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Comparative Anatomy and Physiology of Chemical Senses in Amphibians

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In this chapter, we first introduce the chemosensory systems of tetrapods, then focus on their structure and function in amphibians. Tetrapods possess three major chemosensory systems, the olfactory, vomeronasal, and gustatory (taste) systems. These are defined anatomically. The olfactory epithelium is found in the nasal cavity, and the axons of the olfactory receptor (OR) neurons project to the olfactory bulb at the rostral pole of the telencephalon. Most tetrapods also possess a vomeronasal system, an accessory olfactory system, the sensory epithelium of which is usually located in an organ that is distinct from the main nasal cavity. The axons of the vomeronasal receptor neurons project to the accessory olfactory bulb, a histologically distinct structure adjacent to the olfactory bulb. In contrast, the taste buds are found on the tongue, palate, and pharynx and are innervated by sensory neurons of cranial nerves VII, IX, X, which convey taste information to the hindbrain. In general, the olfactory system is involved in detecting chemicals emanating from distant sources, whereas the taste and vomeronasal systems are involved in detecting chemicals at close range (Eisthen and Schwenk, chapter 3 in this volume).

Tetrapods also possess chemosensors in the respiratory, circulatory, and digestive systems

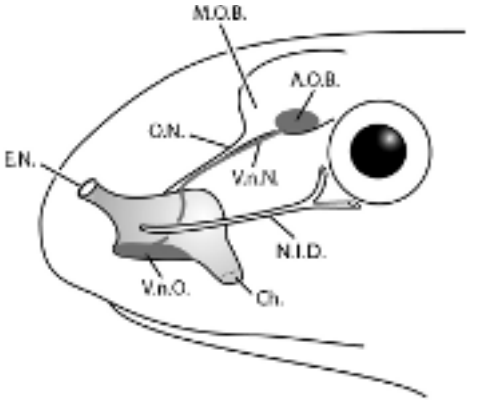


FIGURE 4.1. Schematic illustration of the olfactory organs and rostral telencephalon in a generalized terrestrial tetrapod. The vomeronasal organ is represented by a trough along the ventral side of the main olfactory organ, the condition seen in turtles and amphibian embryos. The olfactory nerve projects to the main olfactory bulb, and the vomeronasal nerve to the accessory olfactory bulb. Abbreviations: A.O.B., accessory olfactory bulb; Ch., choana; E.N., external naris; M.O.B., main olfactory bulb; N.l.D., nasolacrimal duct; O.N., olfactory nerve; V.n.O., vomeronasal organ; and V.n.N., vomeronasal nerve.

that detect gasses, ions, and nutrients. In general, these chemosensory systems consist of scattered, isolated sensory cells that project to the spinal cord and hindbrain. These systems are not considered further here.

OLFACTION

The peripheral olfactory system in tetrapods, including all amphibians, consists of paired nasal sacs (Fig. 4.1), each of which has an incurrent external naris and an excurrent internal naris, or choana, opening into the buccal cavity (Parsons, 1967; Bertmar, 1969). The lining of the nasal sac consists of one or more regions of olfactory epithelium, with the remainder respiratory epithelium. The olfactory sensory epithelium contains three main cell types: receptor cells; sustentacular (supporting) cells; and basal cells, the progenitors of the receptor and sustentacular cells. The receptor cells are bipolar neurons, with dendrites that extend to the lumen of the nasal sac and axons that form an olfactory nerve projecting to the olfactory bulb at the rostral pole of the telencephalon. The odorant receptor proteins, members of a large family of G-protein-coupled receptors that possess seven membrane-spanning regions (Mombaerts, 2004), are localized to the dendrites of receptor cells, which terminate in cilia and/or microvilli, increasing the amount of membrane available for odorant transduction. It has recently been shown that vertebrate OR proteins form a number of well-distinguished phylogenetic groups, groups α through κ (Niimura and Nei, 2005, 2006). Two of these, groups α and γ (class II of Freitag et al. [1995, 1998]), are particularly well developed in tetrapods, and it has been suggested that they are specialized for olfaction in air. The cilia and microvilli carrying ORs are embedded in a layer of mucus that covers the surface of the sensory epithelium. This mucus is secreted by numerous simple Bowman's glands, and these glands are scattered throughout the epithelium. Accessory compound nasal glands are also frequently present. Both types of glands are lacking in fishes and in some aquatic amphibians. In many terrestrial taxa the nasal epithelia are borne on one or more conchae, which serve to increase surface area for olfaction and/or for humidifying the inspired air; in endotherms, the conchae may also warm the air.

VOMERONASAL SYSTEM

The vomeronasal system is a specialized olfactory subsystem found only in tetrapods. It has been suggested that it is specialized for detection of nonvolatile stimuli, including some pheromones, though this hypothesis does not account for all available data (Baxi et al., 2006). The relative functions of the vomeronasal and olfactory systems therefore remain unclear. In amphibians, the vomeronasal, or Jacobson's, organ consists of a diverticulum off the main nasal cavity, but in amniotes the vomeronasal organ can be directly connected to the nasal or

oral cavity, or indirectly connected to both via a nasopalatine duct. The vomeronasal organ is secondarily absent in many taxa, including birds and crocodilians, many bats and cetaceans, and Old World primates, including humans. The vomeronasal sensory epithelium generally resembles the olfactory epithelium, although the dendrites of the vomeronasal receptor neurons terminate exclusively in microvilli. Axons from the vomeronasal receptor neurons project to an accessory olfactory bulb, which is distinct from the main olfactory bulb; higher-order projections also differ. Finally, vomeronasal receptor neurons use different transduction mechanisms than do OR neurons, including a different ion channel and different families of G-protein-coupled receptors (Liman et al., 1999; Mombaerts, 2004).

Supporting the idea that the vomeronasal system detects nonvolatile stimuli, odorant access to the vomeronasal organ in the terrestrial environment frequently involves direct transfer and/or fluid-pumping mechanisms. For example, in squamates the tongue is used to sample chemicals from the environment, which are then transferred to the vomeronasal organ. In many mammals the vomeronasal organ can acquire stimuli by means of the flehmen reflex, by vascular pumping, or due to contraction of the entire organ (Meredith, 1994).

The acquisition and loss of the vomeronasal system in tetrapods has long been suggested to be associated with transitions between aquatic and terrestrial habitats. Broman (1920) used his observation of fluid in the vomeronasal lumen to argue that the vomeronasal organ is specialized for smelling dissolved substances and represents a remnant of the piscine olfactory system, with the main olfactory system newly developed in tetrapods. In contrast, Bertmar (1981) suggested that the vomeronasal system arose in early tetrapods as an adaptation to terrestrial life. However, data from recent studies suggest that the "olfactory epithelium" of teleost fishes is probably a hybrid olfactory and vomeronasal epithelium: different morphological classes of receptor neurons express the olfactory and vomeronasal receptor genes and transduction elements, and these two populations of neurons project to different regions of the olfactory bulb (Cao et al., 1998; Naito et al., 1998; Hansen et al., 2003, 2004, 2005; Sato et al., 2005). The most likely scenario is that the vomeronasal system evolved in tetrapods by partitioning preexisting classes of ORs into distinct main olfactory and vomeronasal epithelia. The question then becomes whether this partitioning was associated with the assumption of terrestrial habits.

Although the vomeronasal system could be independently derived in amphibians and amniotes, this seems unlikely, as the major projections of the main and accessory olfactory bulbs are the same in both groups (Eisthen, 1997). Recent paleontological evidence suggests that amphibians and amniotes became terrestrial independently of each other, and that the last common ancestor was fully aquatic (Clack, 2002). In addition, the vomeronasal system is present in amphibians throughout life and does not arise at metamorphosis, as one might expect if the feature is an adaptation to terrestriality (Eisthen, 1997). Thus, the intermixed olfactory and vomeronasal systems in fishes must have become separated into distinct systems before tetrapods became terrestrial.

