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Organisation of the palate in a spontaneously transforming Mexican axolotl (*Ambystoma mexicanum*): a case report

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Abstract

We describe the organisation of the palate in a specimen of the Mexican axolotl (*Ambystoma mexicanum*), which spontaneously started metamorphosis in an advanced age after several breeding cycles, but had not yet completed transformation at an age of > 7 years when it was euthanized. The palate shows a mosaic of paedomorphic (absence of the edentate vomerine plate, monocuspid pedicellate teeth) and transformed traits (separation of palatine and pterygoid, fusion of the vomer and anterior parts of the palatine (vomeropalatinum), bicuspid teeth at least on the upper jaw). The vomeropalatinum has a broad pars palatina along its inner side (typical for paedomorpic specimens). We think that the transformation process has started after the specimen has reached the full paedomorphic status.

Kurzfassung

Wir beschreiben die Organisation des Gaumens eines Axolotls (*Ambystoma mexicanum*), der sich erst in relativ hohem Alter spontan umzuwandeln begann, die Metamorphose jedoch in einem Alter von > 7 Jahren, in dem er euthanasiert worden war, noch nicht beendet hatte. Der Gaumen zeigt ein Mosaik von pädomorphen (Fehlen einer unbezahnten Vomerplatte, monocuspide Zähne mit Ringnaht) und transformierten Merkmalen (Trennung von Palatinum und Pterygoid, Verschmelzung von Vomer und anterioren Anteilen des Palatinum (Vomeropalatinum), bicuspide Zähne zumindest auf dem Oberkiefer). Das Vomeropalatinum besitzt eine breite pars palatina auf der Innenseite (lingual), wie sie typisch für pädomorphe Individuen ist. Wir gehen davon aus, dass die Umwandlung eingesetzt hat, nachdem der Gaumen die für pädomorphe Individuen übliche Organisation erreicht hat.

Key words

palate, spontaneous metamorphosis, vomeropalatinum.

Introduction

The Mexican axolotl (*Ambystoma mexicanum*), a member of the tiger salamander complex e.g. Weisrock *et al*., 2006 and further readings therein) is an obligate paedomorphic species (i.e. specimens retain larval traits in a sexually mature form). Although obligate paedomorphic, axolotls may spontaneously transform, but such events are extremely rare (see Wistuba & Bettin, 2003; Johnson & Voss, 2013). However, axolotls can be induced to undergo metamorphosis by administration of thyroxin hormones (TH), namely T_4, which technique has already been used for a long time (e.g. Rosenkilde & Ussing, 1996; Denver *et al*., 2002; Page *et al*., 2008, 2009; summarized in Johnson & Voss, 2013).

As with Urodela (= Caudata) in general, one of the most conspicuous hormonally regulated remodelling during metamorphosis takes place in the skull, namely in the dentate palate. In principle, development of the skull and tooth systems in the paedomorphic axolotl and their remodelling during induced metamorphosis is known showing a sensitivity to TH, which varies according to the developmental stage (Keller, 1946; Clemen & Greven, 1977; Lebedkina, 1979, 2004; Smirnov & Vassilieva 2005; for review see Rose, 2003). Tooth systems of the palate in the "fully" developed larva comprise vomer and palatine on each side, which both bear monocuspid non-pedicellate teeth arranged in patches. Vomer and palatine are separated from each other by a gap, which also holds for their dental laminae accompanying the inner (lingual) face of the dentigerous bones. The palatine is still connected with the pterygoid forming the palatopterygoid. Principally, juveniles and paedomorphic specimens retain this pattern, but tooth patches are reduced to a single row of monocuspid, pedicellate teeth (see Tab. 1), whereas in the upper jaw monocuspids have been replaced by (transformed) bicuspid teeth. Presence of bicuspid teeth (Clemen & Greven, 1977) and some other morphological, physiological and molecular traits indicate a partial or "cryptic" metamorphosis (e.g. Rosenkilde & Ussing, 1996; Johnson & Voss, 2013). After induced transformation, the palate contains the enlarged vomeres (vomerine plate) approaching anteriorly the upper jaw arcade; the fate of the palatine, however, is a matter of debate. Contrary to the prevailing opinion that expects a total degradation of the palatine during metamorphosis in all transforming Urodela (see the discussion in Rose 2003; Smirnov & Vassilieva, 2005), Lebedkina (1979, 2004) and Clemen (1979) documented a postmetamorphic fusion of anterior parts of the palatine with the posterior edge of the vomer in the axolotl resulting in a vomeropalatinum bearing a single transversally arranged row of pedicellate teeth that are initially monocuspid, but finally become bicuspid (Clemen & Greven, 1977). Formation of a vomeropalatinum, however, was considered an "artefact" caused by the thyroxin administration (see Smirnov & Vassilieva 2005).

