The Development of the Olfactory Organ of Kaloula borealis (Barbour) as compared with that of Rana nigromaculata Hallowell

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With 7 Text-figures

In a previous paper on the development of the olfactory organ in Rana nigromaculata by one of the present authors (Tsui, 1946a) questions were raised about the significance of the ephemeral existence of the lateral appendix and about the functions of different kinds of nasal glands and of Jacobson's organ or the recessus medi alis. To elucidate them experimental studies will be necessary. Unfortunately the species nigromaculata which is abundant in North China is very rare in this part of the country. Kaloula borealis, on the other hand, is very common here. In order to use it as experimental material, familiarity with the developmental history of its nasal organ is prerequisite. In addition, it is of interest to compare the development of this organ in Rana nigromaculata and Kaloula borealis, and to see whether certain findings in the former species concerning certain controversial points will be confirmed in the latter.

The results of a complete study of the development of the nasal organ in Kaloula borealis and of its comparison with that of Rana nigromaculata are embodied in the present paper.

For the sake of shortness the two species will be generally referred to by their generic names only.

Material and Technique

Kaloula borealis is very abundant in Kunming. The animals stay in their subterranean burrows in the dry months.
of the year but come out to breed in the rainy season (June to August). Their eggs are found on the surface of shallow and temporary pools but are very rare in the deeper ponds or paddy fields where eggs of toads and other frogs are commonly found. They form loose masses and are easily separated from one another by a water current. They are easily distinguishable from other amphibian eggs by the cap-shaped jelly layer at the animal pole. From eggs hatched in the laboratory a series of young larvae, 2–10 mm. in body length, was preserved. Larvae of later stages were collected from the field and immediately preserved. They are identifiable by the absence of the external naris, papillae, horny beak, and horny teeth around the mouth. The larger larvae (24 mm. long or over) are further characterized by possessing a fine whitish line running from the nose to the eye on each side of the head (Boring, Liu and Chou, 1932). This characteristic feature makes the identification easier, as it can be seen with the naked eye. For fixation, Spuler’s fluid gave most satisfactory results. Bouin’s fluid caused some shrinkage and was not used after a preliminary trial. Serial cross sections were cut 8μ in thickness and stained with Delafield’s hematoxylin and eosin. For the purpose of differentiating the ectoderm and endoderm in the primitive oral cavity, serial sagittal sections were cut 5μ in thickness, and stained with Heidenhain’s hematoxylin.

**Developmental Anatomy**

A. Olfactory Cavities.

The development of the olfactory organ in *Kaloula borealis* is similar in general outline to that of *Rana nigromaculata*. It is, however, comparatively simple and may be conveniently divided into three stages of four as in the case of *Rana*.

1. First Stage.—*From the inception of the olfactory anlage to the formation of the primitive choana (length of larva, 3–7.5 mm.).* The method of formation of the olfactory anlage is similar to that in *Rana*, only it appears a little later in life (length of larva, 3mm.). Following this the olfactory pit appears. In the same
manner as in *Rana* the pit extends upward and inward to form the dorsal and middle lumen. The neighbouring cells behind the dorsal lumen are then differentiated to form the lateral appendix. As in *Rana*, posterior to the lateral appendix the olfactory placode is divisible into two portions—a thin lateral one and a thick median one—between which there is a series of clefts. These merge later with one another to form a narrow cavity which becomes continuous with the middle lumen. This cavity corresponds in position to the ventral lumen in *Rana*, but the inward projection which marks off the middle lumen from the ventral lumen in *Rana* is wanting in this species. Therefore, this cavity can be regarded only as a posterior extension of the middle lumen.

The so-called oro-nasal groove which Kurepina (1931) claims to be present in the amphibian larvae was not found in *Rana*. In *Kaloula* there is also no such structure.

The mode of fusion of endoderm and ectoderm at each side of the pharyngeal membrane in the roof of the primitive oral cavity is exactly the same as found in *Rana*. The primitive choana opens into the endodermal part of the oral cavity (when the larva is 6 mm. long) in the same manner as in that species.