Interestingly, a distinct vomeronasal system has been secondarily lost in many amniotes that are aquatic or arboreal, as well as in the proteid family of salamanders, all of which are fully aquatic and paedomorphic as adults (Eisthen, 1997, 2000). Loss in arboreal animals is consistent with the hypothesis that the vomeronasal system is a specialization for detecting nonvolatile stimuli, but loss in aquatic animals appears harder to explain on this basis.

TASTE

The sensory organs of the taste system are the taste buds, which in tetrapods are generally found on the tongue, palate, and pharynx

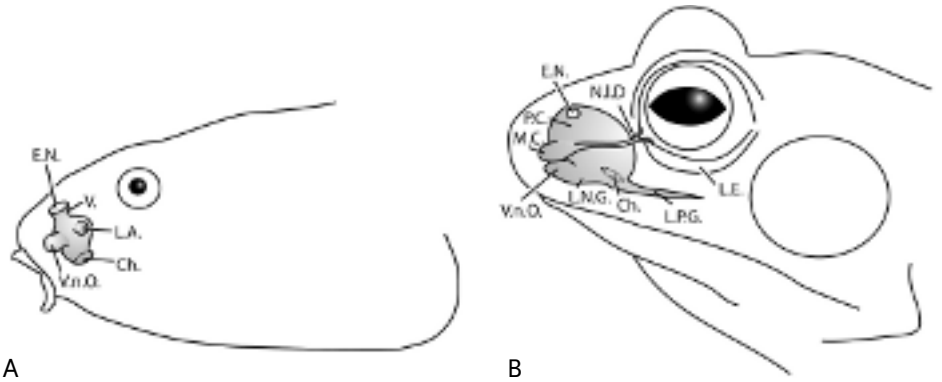


FIGURE 4.2. Schematic illustration of the olfactory organs and associated structures in an anuran tadpole (A) and adult (B), based largely on conditions in the midwife toad, *Alytes obstetricans* (Rowedder, 1937; Yvroud, 1966). Abbreviations: Ch., choana; E.N., external naris; L.A., lateral appendix; L.E., lower eyelid; L.N.G., lateral nasal groove (also called the lateral recess); L.P.G., lateral palatal groove; M.C., middle cavity; N.l.D., nasolacrimal duct; P.C., principal cavity; V., vestibule; and V.n.O., vomeronasal organ (also called the medial recess).

(Northcutt, 2004). Each taste bud consists of taste receptor cells, supporting cells, and basal cells. At the molecular level, taste receptors are heterogeneous, including some that are simple ion channels and others that are members of distinct subfamilies of G-protein-coupled receptors (Bigiani et al., 2003; Mombaerts, 2004).

CHEMOSENSORY SYSTEMS IN AMPHIBIANS

Among tetrapods, amphibians are unique in primitively having an aquatic larval stage, followed by metamorphosis to a more terrestrial adult. Superimposed on this basic biphasic lifecycle, secondarily aquatic adults are found in each of the three groups of living amphibians. Amphibians can become secondarily aquatic in one of two ways (Duellman and Trueb, 1986). In many salamanders, the overall larval morphology is maintained while the gonads mature, an evolutionary process termed neoteny or paedomorphosis. On the other hand, in some frogs (e.g., pipids), salamanders (e.g., newts), and caecilians (e.g., typhlonectids) metamorphosis occurs but is not accompanied by a change in habitat (pipids, typhlonectids) or is followed by a second metamorphosis associated with reentry into the aquatic environment (newts). Intermediate conditions abound, with partially aquatic adults in many taxa, in part because in most forms with aquatic larvae, adults return to water to breed. We first examine the changes that occur during metamorphosis from the aquatic to the terrestrial phase in taxa with a biphasic life history, then examine those evolutionary changes occurring in taxa in which metamorphosed adults have secondarily adopted an aquatic lifestyle.

OLFACTION AND VOMERONASAL CHEMORECEPTION

The morphology of the olfactory organ in adult amphibians has been reviewed by a number of authors (Seydel, 1895; Matthes, 1934; Jurgens, 1971; Saint Girons and Zylberberg, 1992a, 1992b), but the larval condition is less widely known. After a review of the morphology of the larval and adult olfactory organ in each group, we examine general issues of function in aquatic and terrestrial environments.

FROGS

In the larvae of frogs (Anura, Fig. 4.2A), the olfactory organ is highly specialized in association with the small mouth opening (Born, 1876; Hinsberg, 1901; Rowedder, 1937; Yvroud, 1966; Khalil, 1978; Jermakowicz et al., 2004). The nasal sac typically runs almost vertically from external naris to choana. The vomeronasal

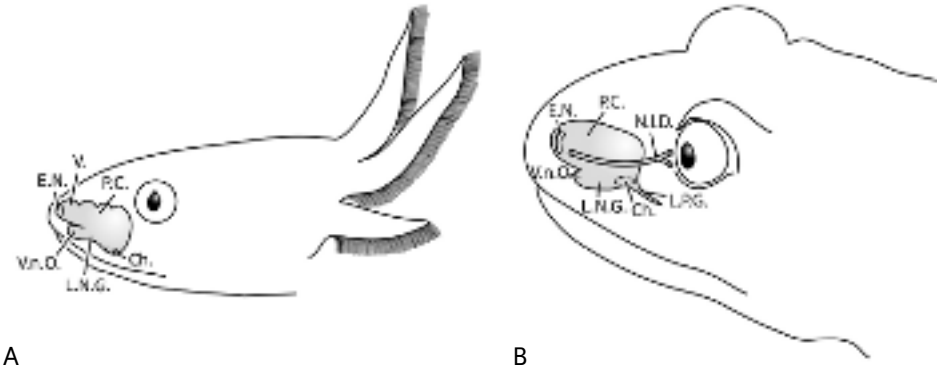


FIGURE 4.3. Schematic illustration of the olfactory organs and associated structures in a salamander larva (A) and adult (B), based largely on conditions in the Coastal Giant Salamander, *Dicamptodon tenebrosus* (Stuelpnagel and Reiss, 2005). Abbreviations: Ch., choana; E.N., external naris; L.N.G., lateral nasal groove; L.P.G., lateral palatal groove; N.l.D., nasolacrimal duct; P.C., principal cavity; V., vestibule; and V.n.O., vomeronasal organ.

organ is represented by a bean-shaped outpocketing on the anterior (morphologically ventral) surface, while several other outpocketings typically occur, including a lateral appendix of unknown function. A nonsensory vestibule is present at the external naris, as is a valve guarding the choana (Gradwell, 1969). Of multicellular glands, only the vomeronasal gland is well developed. Much variation in form of the nose occurs among anuran tadpoles; for example, some have exposed areas of olfactory epithelium within the oral cavity, while others lack external nares (Altig and McDiarmid, 1999).

During metamorphosis, extreme remodeling occurs, as illustrated in Figure 4.2B: the vestibule is reduced or lost, the choana shifts posteriorly and loses its valve, and the vomeronasal organ (medial recess) connects to a lateral nasal groove (lateral recess) that runs posteriorly through the choana to continue as the lateral palatal groove (sulcus maxillopalatinus). The nasolacrimal duct develops and connects to the newly formed middle cavity of the nose. The postmetamorphic olfactory organ thus consists of three interconnected chambers: a principal (superior) cavity, a middle cavity, receiving the nasolacrimal duct; and an inferior cavity, with its lateral and medial recesses. Interestingly, the olfactory eminence in the floor of the principal cavity is best developed in more terrestrial and especially fossorial forms (Jurgens, 1971). In most anurans, a special mechanism involving the lower jaw and the submentalis muscle develops to close the external nares during lung inflation (Gaupp et al., 1904; Nishikawa and Gans, 1996; Jorgensen, 2000). Bowman's glands usually appear only during metamorphosis, as do the rostral (internal oral) and lateral nasal glands.