In the present note we describe the organisation of the palate of a specimen of *A. mexicanum* that started to metamorphose spontaneously at a relatively high age. The transforming process progressed very slowly and even appeared to be ceased, because it was not finished, when the specimen was euthanatized at the age of 7 ½ years. In the context of our studies on the transformation of the urodele palate during metamorphosis and on delayed metamorphosis (e.g. Clemen & Greven, 1995, 2007; Greven *et al*., 2015), we had a closer look on the palate of this specimen.

Material and Methods

The specimen examined came from a pure-bred stock of *Ambystoma mexicanum* held by the late Dr. A.-G. Johnen (University of Cologne). Here axolotls have been bred for years and were fed with horse and beef meat (heart). During this entire period only one approximately six-year-old male of 21 cm length showed signs of metamorphosis in 1969. We do not know exactly when metamorphosis began; however, the male has reproduced several times. Tail and overall appearance of the body resembled that of transformed specimens; the head was almost square and apart from small stumps the gills were reduced. After the next 1 ½ years the axolotl was held in shallow water (3 – 4 cm), where he often put down the head outside. The axolotl was fed mainly with *Tubifex* sp. and maggots. During this time no further metamorphic progress was observed. Finally, the axolotl was killed with an overdosis of MS 222 and decapitated. The skull (without the lower jaw) was fixed in 4 % formalin and stored approximately 30 years in methyl salycilate. In 2002 the preparation was stained in toto with an aqueous Alizarin red-solution, photographed and then stained with Alizarin red and cleared (KOH, pancreatin) (Dingerkus & Uhler, 1977). Probably due to the long storage in methyl salycilate, staining was not entirely satisfactory.

Results

Table 1 summarizes the characteristics of the dentigerous bones in the palate of the typical larva, the paedomorphic adult, the fully (artificially) transformed specimen and the spontaneously transforming specimen described herein (see Fig. 1). It is evident that the palate shows a mosaic of paedomorphic and transformed features (see Fig. 1 E).

Typical paedomorphic traits are the absence of the edentate vomerine plate (Fig. 1 B – E) and the presence largely monocuspid pedicellate teeth (Fig. 1 C). Transformed features are the wide separation of palatine and pterygoid (Fig. 1 A, B, E), the fusion of the vomer and the anterior parts of the palatine (vomeropalatinum) (Fig. 1 B, D, E), the gap in the tooth row of the vomeropalatinum (Fig. 1 A, D, E), and bicuspid teeth on the upper jaw (not shown). A broad pars palatina (not adequately stained after Alzarin red) is seen along the inner side of the dentigerous bones (Fig. 1 C). The choana, bounded in part by the vomeropalatinum has a slot-like appearance like in paedomorphic specimens (Fig. 1 A; see Abb. 1 in Clemen & Greven, 1977).

Discussion

Broadly speaking paedomorphosis, i.e. the metamorphic block, in the Mexican axolotl (*Ambystoma mexicanum*) is associated with low hypothalamic-pituitary-thyroid (HPT) activity. Induction of metamorphosis by TH suggests that intracellular thyroid hormone receptors and deiodinase enzymes that convert T4 to T3 at the target tissues are functional. However, also other hormones

Table 1. Main characters of the palate of the larva, the paedomorphic adult, the fully transformed specimen, and the herein described spontaneously transforming specimen of *Ambystoma mexicanum*. *Data from Keller (1946), Clemen & Greven (1977, 1994), Lebedkina (1997, 2004), and Vassilieva & Smirnow (1997).