During the opening of the primitive choana the olfactory placode extends posteriorly to form a prolongation beyond the primitive choana. It is small and solid at first, but soon lengthens and becomes hollow containing a lumen which constitutes a further extension of the posterior part of the middle lumen.

The external naris is formed immediately after the formation of the primitive choana (length of larva, 6-5 mm.). As in *Rana* there is also a short entrance canal which connects the olfactory organ with the external naris. This opening exists, however, only for a short while in *Kaloula* and closes up when the larva is only 7-5 mm. long. During the greater part of larval life there is no external opening to the nasal organ. The communication of the nasal organ with the outside is through the mouth only. As will be described later, external nares are formed afresh at the beginning of metamorphosis.

2. Second Stage.—Opening up of the middle lumen and formation of the blind sacs (length
of larva, 7.5–48 mm.). The olfactory placode enlarges and its floor becomes longitudinally split in the middle. This separation begins from the end next to the primitive choana and extends anteriorly to the front-most part of the placode (Text-figs. 1A and 2). As a result, the middle lumen now opens up along its entire ventral side into the oral cavity and forms together with the primitive choana a simple opening at this stage. This large and compound opening is designated as the choana, since through it the olfactory organ communicates with the oral cavity. The posterior prolongation described in a preceding paragraph remains as a blind sac behind the choana (Text-fig. 1B).

In Rana the primitive choana is the terminal opening of the ventral lumen. This position remains unchanged throughout the whole of its embryonic life. The position of the primitive choana in Kaloula corresponds to that in Rana at an early stage. However, as soon as the posterior prolongation lengthens out from the olfactory placode and becomes a blind sac, its position is no longer terminal. Then, too, the opening of the middle lumen into the oral cavity obscures its identity, making the compound opening (the opened middle lumen plus the primitive choana) look like an enlarged primitive choana. Thus, the existence of a sac posterior to it and its confluence with the middle lumen tend to make the primitive choana appear shifted forward and extended anteriorly, but in reality it remains in its original position as in the case of Rana.

To our knowledge, the opening of the middle lumen direct into the oral cavity is found only in this species. The significance of it is unknown, but it is interesting to note that this change which enables water in the oral cavity to come in easy contact with the olfactory placode is synchronous with the closing up of the external naris.

There are also three embryonic blind sacs at the end of this stage—the anterior lower sac, upper sac, and posterior lower sac.

The anterior lower sac arises from the ventro-anterior region of the olfactory placode (length of larva, 10 mm.; Text-fig. 2) as found in Rana. The upper sac, and the posterior lower sac arise in the following manner.
A. Cross section through the olfactory organ, showing the opening of the middle lumen into the oral cavity (length of larva, 8 mm.). The section is slightly oblique; the left olfactory organ is cut through the most anterior region and the right one through the middle region. Cho, choana; Dori, dorsal lumen; Ethch, ethmoidal part of chondrocranium; Latap, lateral appendix; Midl, middle lumen; Olfpl, olfactory placode; Orep, oral epithelium; Sk, skin. x120.

B. Cross section through the same specimen as A but posterior to it. Midl, posterior extension of the middle lumen; Olfpl, posterior prolongation of the olfactory placode. Other lettering as in A. x120.
At the end of the first stage when the naris is closed up the lumen of the distal part of the entrance canal is obliterated, but its wall remains intact persisting as a fine cord (Text-fig. 2). The proximal part of the canal, which is connected with the olfactory placode, remains open, though the lumen is very small. When the larva reaches the length of 11 mm. this part together with the adjacent part of the placode enlarges to form a small dorsal evagination (Text-fig. 3). The closed portion of the canal persists and is connected with the dorsal evagination. As the dorsal evagination enlarges, it forces open again a portion of the closed entrance canal, so that its length decreases accordingly (Text-fig. 4 A).