SALAMANDERS

Among larval salamanders (Caudata), the olfactory organ typically consists of a tubular sac, extending from the external naris to the choana, as shown in Figure 4.3A (Seydel, 1895; Schuch, 1934; Stuelpnagel and Reiss, 2005). As in frogs, a nonsensory vestibule leads into the principal cavity of the olfactory organ, and a nonmuscular choanal valve is usually present at the medial border of the choana, presumably preventing reverse flow of water through the nose (Bruner, 1914a, 1914b). The olfactory epithelium is found in troughs separated by folds of nonsensory epithelium; this may be a primitive feature, because it resembles the condition seen in young lungfish larvae. The vomeronasal organ is usually a ventrolateral diverticulum of the main nasal sac (Seydel, 1895). Bowman's glands are present in most larval salamanders

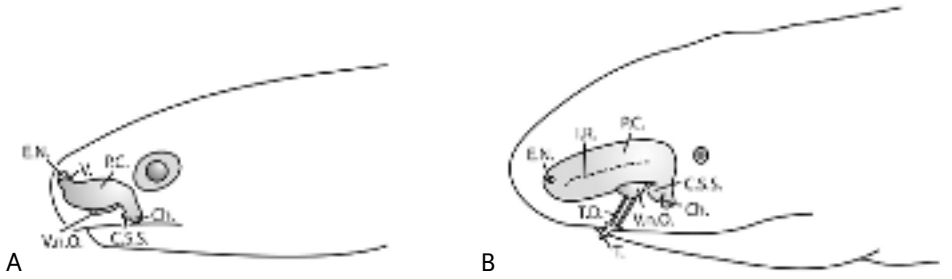


FIGURE 4.4. Schematic illustration of the olfactory organs and associated structures in a caecilian larva (A) and adult (B), based largely on conditions in the genus *Ichthyophis* (Sarasin and Sarasin, 1890; Badenhorst, 1978). Abbreviations: Ch., choana; C.S.S., choanal slime sac; E.N., external naris; I.R., internal ridge separating the medial and lateral portions of the principal cavity; P.C., principal cavity; T., tentacle; T.D., tentacle (nasolacrimal) ducts; V., vestibule; and V.n.O., vomeronasal organ.

but are not abundant and may not be functional; by contrast, the vomeronasal gland is well developed.

During metamorphosis the vestibule is lost, a muscular mechanism for closing the external naris develops (Bruner, 1901; Nikitkin, 1986), the nasal sac widens, the nonsensory folds are greatly reduced or disappear completely, and the choana widens and loses its choanal valve (Fig. 4.3B). The responsiveness of the olfactory epithelium changes: sensitivity to dissolved odorants decreases, while that to volatile odorants increases (Arzt et al., 1986). Much as in anurans, the vomeronasal organ acquires a connection to the oral cavity by extension of the lateral nasal groove posteriorly along the lateral edge of the choana and into the mouth as the lateral palatal groove. The nasolacrimal duct develops, connecting the medial angle of the eye to the lateral nasal sac, just anterior to the vomeronasal organ. The Bowman's glands enlarge and proliferate, and in hynobiids and salamandrids the compound lateral nasal glands develop (Saint Girons and Zylberberg, 1992a). In plethodontid salamanders, the vomeronasal organ shifts forward to acquire a functional connection with the newly developed nasolabial groove (Wilder, 1925). Although some have suggested that ciliated and microvillar OR neurons are specialized for detecting odorants in air and water, respectively, both types of cells are found in salamanders at all stages of development, including in neotenic adults (Eisthen, 1992, 2000).

CAECILIANS

In caecilians (Gymnophiona), the larval olfactory organ is a simple, triangular sac, as depicted in Figure 4.4A (Sarasin and Sarasin, 1890; Badenhorst, 1978). The vomeronasal organ is represented by a diverticulum of the nasal sac; it lies laterally for most of its length but shifts medially at the level of the choana. As in frogs and salamanders, a vestibule and (in at least some species) a choanal valve are present. During metamorphosis the vestibule is lost, and the principal cavity acquires a large ridge on its floor, somewhat resembling the olfactory eminence of anurans (Fig. 4.4B); this divides the nasal cavity into a medial, sensory part, and a lateral, respiratory part (Schmidt and Wake, 1990). The vomeronasal organ changes position, coming to lie transversely, and its distal tip connects with the newly formed nasolacrimal (tentacular) ducts. The tentacle, including the tentacular (Harderian) gland and the retractor muscle, forms, and its lumen connects with the tentacular ducts (Badenhorst, 1978; Billo and Wake, 1987). The choana widens, and the choanal slime sac greatly enlarges, but, unlike in frogs and salamanders, no lateral palatal groove forms. As in frogs and salamanders, the vomeronasal gland is present in larvae, but Bowman's glands and the lateral nasal gland form only during metamorphosis (Badenhorst, 1978).

FUNCTION IN WATER AND AIR

Because of their large cells and simple nasal cavity architecture, amphibians have long been used as model animals for neurobiological research in olfaction, and much detailed information is available concerning mechanisms of olfactory system function at the level of both the sensory receptor cells and the olfactory bulb (Kauer, 2002). Nevertheless, studies of function of the chemical senses in amphibians are spotty, with much information available in some areas, while others are almost completely unexplored. In general, for animals that clearly must deal with both, surprisingly little attention has been paid to differences between function in aquatic and terrestrial environments.

In larvae and neotenes, the nasal sac is typically irrigated with water by the respiratory pump, aided by ciliary action. The flow in larvae is usually unidirectional inward, with backflow being prevented by the choanal valves. As in fishes, air used to inflate the lungs is gulped through the mouth, not taken through the nose. In neotenic salamanders the situation is more variable, with some taking only water through the nose, but others inspiring air as well; a choanal valve may be present or absent (Bruner, 1914b). In larval amphibians, the chemical senses have been shown to be behaviorally important for feeding, predator avoidance, and kin recognition (reviewed in Dawley, 1998), although many of these studies have not distinguished among the roles of the olfactory, vomeronasal, and taste systems. Interestingly, the ability to smell in the aquatic environment is retained after metamorphosis in at least some species. For example, blinded tiger salamanders *(Ambystoma tigrinum)* will preferentially nose tap and bite bags containing earthworms underwater; occlusion of the nares eliminates this response (Nicholas, 1922). Likewise, pheromones involved in aquatic reproduction are widely used in both salamanders (reviewed in Arnold, 1977; Dawley, 1998) and frogs (Wabnitz et al., 1999).

In the terrestrial environment, the olfactory system typically serves to sample air. In all three groups of amphibians, oscillations of the buccal floor with the mouth closed serve to continually bring fresh air into the nasal sac and oral cavity, and airflow is bidirectional (reviewed in Jorgensen, 2000). Lung inflation occurs intermittently by closing the external naris and compressing the floor of the buccal cavity. Sensing of airborne chemicals is important for feeding, homing behavior, and reproduction (reviewed in Dawley, 1998). In plethodontid salamanders, nonvolatile chemicals are transported along the nasolabial groove to the vomeronasal organ (Dawley and Bass, 1989), and in caecilians the tentacular ducts may serve a similar function (Schmidt and Wake, 1990; Himstedt and Simon, 1995). In frogs and salamanders, the vomeronasal organ may also serve to sample fluid transported from the lateral palatal groove forward through the choana (Seydel, 1895).