	Larva*	**Adult paedomorph***	**Spontaneously transforming specimen**	**Fully transformed specimen***
Vomer				
Position	Posterior edge at the level of the posterior edge of choana; parallels the upper jaw	As in the larva	Ends at the first third of the choana and is fused with the palatinum forming a vomeropalatinum (see below); parallels the upper jaw	As in the spontaneously transforming specimen; covers the area between upper jaw and anterior edge of the chona until the middle
Appearance	Broad; fully occupied with teeth	Relatively large with broad pars palatina	Small and banana-like, fused with the palatine; broad pars palatina showing a recess between vomer and palatine	With a large anterior outgrowth (= vomerine plate); pars palatina small
Dentition	Tooth patch of monocuspid, non-pedicellate teeth	Single labial tooth row with monocuspid pedicellate teeth	Single row of pedicellate monocuspid (?) teeth arranged approximately in the middle of the vomer and the anterior part of the palatine	As in the spontaneously transforming specimen, but the tooth row is situated lingually and arranged transversally; teeth are pedicellate and bicuspid
Palatopterygoid				
Position	Posterior of the choana; paralles the maxillare	As in the larva	As in the pädomorphic species	Sligthly curved behind the choana
Appearance	Intact, i.e. palatinum and pterygoid not separated	As in the larva; anterior edge fissured	Palatine disintegrated, but its anterior part is fused buccally with the vomer (= vomeropalatinum); pterygoids near the quadrata and the prootic region	As in the spontaneously transforming specimen; palatine and pterygoid widely separated
Dentition	Tooth patch on the palatinal portion with monocuspid non-pedicellate teeth	As single tooth row of pedicellate monocuspid teeth at the buccal edge	Single row of pedicellate, monocuspid (?) teeth in the middle of the small pars dentalis in continuation with the vomerine tooth row	As in the spontaneously transforming specimen, but teeth are bicuspid, and situated lingually

such as corticosteroids influence the rate of metamorphosis by controlling TH production and action on target tissues (e.g. Rosenkilde & Ussing, 1996; Denver *et al*., 2002; Kuhn *et al*., 2005; Laudet, 2011; Johnson & Voss, 213; Denver, 2013).

Spontaneous transformations of the axolotl are rare and often attributed to the influence of hybridisation with the related paedomorphic and metamorphic tiger salamander *A. tigrinum* or with (unintential) feeding with thyroxin-containing food (see Wistuba & Bettin, 2003; Johnson & Voss, 2013). Johnson & Voss (2013) report on frequencies of spontaneous metamorphosis of 1–2% in the *Ambystoma* genetic Stock Center, which may increase to 10% in stressed axolotls. Voss & Shaffer (2000) explain the relative rareness of spontaneous metamorphoses in laboratory axolotls compared to wild caught axolotls with an altered genetic composition of the former.

In a rather well-documented case of spontaneous metamorphosis a larva began to transform at the age of 7 months and a total length of 13 cm leaving the water a short time later (Böhme, 2001). The herein described specimen was the only transforming individual emerged from the mass of pure axolotls bred for decadess at the University of Cologne. Therefore an induced metamorphosis was most unlikely. Regarding the external features such as disappearance of the dorsal fin and reduction of the external gills, our specimen was in a transitional stage between midmetamorphosis and the end of metamorphosis (e.g. Lebedkina, 2004).

As with transforming Urodela in general, the palate of the transforming axolotl is remodelled considerably (see above) and the tissues comprising these structures show a remarkable variations in TH sensitivities (Smirnov & Vassilieva, 2005). The herein examined specimen shows both, traits typical for paedomorphic and traits typical for transformed specimens. The most conspicuous characteristics are the absence of the large ossified edentate vomerine plate in front of the dentition and the presence of already pedicellate, largely monocuspid teeth. However, as bicuspidity develops gradually (see Davit-Béal *et al*., 2007), we can not exclude the presence of incipient bicuspid teeth with very small cusps. Ossification of the vomerine plate and the gradual replacement of palatal monocuspids take place during metamorphosis (e.g. Clemen & Greven, 1977; Lebedkina, 2004; Smirnov& Vassilieva 2005; Davit-Béal *et al*. 2007). The buccal position of the single row of teeth is the same for paedomorpic specimens and the transforming specimen. The pars palatina, although insufficiently stained by Alizarin-red, but still present after pancreatin-treatment that is required for clearing and staining according to Dingerkus & Uhler (1977), is broad along the entire vomeropalatinum with a recess at the point where a gap is seen in the tooth row. Broad ossified partes palatinae are present in mature paedomorphic axolotls (Clemen & Greven, 1977)