Later (length of larva, 17 mm.) the posterior part of the dorsal evagination together with the upper part of the olfactory placode extends medially, and there appears a median groove between the upper and lower part of the placode (Text-fig. 4 B). This groove is at first limited to the anterior portion, but later, as the placode enlarges, it extends posteriorly and at the same time widens into a trough which will be called the median trough.
Finally (when the larva is 40 mm. long), it extends to the end of the posterior prolongation of the placode.

During the extension of the median trough the lower part of the placode expands laterally (length of larva, 27 mm.). As a result, a trough on the other side or a lateral trough is formed (Text-fig. 5 A and B). It extends also posteriorly and meets the median trough at the end of the posterior prolongation of the placode, thus dividing the placode into two compartments, which are henceforth known as the upper sac and the posterior lower sac with the choana as the opening of the latter (Text-fig. 5 A and B).

The formation of the upper and posterior lower sacs in the embryonic olfactory organ of Kaloula as described above is similar to that found in Rana. But in Rana there appears at a very early stage an inward projection which later forms a lateral groove dividing the olfactory placode early into upper
TEXT-FIG. 4 A and B.

A. Cross section through the anterior region of the olfactory organ, showing the enlargement of the dorsal evagination. The arrows indicate the direction of its extension immediately posterior to this section; the dotted lines show the position of the closed entrance canal anterior to this section (length of larva, 29 mm.).

**Antlos**, anterior lower sac; **Dorev**, dorsal evagination; **Medgl**, median nasal gland. Other lettering as in Text-fig. 1 A. $\times 60$.

B. Cross section through the middle region of the olfactory organ, showing the median trough (same specimen as A but posterior to it). **Medgl**, median nasal gland; **Medtr**, median trough; **Ups**, upper sac. Other lettering as in Text-fig. 1 A. $\times 60$. 
A. Cross section through the anterior region of the olfactory organ (length of larva, 42 mm.). Latgl, lateral nasal gland; Lattr, lateral trough; Medgl, median nasal gland; Medtr, median trough; Nascap, cartilaginous nasal capsule; Naso duct, naso-lachrymal duct; Postlos, posterior lower sac; Ups, upper sac. Other lettering as in Text-fig. 1 A. × 40.

B. Cross section through the posterior region of the olfactory organ of the same specimen as A. Midl, posterior extension of the middle lumen; Phagl, pharyngeal gland. Other lettering as in A, and in Text-fig. 1 A. × 40.
and posterior lower sac on the outer side. In Kaloula no such lateral groove is found. The upper sac is not marked off from the posterior lower one until the median trough appears. The lateral trough appears still later, which corresponds to the supralateral groove in Rana. In that species when the upper sac enlarges there is also formed below it a similar medial trough. It is clearly shown in the model depicted in figs. 20–2 of the previous paper (Tsui, 1946a), but unfortunately the median view of the model which would have shown the median trough was not depicted.

In Rana there exists a ledge, between the two grooves—the lateral and superlateral grooves. This ledge together with the lateral groove below it is absent in Kaloula. The ledge in Rana is the anlage of the recessus lateralis, which in the case of Kaloula grows out directly from the side extension of the cavum principale during the metamorphosis.

When the larva grows to the length of 33 mm. there arises another small evagination in the antero-median region of the upper sac (Text-fig. 6). This region corresponds to the lower part of the enlarged entrance canal in Rana at a point above the anterior lower sac. The evagination lengthens medially and laterally (Text-fig. 7a and b), and becomes the cavum medium in the adult stage. It arises in the same manner as the same structure in Rana.

It may be pointed out here that the mode of formation of all the blind sacs in the embryonic olfactory organ of these two species is almost the same. Only, in Kaloula the closure of the external naris and of the distal part of the entrance canal and the absence of the lateral groove together with the ledge above it are responsible for minor differences.