TASTE

The biology, anatomy, physiology, and development of the taste system of amphibians has recently been reviewed by Barlow (1998) and Zuwala and Jakubowski (2001b). In frogs, significant differences occur between larvae and metamorphosed adults (Fig. 4.5). In larvae, taste buds are present on papillae throughout the oral epithelium. During metamorphosis, the fleshy secondary tongue arises, and the larval taste buds are replaced by taste discs, which are found on the secondary tongue as well on as the oral and pharyngeal epithelium. Taste discs differ from taste buds in cellular composition as well as morphology. A similar change from taste buds to taste discs at metamorphosis has recently been shown to occur in salamanders as well (Takeuchi et al., 1997; Zuwala and Jakubowski, 2001a; Zuwala et al., 2002). In contrast, in caecilians taste buds have been described only in larvae and adults of the secondarily aquatic *Typhlonectes* and may be lost in most metamorphosed forms (Wake and Schwenk, 1986). While the morphological changes in the taste periphery at metamorphosis are profound, the functional significance of these changes remains completely unclear

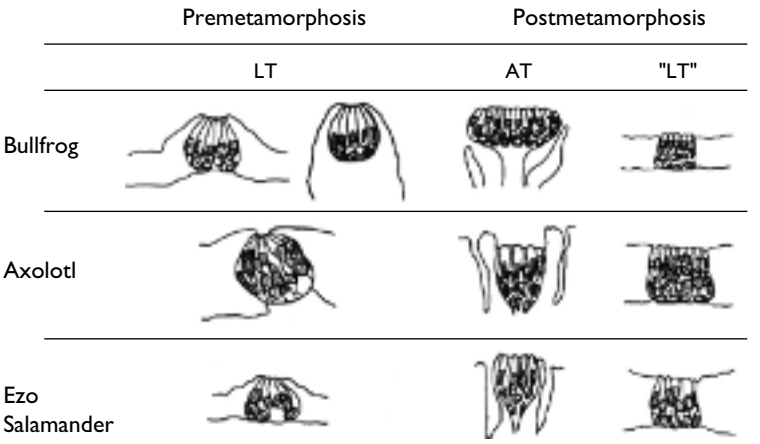


FIGURE 4.5. Overview of tastebud development in bullfrogs *(Rana catesbeiana)* and two species of salamanders, the axolotl *(Ambystoma mexicanum)* and the Ezo salamander *(Hynobius retardatus)*. Barrel-shaped taste buds on the larval tongue (LT) are replaced by fungiform taste disks on the adult tongue (AT), and by taste disks embedded in the oral epithelium on the more posterior "larval tongue remnant" ("LT"). Figure modified after Takeuchi et al. (1997) (permission granted by T. Nagai, Department of Biology, Keio University School of Medicine).

(Barlow, 1998). In particular, although responses to sweet, sour, salty, bitter, and amino acid tastes have been demonstrated electrophysiologically in larvae, neotenes, and adults, there is no evidence for any differences functionally correlated with feeding in aquatic versus terrestrial habitats. However, the results of a recent study (Nagai et al., 2001) suggest that salt taste is mediated by amiloride-sensitive sodium channels in metamorphosed salamanders but not in larvae or neotenes. This indicates that significant physiological differences may exist.

COMPARATIVE CHEMORECEPTION IN SECONDARILY AQUATIC AMPHIBIANS

Adult amphibians often cross the threshold between terrestrial and aquatic environments, but here we focus on three prominent cases in which metamorphosed adults have secondarily adapted to semipermanent or permanent residence in the aquatic environment: pipid frogs, newts (salamandrids), and typhlonectid caecilians. Because the taste system of amphibians has not been extensively explored, our examples focus on olfaction.

FROGS

Pipid frogs (family Pipidae) are the best-studied example of secondarily aquatic amphibians, largely due to the widespread use of the African clawed frog *Xenopus laevis* in laboratory research. Adult *Xenopus* are almost completely aquatic; excursions onto land appear to occur rarely (Tinsley et al., 1996), although terrestrial prey capture has been reported (Measey, 1998). As illustrated in Figure 4.6, adult *Xenopus* possess three main nasal cavities, as in typical terrestrial frogs. These are known as the principal cavity or medial diverticulum, middle cavity or lateral diverticulum, and vomeronasal organ, which comprises the entire inferior cavity (Föske, 1934; Paterson, 1939a, 1939b, 1951; Saint Girons and Zylberberg, 1992a, 1992b; Hansen et al., 1998). The principal cavity is tubular, with no sign of the olfactory eminence developed in terrestrial frogs. Moreover, while in typical frogs the middle cavity is nonsensory, in *Xenopus* the middle cavity contains a well-developed sensory epithelium. Studies by Altner (1962) provide evidence that the principal cavity is used to detect airborne chemical stimuli, while the blind-ended middle cavity is used to detect waterborne stimuli: a flap valve in the

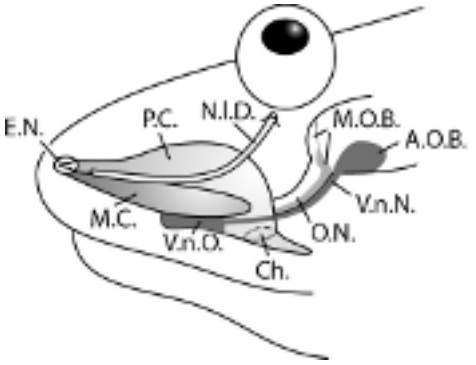


FIGURE 4.6. Schematic illustration of the nasal cavity of the African clawed frog *(Xenopus laevis)*. The axons of the receptor cells in the principal cavity ("air nose") project to the dorsal portion of the main olfactory bulb, and those of the middle cavity ("water nose") project to the ventral portion of the main olfactory bulb. Note the valve in the external naris separating the entrance to the middle and principal cavities, and the papilla under the eye bearing the opening of the nasolacrimal duct. Abbreviations: A.O.B., accessory olfactory bulb; Ch., choana; E.N., external naris; M.C., middle cavity; M.O.B., main olfactory bulb; N.l.D., nasolacrimal duct; O.N., olfactory nerve; P.C., principal cavity; V.n.O., vomeronasal organ; and V.n.N., vomeronasal nerve.

external naris allows access to one or the other chamber depending on the external medium. Water flow in and out of the middle cavity correlates with pulsations observed on the lateral side of the snout, apparently due to rhythmic contractions of the submentalis muscle, which helps close the naris in terrestrial frogs (Altner, 1962). As in typical frogs, the vomeronasal organ is connected to the oral cavity by the lateral nasal groove. A nasolacrimal duct is present, but its entrance is at the tip of a short papilla below the eye, rather than on the lower eyelid as in most frogs. The medial nasal (vomeronasal) gland is present, as is the rostral (internal oral) nasal gland, but the lateral nasal gland is absent.

