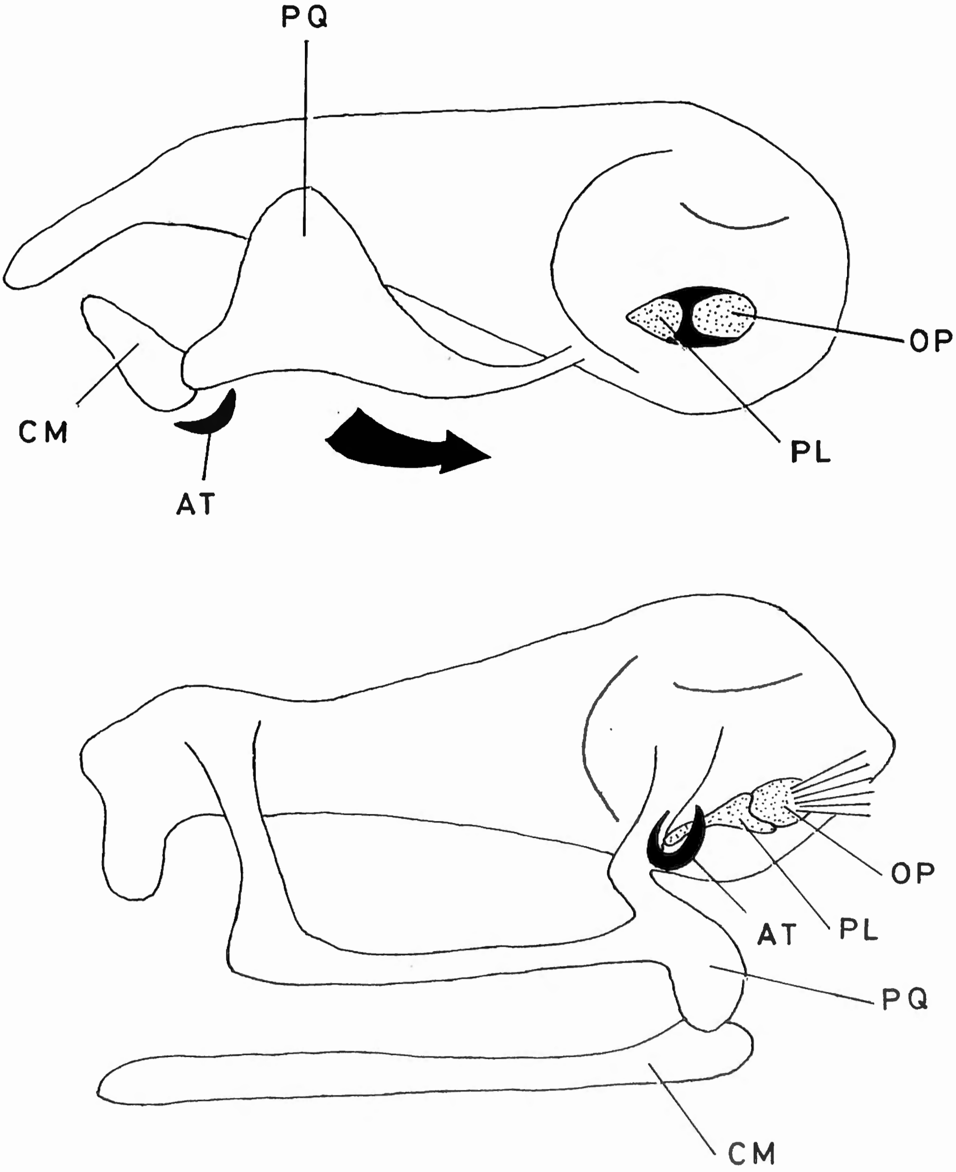

Fig. 3. — Metamorphic changes in the anuran middle ear and jaw mechanism (Modified from Bolt and Lombard, 1985).

A - late larva ; B - postmetamorphic juvenile ; AT - annulus tympanicus ; CM - cartilago Meckeli ; OP - operculum ; PL - plectri ; PQ - palato-quadratum.

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