3. Third Stage.—From the beginning of the metamorphosis to the adult stage (48 mm. larva to young frog). When the tadpole reaches 48 mm. in length, its tail begins to be absorbed. The shrinkage in size both of the body and of the olfactory organ is more pronounced than in Rana. There is also a similar shifting of the embryonic nasal organ to the tip of the head.
The first remarkable change is the formation of the external naris. At the end of the second stage the dorsal evagination grows actively upwards (dorsad, Text-fig. 6) until it reaches the skin at a point median to the former (now obliterated) external naris (Text-fig. 7 A). At the beginning of present stage the point of contact between the dorsal evagination and the skin is broken through; thus a new external naris is formed (Text-fig. 7 B). That the external naris is formed de novo and is not the old

Cross section through the anterior region of the left olfactory organ, showing that the closed entrance canal is now ruptured. Its distal end is attached to the skin at the spot where the external naris existed. Its proximal part is now connected with the naso-lachrymal duct. The section is slightly oblique, so that the distal end of the closed entrance canal, which is a little bit anterior to the proximal end, is shown in the same section; the dotted lines indicate the epidermal ridge (anlage of the naso-lachrymal duct) posterior to this section. It runs along the skin but begins to get detached at its proximal end (length of larva, 38 mm.). Antlos, anterior lower sac; Cavmed, cavum medium; Entca, closed entrance canal; Midgl, middle nasal gland. Other lettering as in Text-figs. 1 A and 5 A. x 60.
one now reopened is clearly demonstrable. During the second stage the spot in the skin where the former external naris existed can be located by its connexion with the remnant of the

Cross section through the anterior region of the olfactory organ, showing the dorsal evagination extending upward to the skin and the advanced nasal sacs at the end of the larval stage (length of larva, 43 mm.). Cavinf, cavum inferius; Cavmed, cavum medium; Cavprinc, cavum principale; Dor.ev, dorsal evagination; Ethch, ethmoidal part of chondrocranium; Medgl, medial nasal gland; Midgl, middle nasal gland; Nassept, cartilaginous nasal capsule; Naseduct, naso-lachrymal duct, Orep, oral epithelium; Recmed, recessus medialis; Sk, skin; the hatched area lateral to the dorsal evagination indicates the former external naris where the distal end of the closed entrance canal was attached. ×60.

entrance canal. Towards the end of this stage, this entrance canal is ruptured; its proximal part becomes connected with the naso-lachrymal duct whose formation will be described later, and the distal end first appears as a peg formed beneath and then fused with the bit of skin which sealed off the former external naris (Text-fig. 6). The spot where the external naris once existed
is still recognizable up to the time of the appearance of the new naris. This new opening is median to this spot (Text-fig. 7 A).

Noble (1931, p. 62) states that in the Brevicipitidae 'the external nares do not break through until late in larval life'. In Kaloula borealis obviously functional external nares exist in the young larva, though they soon close up. One wonders if other species of the Brevicipitidae may not undergo similar developmental changes resulting in having external nares at two periods in their life-history. The fact that during the major part of its larval life Kaloula is without external nasal opening evidently escaped the notice of Boring, Liu and Chou (1932), for in their book they mention and figure nostrils in the late-stage larva of the same species.

Recessus alaris, infundibulum, and recessus sacciformis are differentiated from the principal portion of

**Text-fig. 7 B.**

Cross section through the anterior region of the olfactory organ, showing the opening of the external naris (beginning of metamorphosis). **Ext.naris**, external naris; **Lat.gl.,** lateral nasal gland; **Reclat,** recessus lateralis. Other lettering as in Text-fig. 7 A. × 60.
the dorsal evagination. The formation of these three sacs is identical with that in *Rana*, though here the process is more or less indirect. In that species these sacs arise directly from the upper part of the enlarged entrance canal. In *Kaloula* as described in a preceding paragraph, the proximal part of the entrance canal together with a part of the placode first forms a dorsal evagination. The three sacs arise in turn from it.

The formation of the cavum principale, cavum medium, cavum inferius, and recessus medialis occurs in the same manner as in *Rana*. In the absence of the ledge (see p. 308) on the lateral wall of the embryonic nasal organ, the mature recessus lateralis is simply derived from the side extension of the cavum principale.

The choana opens very widely. As soon as the upper sac together with the cavum medium and anterior lower sac extends anteriorly at the beginning of this stage, the choana is shifted posteriorly. Its mature position is in the ventro-posterior part of the olfactory organ—as in *Rana*.