The olfactory epithelia of the principal and middle cavities in *Xenopus* show profound differences at both morphological and molecular levels. Bowman's glands and associated olfactory binding proteins are present in the principal cavity, but lacking in the middle cavity (Millery et al., 2005). Receptor neurons of the principal cavity are ciliated, and supporting cells are secretory. By contrast, the middle cavity contains both microvillar and ciliated receptor cells, and both secretory and ciliated supporting cells (Weiss, 1986; Saint Girons and Zylberberg, 1992b; Hansen et al., 1998; Oikawa et al., 1998). In this respect the epithelium of the adult middle cavity closely resembles that of the larval principal cavity. Primary and secondary projections of the principal and middle cavity are also distinct (Weiss, 1986; Reiss and Burd, 1997; Gaudin and Gascuel, 2005). At the molecular level, middle cavity receptor cells express at least group ε (class I) odorant receptors, whereas principal cavity receptor cells express group γ (class II) odorant receptors; these have been suggested to be functionally correlated with olfaction in water versus air, respectively (Freitag et al., 1995, 1998; Mezler et al., 1999, 2001). Transduction mechanisms also differ (Mezler et al., 2001). Here too the adult middle cavity resembles the larval principal cavity, which (at least in early larval stages) is known to express only group ε receptors. By contrast, the vomeronasal epithelium resembles that of other anurans both morphologically and in containing microvillar receptor cells and ciliated supporting cells, and shows no striking changes during metamorphosis. It is worth noting that the "posterolateral epithelial area of the principal cavity" recently identified as expressing V2R vomeronasal receptor genes (Hagino-Yamagishi et al., 2004) is merely the posterior part of the vomeronasal organ itself, which is unusually extensive in *Xenopus* compared with terrestrial frogs.

The taste system of *Xenopus*, which lacks a tongue, has been examined by Toyoshima and Shimamura (1982) and Witt and Reutter (1994); as in terrestrial metamorphosed frogs, taste discs, rather than taste buds, are present in the oral epithelium. However these taste discs are not raised on fungiform papillae, as are the lingual taste buds of terrestrial species. Physiological studies have shown that the taste system of *Xenopus* is particularly sensitive to amino acids and bitter substances (Yoshii et al., 1982), and a recent genomics study (Shi and

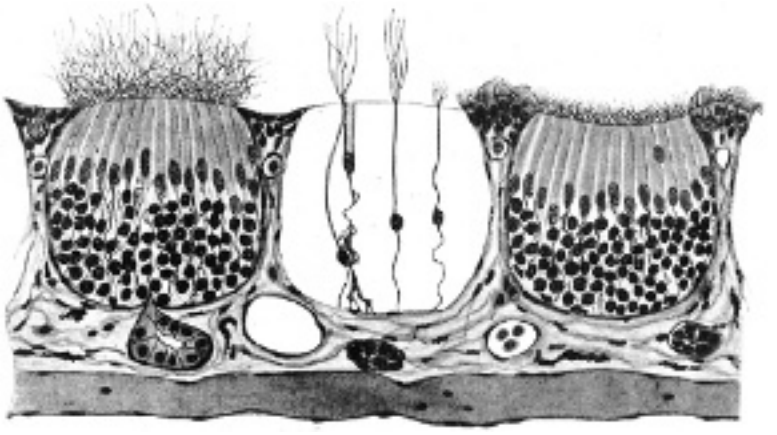


FIGURE 4.7. Diagrammatic comparison of the olfactory epithelium in land-phase *(left)* and water-phase *(right)* metamorphosed salamandrid, the newt *Triturus*. Note the much longer olfactory cilia in the land-phase animal, the elevation of the olfactory epithelium into a slight bulge, and the well-developed Bowman's gland at the base of the epithelium. By contrast, the water-phase animal shows much shorter cilia, depression of the olfactory epithelium into a groove, and ciliated respiratory epithelium between the "buds" of olfactory epithelium. The central region shows individual cells. From left to right these are an olfactory receptor cell with supporting cell from a land-phase animal, a receptor cell from a land-phase animal, and a receptor cell from a water-phase animal (from Matthes, 1927).

Zhang, 2006) has shown that *X. tropicalis* has 49 T2R (bitter) taste receptor genes but apparently no T1R (sweet/umami) receptors. The lack of comparative data makes the significance of these findings unclear.

SALAMANDERS

Among salamanders, newts (family Salamandridae) are notable for their frequent resumption of aquatic habits after metamorphosis. Studies of the smooth newt *(Triturus vulgaris)* and alpine newt *(T. alpestris)* provide the most complete information on changes in olfactory structure and function, due largely to the work of Matthes (1924a, 1924b, 1924c, 1926, 1927) and Schuch (1934). These European newts develop in ponds and have a typical metamorphosis to a terrestrial stage. After emerging in the fall, they return to the ponds to breed the following fall or spring and remain there for three months or more. In doing so, they undergo a secondary metamorphosis, marked most prominently by the development of a large tail fin, particularly in males. In the aquatic environment, olfaction functions both in food localization (Matthes, 1924a, 1927) and in courtship behavior (Halliday, 1977). The olfactory organs of land- and water-phase newts are morphologically distinct, with the olfactory epithelium of water-phase newts having larger folds separating the grooves of olfactory epithelium, greatly reduced numbers of goblet cells in the respiratory epithelium, and much shorter cilia on the OR cells (Fig. 4.7) (Matthes, 1927). Blinded land-phase animals placed in the water can immediately find food, but blinded water-phase animals require several days before they can find food on land. These behavioral results correlate with morphology: olfactory cilia shorten immediately upon placing land-phase animals in water, perhaps due to osmotic effects, but the cilia of water-phase animals transported onto land take several days to lengthen. Matthes also showed that food-finding in water or on land did not depend on the vomeronasal organ, as blinded animals in which the vomeronasal nerve had been sectioned found food as easily as those in which it was intact. Unfortunately, these pioneering studies have not been confirmed or followed up

by more recent workers. Nothing is known of the taste system in *Triturus*.

CAECILIANS

Among caecilians (Gymnophiona), the family Typhlonectidae has entirely aquatic adults and juveniles; typhlonectids have live birth, with no gilled larval stage. *Typhlonectes compressicauda* and *T. natans* have been common animals in the pet trade and thus have been the subject of a number of morphological and physiological investigations. Although aquatic, the species of *Typhlonectes* breathe air. Unlike more primitive caecilians, but like many other derived but terrestrial forms, *Typhlonectes* has a single, undivided nasal cavity (Schmidt and Wake, 1990). A short tentacle is present, and its duct communicates with an exceptionally well-developed vomeronasal organ (Schmidt and Wake, 1990). Interestingly, two distinct types of olfactory epithelium are present in the nasal cavity: the anteroventral region has a very thick epithelium containing both ciliated and microvillar receptor neurons and lacks Bowman's glands; and the posterodorsal region has a thin epithelium containing only ciliated receptor neurons (Saint Girons and Zylberberg, 1992b; although Schmidt and Wake [1990] reported this region as nonsensory). By analogy with *Xenopus*, it appears that the anteroventral region may be specialized for aquatic olfaction, and the posterodorsal for aerial olfaction. Unfortunately, no study has examined epithelial ultrastructure in a terrestrial caecilian, so no comparison can be made.