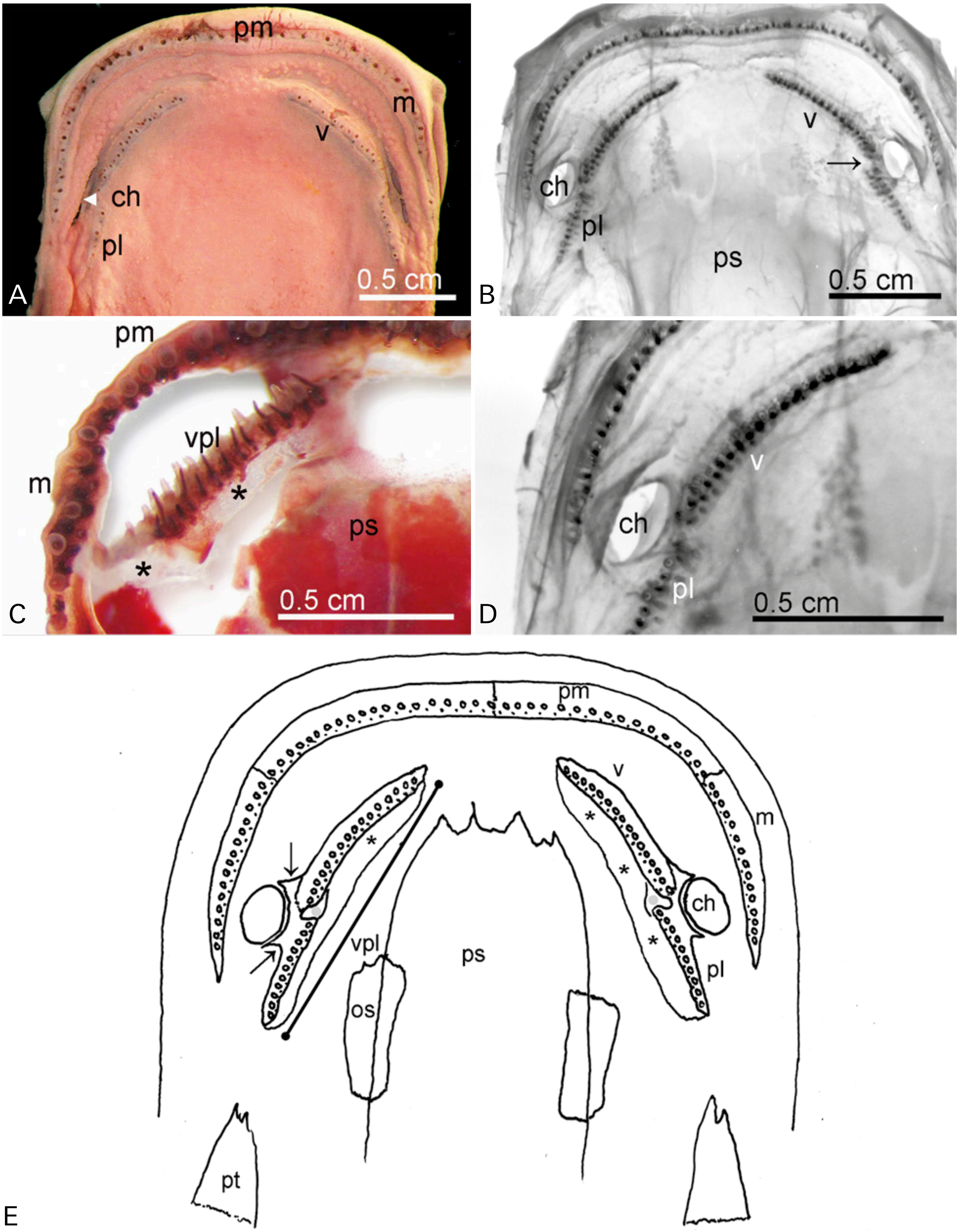

Fig. 1. A Mouth roof of the spontaneously transforming *A. mexicanum*. A. After slight staining with Alizarin red. **B** The same preparation in methyl salycilate. **C** Isolated part of the vomeropalatinum. Most parts of the palatinal portion have got lost. Note the large pars palatina (asterisks). **D** Section from figure B. Note the gap between the tooth row of the vomerine and the palatinal portion and their ossified connection. **E** Schematic overview; the line indicates the extension of the vomeropalatinum. Abbreviations and symbols: arrows point to the zone of fusion; asterisk = pars palatina; grey large dots = recess; small circles = functional teeth; points = developing teeth. ch = choana, m = *maxillare* (maxilla); pl = *palatinum* (palatine); os = *orbithospenoidum* (orbithosphenoid); pm = *praemaxillare* (premaxilla); ps = *parasphenoidale* (parasphenoid); pt = *pterygoideum* (pterygoid); v = *vomer* (vomer); vpl = vomeropalatinum.

and other adult paedomorphic urodeles such as *Eurycea* spp. (Clemen & Greven, 2000; Clemen *et al*., 2009), but not in transformed specimens (for *A. mexicanum* see Fig. 1 b, c in Clemen & Greven, 1977). Broad partes palatinae are also present in *Lissotriton vulgaris* during delayed metamorphosis (Greven *et al*., 2015).

Distinctive postmetamorphic characters in the transforming axolotl are (1) the wide separation of the palatine and the pterygoid, which form a single bone in larva and paedomorphs, the palatopterygoid, (2) the obvious fusion of the vomer and anterior parts of the palatine (vomeropalatinum), and (3) bicuspid teeth at least on the upper jaw (not shown), which are, however, already present in paedomorphic specimens (see above; Clemen & Greven, 1977). The disintegration of the bony connection of the palate and the pterygoid is clearly TH-dependent and its onset is an important morphological criterion for the onset of metamorphosis (e.g. summarized in Rose 2003). The vomeropalatinum, whose formation is not necessarily TH-dependent (see below), extends far beyond the choana and shows a clear gap in the tooth row (and in the accompanying dental lamina; see Clemen & Greven, 1977).

From the organisation of the palate it can be concluded that the herein described transforming axolotl has reached the mature paedomorphic stage, when metamorphosis has started.