B. Lateral Appendix, Accessory Glands, and Naso-lachrymal Duct.

Lateral appendix.—As in *Rana* the lateral appendix is here also an embryonic structure. Its formation, change of position and degeneration in the larval olfactory organ of *Kaloula* are, in general, similar to those found in *Rana* (Tsui, 1946b).

When the appendix reaches the height of its development (larva length, 9 mm.), it contains about three layers of epithelial cells. After this period the appendix begins to degenerate by diminishing its cell layers. This process goes on very slowly. It occupies the whole length of the second stage. In the beginning of the metamorphosis the appendix consists of only a single layer of high columnar cells. There is, however, little diminution in the size of the organ. At the end of this developmental stage its size is rapidly reduced and it becomes embedded in the olfactory epithelium as a small vestige. It disappears completely in the nasal organ of the young frog.

Glands.—The origin and development of the pharyngeal gland, and the median and lateral nasal glands are similar to
those in *Rana*. The larval lengths at the time of origin of these glands are 40 mm. for the pharyngeal gland, 17 mm. for the median, and 42 mm. for the lateral nasal gland. The first two arise later than in *Rana*, while the last one appears much earlier. Bowman’s gland appears also earlier (larval length, 48 mm.). Its mouth part appears at the same time as its body, while in *Rana* the mouth appears earlier than the body. Moreover, all Bowman’s glands make their appearance simultaneously in the olfactory epithelium of the *cavum principale*. In *Rana* they appear earlier in the posterior than in the anterior part of that cavity.

There is another nasal gland in *Kaloula* which is not found in *Rana* nor, to our knowledge, in any other species of Amphibia. It arises in the median wall of the anterior part of the upper sac above the anlage of the *cavum medium* (length of larva at the time of its appearance, 38 mm.; Text-fig. 6). It grows slowly at the second stage and at the beginning of the metamorphosis. Later, as the upper sac enlarges to form the *cavum principale*, its olfactory epithelium thickens; the gland becomes embedded in the epithelium (Text-fig. 7A and B). At the end of this stage this gland bifurcates and increases suddenly in size. As a result of the bifurcation it possesses two ducts. In the adult stage it lies between the *cavum principale* and *cavum medium*. Its two ducts open one after the other into the ventral wall of the *cavum principale*. We shall designate it as the middle nasal gland because its position is between the median and lateral nasal glands.

**Naso-lachrymal Duct.**—As described in a preceding paragraph (see p. 304) the lumen of the distal part of the entrance canal is obliterated after the closure of the external naris. The epithelial cells at the junction of the distal end of the closed entrance canal with the skin begin to change their shape. These cells which were flat now becomes first cuboidal and then perpendicularly elongated to form a little knob below the epidermis. From this starting-point the wave of differentiation progresses in a latero-posterior direction until it reaches the lower eyelid. In this manner an epidermal ridge is formed running from the closed naris to the lower eyelid. This ridge is the
anlage of the naso-lachrymal duct. Later it becomes separated from the epidermis; the separation also starts near the closed entrance canal (Text-fig. 6) and progresses distally until the ridge is detached along its whole length. This process goes on very slowly, beginning when the larva is 38 mm. long and not being completed until the beginning of the metamorphosis.

At the time when the ridge starts to get detached from the epidermis, its loose proximal end grows medio-anteriorly for a short stretch to become connected with the proximal part of the closed and now ruptured entrance canal. That part is thus transformed into the proximal end of the future naso-lachrymal duct (Text-fig. 6). Since the entrance canal is attached to the lateral wall of the dorsal evagination at this stage, the epidermal ridge by virtue of this transformation becomes connected with it (Text-fig. 6). At the end of the second stage, the cavum m e d i u m enlarges and extends laterally, until the part of the dorsal evagination to which the epidermal ridge is attached becomes a portion of its lateral wall. As a result the proximal end of the future naso-lachrymal duct is now attached to the latero-posterior part of the cavum m e d i u m. Following this the lumen appears in the duct (Text-fig. 7 a and b). Its distal end bifurcates and the two branches open into the lower eye-lid. Thus the naso-lachrymal duct comes into being at the beginning of the metamorphosis. It undergoes no further change.