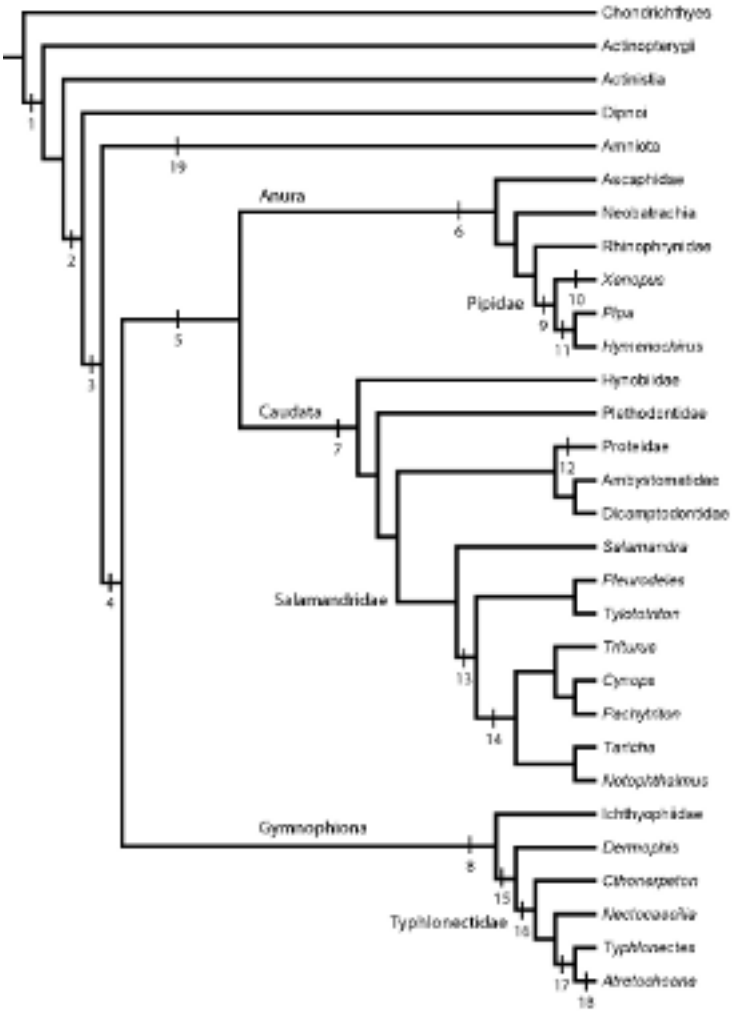
Both inspiration and expiration in *T. natans* occur through the nares. The choanae are protected by valves and are usually closed when the animals are filling the lungs or underwater (Prabha et al., 2000), but buccal floor oscillations still occur and increase in frequency when food is introduced to the aquarium, suggesting that pressure changes transmitted across the choanal valve are used to move water in and out of the nose (Wilkinson and Nussbaum, 1997). Recent work has shown that *T. natans* is able to use waterborne chemical cues to distinguish sex and kinship relations of conspecifics (Warbeck and Parzefall, 2001). Finally, as noted above, *Typhlonectes* is the only adult caecilian in which taste buds have been reported (Wake and Schwenk, 1986); this may be correlated with its aquatic habits.

EVOLUTION OF THE CHEMICAL SENSES IN SECONDARILY AQUATIC AMPHIBIANS

For each of the species described in the previous section, incomplete data concerning both the exemplar species and the distribution of characters in other taxa make impossible a full characterization of the adaptations to a secondarily aquatic lifestyle. Nevertheless, some tantalizing hints allow us to piece together an evolutionary scenario for the origin and diversification of the chemical senses in the group to which each belongs (cf. Fig. 4.8).

PIPID FROGS

Pipids provide the best case to study evolution of chemosensation, as we know much about their morphology and physiology, as well as their evolutionary relationships. Current phylogenies (Cannatella and Trueb, 1988; Roelants and Bossuyt, 2005; Frost et al., 2006) agree that the sister group of the aquatic pipids is the Mexican burrowing toad, *Rhinophrynus dorsalis* (Rhinophrynidae) (Fig. 1.3 in this volume), which as an adult has typical anuran nasal cavities (Trueb and Cannatella, 1982). By contrast, the nasal cavities of the aquatic pipids have long been known to be strange and have been the subject of a number of investigations (reviewed in Helling, 1938; Paterson, 1951). *Xenopus* is the least specialized of the pipids: to derive its nose from a more typical anuran type, it would seem that the normally nonsensory middle cavity would only have to acquire a sensory epithelium of the larval type, and a valve separating it from the principal cavity. However, the homology of the "middle cavity" of *Xenopus* with that of other frogs is not entirely clear (Föske, 1934; Helling, 1938; Paterson, 1951). Helling (1938) argued that the "recessus olfactorius" (a small region of



olfactory epithelium lacking Bowman's glands at the rostral end of the principal cavity in many frogs) is in fact the homolog of the middle cavity of *Xenopus*, and it has been suggested that in all frogs the anterior principal cavity may be exposed to waterborne odorants (Døving et al., 1993). Supporting this view, Benzekri (2006) has recently found that the primitive anuran *Ascaphus truei* has two distinct types of epithelium in the principal cavity, an anterolateral one resembling the middle cavity epithelium of *Xenopus*, and a posteromedial one resembling the principal cavity epithelium of other frogs. Whether the middle cavity of *Xenopus* derives from that of other frogs, from a region of the principal cavity, or both, the presence of anatomically and functionally distinct nasal chambers, one used for aerial, the other for aquatic olfaction, is clearly associated with the assumption of a secondarily aquatic lifestyle.

Conditions in other pipids differ even further from the typical anuran type but are easily derived from that of *Xenopus* (Helling, 1938; Paterson, 1951). In *Pipa* and *Hymenochirus* the nasal sac consists of parallel medial and lateral nasal canals, connected by a narrow isthmus along their entire length. The medial nasal canal corresponds to the principal cavity of *Xenopus* and other anurans, while the lateral nasal canal corresponds to the middle cavity of *Xenopus* (Helling, 1938; Paterson, 1951; Meyer et al., 1997). A narial valve separating the two