Finally, a few comments to the vomeropalatinum: Findings of Lebedkina (1979, 2005) Clemen (1979), Smirnov & Vassilieva (2005) as well as the present results demonstrate that in the axolotl a vomeropalatinum occurs after induced and after spontaneous metamorphosis. A similar phenomenon was observed by one of us in very old (ca. 8 years) paedomorphic axolotls (G.C. unpublished). We previously hypothesized that the fusion of the vomer and anterior parts of the palatine may also be present in other ambystomatids and, if so, its presence might be used as systematic character (Clemen & Greven, 1994). Smirnov & Vassileva (2003) also found a vomeropalatinum in *Lissotriton vulgaris* treated with a high dosage of TH. With regard to the axolotl authors discuss the possible teratological nature of the vomeropalatinum and doubt its presence in naturally transforming ambystomatids (Smirnov & Vassilieva, 2005; see also Rose, 2003). We will look at this issue more closely in the near future.

In this context, it should be noted that not only vomer and palatine (after separation from the pterygoid) may fuse, but in several Urodela even vomer and palatopterygoid, i.e. a fusion takes place before splitting the latter in palatine and pterygoid. However, the position of the fusion site, indicated morphologically by a gap or recess in the bone, may vary. In the axolotl this recess lies always lingually, in the other urodeles mentioned below always buccally. The causes for that are largely unknown and may differ between taxa, but generally such a fusion may be promoted by the close proximity of the two elements (see discussion in Greven *et al*., 2015). Presence of a vomeropterygopalatinum was shown in the paedomorphic *Eurycea rathbuni* (Clemen *et al*., 2009), TH-treated axolotls (Smirnow & Vassilieva, 2009), and several wild-caught larval salamandrids such as *Lissotriton vulgaris* (Greven *et al*., 2015) and *Ichthyosaura alpestris* (unpublished). In the latter two species fusion was probably caused by a climate-induced delayed metamorphosis. Certainly, these phenomena depict deviations from the "normal" development, but are not always caused by typical teratogenic agents.

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