As stated above (see p. 300), in the larva of later developmental stages (24 mm. in length or over) there is a visible whitish line running from the nose to the eye. This line marks the position of the epidermal ridge. The pigment cells are crowded under the epidermis at these stages, but they are absent or scarce under the ridge, thus leaving a transparent line where the ridge lies. As a matter of fact the ridge begins to appear much earlier in life (when the larva is 14 mm. long). But at that time its presence is not easily detectable externally, because the pigment cells are then uniformly scattered under the epidermis. Under a low-power microscope, however, the ridge itself can be seen as a fine opaque line. When the ridge is completely detached from the epidermis and becomes the naso-lachrymal duct at the
beginning of metamorphosis, the epidermis thickens and the whitish line is no longer visible.

In *Rana*, the naso-lachrymal duct appears much later (at the beginning of metamorphosis) and arises directly from a point in the dorso-lateral wall of the cavum principale behind the external naris and later, owing to transformation of the blind sacs, becomes attached to the latero-posterior part of the cavum medium. In *Kaloula* this duct appears earlier in larval life and arises from the epidermis of the skin. Later, as it gets connected with the lateral wall of the dorsal evagination and subsequently with cavum medium (the lateral wall of the dorsal evagination has become the latero-posterior wall of the cavum medium), the point of attachment of the naso-lachrymal duct becomes identical in the two species. Even in the point of origin of the duct, it is not so different in the two species as it appears; for the spot where the naso-lachrymal duct arises in *Rana* is actually in the former dorso-lateral wall of the entrance canal. Only, in *Kaloula* the developmental history of the naso-lachrymal duct is longer and round-about, starting in the epidermis and becoming subsequently detached.

This mode of origin of the naso-lachrymal duct in epidermis in *Kaloula* may be in some way connected with closure of the external naris. In the Brevicipitidae, the external naris is said to open (reopen?) late in larval life. It would be interesting to know if in other species of this family the naso-lachrymal duct forms in the same way as in *Kaloula borealis*.

**Summary**

1. The anlage of the olfactory placode of *Kaloula borealis* arises from the sensory layer of ectoderm as in *Rana*, but appears slightly later.
2. There is also no such structure as the oro-nasal groove.
3. There are present only the dorsal and middle lumina in the early developmental stage. A separate ventral lumen and the inward projection present in *Rana* are wanting in *Kaloula*.
4. The primitive choana opens into the endodermal part of the oral cavity as in *Rana*.
5. The external naris closes up after the formation of the primitive choana and is formed de novo at the beginning of the metamorphosis.

6. The floor of the middle lumen opens entirely into the oral cavity. The process which synchronizes with the closure of the external naris begins from the end next to the primitive choana and extends anteriorly. This compound opening forms the choana in the adult stage.

7. There are also three embryonic nasal sacs in Kaloula. Their mode of origin and prospective rôle in the formation of the mature nasal cavities are essentially identical with those in Rana.

8. The shrinkage of the nasal organ in metamorphosis is more pronounced than in Rana.

9. The lateral appendices in Kaloula and Rana are alike in their manner of formation, change of position, and degeneration.

10. The formation of the median and lateral nasal glands, pharyngeal gland, and Bowman’s gland is similar to that found in Rana.

11. There exists a nasal gland, which is not known in Rana. It is designated as the middle nasal gland.

12. The naso-lachrymal duct differentiates under the skin as an epidermal ridge and is subsequently detached. It is initiated at the distal end of the closed entrance canal, but is later connected with its proximal end. When fully differentiated it opens into the latero-posterior part of the cavum medium at its proximal end.

**Literature Cited**


——— 1946 b.—“Morphological observations on the fate of the lateral appendix in the embryonic olfactory organ of Rana nigromaculata Hallowell”, Ibid., 87.