FIGURE 4.8. A speculative phylogeny of the tetrapod olfactory system. Characters on branches are as follows: 1. Osteichthyes: Olfactory organ with both microvillar and ciliated receptor cells, secretory and ciliated supporting cells. V2R (and possibly V1R) vomeronasal receptor genes expressed on microvillar receptor cells, OR olfactory receptor genes on both microvillar and ciliated receptor cells. 2. Sarcopterygii: Expansion of groups α and γ (class II) OR genes. Olfactory lamellae bear sensory grooves containing receptor cells. 3. Tetrapoda: Further expansion of groups α and γ OR genes. Posterior nostril migrates into mouth to form choana. Receptor cells partitioned into vomeronasal organ expressing V2R and V1R genes, and main olfactory organ expressing OR genes. Vomeronasal gland forms. Larvae use water as olfactory medium, flow is unidirectional inward, have choanal valve. Adults use air (at least sometimes) as olfactory medium, flow is bidirectional. In adults, main olfactory organ divided into water-sensing and air-sensing areas. Bowman's glands form in air-sensing area. Multicellular accessory glands form. Nasolacrimal duct forms, drains lacrimal secretions into nasal sac. 4. Lissamphibia: Adults undergo significant metamorphosis, lose choanal valve and vestibule, reduce sensory grooves in main olfactory organ, develop nasolacrimal duct, Bowman's and accessory glands. 5. Batrachia: Metamorphosed adults develop smooth muscular mechanism to close external nares, palatal groove connecting to vomeronasal organ. 6. Anura: Metamorphosis extreme. Nasal sac approximately vertical in larvae, sensory grooves lost, lateral appendix forms. Adults develop nonsensory middle cavity, recessus olfactorius for detection of waterborne odors, olfactory eminence in principal cavity, mechanism to close nostrils by submentalis muscle. 7. Caudata: Assumed primitive for all features. 8. Gymnophiona: Sensory grooves lost, choanal slime sac forms. In adults, principal cavity divided into lateral (respiratory) and medial (olfactory) parts by ventral ridge, tentacle forms, connects to nasolacrimal duct, drains into vomeronasal organ, choanal slime sac enlarges. 9. Pipidae: Secondarily aquatic adult; overland excursions rare. In adult, great development of middle cavity/lateral diverticulum (probably derived from middle cavity and recessus olfactorius of other frogs) with sensory epithelium devoted to water-sensing. Middle cavity has both microvillar and ciliated receptors that express group δ, ε, and η OR genes. Principal cavity (medial diverticulum) specialized for air-sensing, has exclusively ciliated receptors expressing group α and γ OR genes. Vomeronasal organ and gland enlarged, lateral nasal gland lost (but rostral gland retained). 10. *Xenopus*: Valve in external naris separates middle cavity and principal cavity, middle cavity with enlarged nonsensory accessory sacs used for ventilation, nasolacrimal duct opens beneath eye at tip of tentacle. 11. *Pipa* plus *Hymenochirus*: Tubular vestibule leads to principal (medial nasal canal) and middle (lateral nasal canal) cavities. Lateral nasal canal formed by connection of lateral diverticulum with choana. Nasolacrimal duct lost. 12. Proteidae: Metamorphosis lost; vomeronasal organ lost. 13. Newts: Secondarily aquatic adults, return to water for extended periods after metamorphosis. Water moved through nasal cavity by buccal floor oscillation. 14. Derived newts: Ability to reversibly respond to aquatic environment by losing/gaining goblet cells, lengthening/shortening olfactory cilia. 15. Viviparous caecilians: Loss of aquatic larval stage, live birth. 16. Typhlonectidae: Adults partially aquatic. Main olfactory cavity single, not divided into medial and lateral parts. 17. *Typhlonectes* plus *Atretochoana*: Adults fully aquatic. Enlarged, subtriangular external nares, reduced, nonprotrusible tentacle. Main olfactory cavity divided into anterior and posterior parts, specialized for aquatic and aerial olfaction. Anterior part with ciliated and microvillar receptors, lacking Bowman's glands, posterior part with only ciliated receptors. Vomeronasal organ well developed, choanae large with well-developed, superficial choanal valve. Narial plugs well developed. 18. *Atretochoana*: Lungs lost. Choanae closed by membrane. External nares countersunk and extremely enlarged. 19. Amniota: Loss of aquatic larval stage. Loss of water-smelling ability in main olfactory organ, specialization for detection of volatile molecules. Further expansion of groups and OR genes, loss (or extreme reduction) of all others. Loss (or reduction) of microvillar receptor cells. Further specialization of vomeronasal organ (likely for detection of nonvolatile molecules). Placement of changes on the tree is unconstrained due to lack of information and reflects tentative judgment of the authors. Phylogeny is based on work by Frost et al. (2006) but follows Weisrock et al. (2006) for salamandrids, and Canatella and Trueb (1988) for pipids.

chambers is lacking, however; instead a short *(Hymenochirus)* to long *(Pipa)* vestibule leads to both medial and lateral canals. A well-developed vomeronasal organ and associated gland are present in all of these forms, as is the rostral (internal oral) nasal gland, but the nasolacrimal duct is absent. Unlike *Xenopus*, both the medial and lateral nasal canals communicate with the oral cavity through the choana, and the vomeronasal organ opens into the lateral canal (middle cavity) rather than the medial canal (principal cavity). This suggests that water currents might pass through the lateral nasal canal into the oral cavity. Unfortunately, little is known of the functional significance of olfaction in pipids, though anecdotal observations support an important role for olfaction in food localization and reproduction (reviewed in Elepfandt, 1996), and recent work has shown that female *Hymenochirus* are specifically attracted to pheromones from the breeding glands of males (Pearl et al., 2000).

The olfactory sense, particularly the "water nose," is clearly highly developed in *Xenopus*

and other pipids compared with other frogs. This appears easily explained by habitat: these frogs typically inhabit, feed, and breed in rather muddy ponds, and olfaction is clearly a sense that would be emphasized here, whereas vision, the predominant sense used for terrestrial feeding, is less likely to be useful. What remains unclear is the functional distinction between the vomeronasal and main olfactory system in underwater smelling—do they have different classes of ligands and/or modulate distinct categories of behavior? On the other hand, it is surprising that all pipids appear to retain a well-developed "air nose," suggesting the continued importance of airborne odors, perhaps in locating bodies of water during overland migrations.

SALAMANDRID NEWTS

The situation in newts is less satisfactorily understood. The family Salamandridae is composed of two clades, the "true salamanders," *Salamandra* and its relatives, and the newts, including *Triturus*, *Pleurodeles*, and *Cynops* in the Old World, and *Taricha* and *Notophthalmus* in the New World (Titus and Larson, 1995; Weisrock et al., 2006). The true salamanders are largely terrestrial. By contrast, all of the newts are somewhat aquatic as adults, although the degree to which feeding occurs in the aquatic environment is quite variable (Özeti and Wake, 1969). Among the newts, there is a trend toward increased specialization for aquatic feeding from the basal *Pleurodeles* and *Tylototriton* through the highly derived *Pachytriton*, which is wholly aquatic as an adult. Unlike many terrestrial salamanders, newts characteristically ventilate the nose by buccal floor oscillations underwater (Joly and Caillere, 1983; reviewed in Jorgensen, 2000). Unfortunately, we simply do not have enough comparative information to determine whether the morphological changes in the olfactory organ upon entry into the aquatic environment described by Matthes and Schuch are characteristic only for the genus *Triturus*, for newts as a whole, or even for all salamanders. Saint Girons and Zylberberg (1992b) were unable to find any differences in the olfactory epithelium of land- and water-phase *Pleurodeles* at the light-microscopic level, suggesting that a comparative investigation of olfactory morphology and function in land- and water-phase newts could prove quite interesting.

Schmidt and colleagues (1988) investigated the central projections of the olfactory and vomeronasal organs in two species of salamandrids, including *T. alpestris*, and eight species of plethodontid salamanders. The authors report that the number of lobes in the accessory olfactory bulb is greater in adults of metamorphosing species than in direct developers, suggesting an association between vomeronasal function and the aquatic larval period, or possibly aquatic breeding. Species-specific female-attracting peptide pheromones, sodefrin and silefrin, have recently been isolated and characterized from male abdominal glands of the Japanese newts *Cynops pyrrhogaster* and *C. ensicauda* (Toyoda et al., 2004). Bilateral plugging of the nares and olfactory nerve transection demonstrate that the nasal chemosensory systems are necessary for this attraction response, and electro-olfactogram recordings further suggest that the vomeronasal system may be primarily responsible for mediating responses to these compounds (Toyoda et al., 2004).

Finally, as with the vomeronasal system, we have only tantalizing evidence for secondary adaptation of the taste system to aquatic life. In a recent study, Zuwala and Jakubowski (2001b) showed that the fire salamander *Salamandra salamandra* undergoes a morphological transition from taste buds to taste discs during metamorphosis. However, a previous report on the newt *Cynops pyrrhogaster* noted both "bud-shaped" and "barrel-shaped" taste buds (Toyoshima and Shimamura, 1987), suggesting that this species may retain larval-type taste structures, or that there may be a secondary metamorphosis from adult-type to larval-type structures with resumption of aquatic habits.

The situation in newts shows that not only is the commitment of the olfactory system to aquatic versus terrestrial olfaction able to

change in evolutionary time, it is able to change in ontogeny as well. This argues for the importance of careful attention to husbandry conditions in morphological, physiological, and behavioral studies on the chemical senses of salamanders, and amphibians in general.

TYPHLONECTID CAECILIANS

Within the typhlonectids, an evolutionary series can be constructed from the partially aquatic *Chthonerpeton* and *Nectocaecilia* through the fully aquatic *Typhlonectes* to the fully aquatic, highly specialized, lungless *Atretochoana* (Nussbaum and Wilkinson, 1995; Wilkinson and Nussbaum, 1997, 1999). Along this series we see accentuation of features associated with the nasal cavities, including enlarged external nares, reduced tentacular aperture with nonprotrusible tentacle, enlarged choanae with superficial choanal valves, and enlarged narial plugs on the buccal floor. In *Atretochoana* the choanal valves have fused, so that the choanae are not patent. Wilkinson and Nussbaum (1997) have argued that this suite of features represents a transition to a nose adapted to smelling in water: they postulate that air is drawn into the lungs through the nasal sac and choanae, but water is moved in and out of the nasal sac by buccal floor oscillations with the choanal valve closed. With the loss of lungs in *Atretochoana*, it was possible to close this valve permanently. A more complete examination of olfactory structure and function in *Typhlonectes* could help to support this scenario; it would be of great interest to know whether air is retained in the nasal cavity in submerged animals.

CONCLUSIONS

The brief overview presented here is summarized in Figure 4.8. It is clear that we still have much to learn about the structure and function of the amphibian olfactory and taste systems in general, and their modification in secondarily aquatic amphibians in particular. In the olfactory system apparent morphological adaptations to a secondarily aquatic existence include a reduction in glands (aside from the vomeronasal gland) and the nasolacrimal duct, segregation of distinct epithelia for olfaction in water and air, and the provision of a special mechanism for moving water through the nasal cavities. Unsurprisingly, many of these evolutionary modifications involve the reversal of changes that usually occur in metamorphosis from aquatic larva to terrestrial adult. However, given that amphibians primitively return to water to breed, it may also be that some of these adaptations are more widespread among amphibians than we presently realize. For example, as noted above, peptide pheromones occur in the secondarily aquatic newt *Cynops* (Kikuyama et al., 1995; Toyoda et al., 2004), but also in the terrestrial neobatrachian treefrog *Litoria*, which returns to water to breed (Wabnitz et al., 1999). The possibility that the ability to smell in both water and air is primitive for amphibians is supported by the recent discovery of two distinct regions of olfactory epithelium, apparently associated with aerial versus aquatic olfaction, in the principal cavities of the salamander *Dicamptodon tenebrosus* (Stuelpnagel and Reiss, 2005) (Dicamptodontidae of Fig. 1.3 in this volume; Fig. 4.8) and the frog *Ascaphus truei* (Benzekri, 2006). In amphibians, secondarily aquatic forms have generally evolved from ancestors that were partially aquatic already. Only a broader understanding of the functional diversity of amphibian olfaction, and chemoreception in general, will enable us to place the modifications in secondarily aquatic forms in proper evolutionary context.

A key issue that has not been well examined in the previous literature is the relative functional role of the olfactory and vomeronasal systems in aquatic olfaction. Matthes (1927) showed that food localization in aquatic *Triturus* depends on an intact olfactory, but not vomeronasal, system, and recent work with peptide pheromones in newts (Toyoda et al., 2004) supports the role of the vomeronasal system in detecting these compounds. However, in axolotls *(Ambystoma mexicanum)* the olfactory

and vomeronasal organs respond equally to chemical cues from conspecifics (Park et al., 2004). Moreover, in the terrestrial environment, Placyk and Graves (2002) found that prey detection in *Plethodon cinereus* is facilitated by the presence of an intact vomeronasal system. Thus, it is not yet possible to make any broad distinction between the function of the olfactory and vomeronasal systems in aquatic amphibians.

Data from teleosts are relevant here. The discovery that microvillar OR neurons in teleost fishes express class V2R vomeronasal receptor proteins, while ciliated olfactory receptor neurons express OR proteins (Hansen et al., 2003, 2004, 2005; Sato et al., 2005) suggests that the two cell types may be specialized for different functions. Sato et al. (2005) proposed that the microvillar receptor neurons may be specialized to detect polar molecules, such as amino acids and nucleic acids, while ciliated receptor neurons may be specialized to detect relatively nonpolar molecules, such as bile acids, steroids, and prostaglandins. They note, however, that ciliated receptor neurons have also been shown to respond to amino acids. In addition, studies using different techniques with other teleost species have produced wildly inconsistent results on the relative functions of ciliated and microvillar cells (reviewed in Eisthen, 2004). Regardless of their functions in teleosts, phylogenetic analysis indicates that one cannot deduce a simple relationship between the ciliated receptor cells of teleosts and the ciliated main OR cells of tetrapods, and between the microvillar receptor cells of teleosts and vomeronasal receptor cells of tetrapods (Eisthen, 2004). The evidence from *Xenopus*, the only amphibian for which comprehensive molecular and morphological data are available, makes it clear that microvillar receptor cells do not always express vomeronasal receptor proteins; instead, those located in the main olfactory cavity express group ε ORs (Freitag et al., 1995).

Integrating the evidence from teleost fishes and olfactory genomics (Niimura and Nei, 2005, 2006) with the data here reviewed for amphibians suggests the following, admittedly speculative, scenario for the origin and evolution of the tetrapod olfactory and vomeronasal systems (Fig. 4.8). The common ancestor of all bony fishes had at least three types of receptor cells in the olfactory epithelium: microvillar receptor neurons expressing V1R and V2R receptor proteins, and microvillar and ciliated receptor neurons expressing ORs. All OR groups were likely already present in this common ancestor (Niimura and Nei, 2005, 2006). In the sarcopterygian line leading to tetrapods, neurons expressing V1R and V2R receptor genes and bearing microvilli were segregated into a distinct vomeronasal organ, which maintained a fluid-filled lumen associated with copious mucus secretion from the newly evolved vomeronasal gland. As discussed above, paleontological data suggest that this likely occurred in the aquatic habitat, a hypothesis supported by the fact that both the organ and the gland are well developed in most larval, neotenic, and secondarily aquatic amphibians. The vomeronasal receptor neurons may have retained their ancestral sensitivity to amino acids and peptides. In the main olfactory organ, ciliated neurons expressing group γ (class II of Freitag et al. [1995, 1998]) ORs greatly proliferated, but microvillar and ciliated neurons expressing other groups of ORs were initially present as well. With the evolution of a terrestrial adult stage, a functional separation developed between a region of olfactory epithelium expressing group γ (and α?) ORs and specialized for olfaction in air, and a region expressing other groups of receptors, which continued to function in the aquatic environment. This separation necessarily was associated with the evolution of mechanisms separating water and air within the nasal cavity itself, and the evolution of accessory nasal glands (including Bowman's glands). In living amphibians, we see various evolutionary offshoots of this early specialization for olfaction in water and air, reaching their extreme in secondarily aquatic amphibians, such as the pipid frogs and typhlonectid

caecilians discussed here, which have greatly developed the water nose, while maintaining the air nose. The vomeronasal organ and gland are typically well developed in secondarily aquatic species, although the neotenic and permanently aquatic proteid salamanders provide an interesting case in which the vomeronasal system has been secondarily lost, and the main olfactory epithelium is exclusively involved in aquatic olfaction. Finally, in the amniote line we see a further specialization with the loss of water-smelling ability in the main olfactory system (correlated with the loss of all but group α and γ ORs), and perhaps greater differentiation of function between the olfactory and vomeronasal systems. In amniotes, it is the vomeronasal system that becomes specialized for aquatic olfaction; in its absence, aquatic olfaction is apparently lacking (see Schwenk, chapter 5 in this volume). This scenario is testable by additional data on the diversity of olfactory structure and function in fishes (including lungfish), amphibians, and amniotes.

As in the olfactory system, profound morphological changes in the gustatory system at metamorphosis suggest that significant functional differences exist between taste in aquatic and terrestrial environments. Clearly, there is much yet to be learned about vertebrate chemoreception across the water-air threshold.

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