



SYMPOSIUM

Perspectives on the Convergent Evolution of Tetrapod Salt Glands

Leslie S. Babonis^{1,*} and François Brischoux[†]

*Kewalo Marine Laboratory, PBRC/University of Hawaii, 41 Ahui Street, Honolulu, HI 96813, USA; [†]Centre d'Etudes Biologiques de Chizé, CEBC-CNRS UPR 1934, 79360 Villiers en Bois, France

From the symposium “New Frontiers from Marine Snakes to Marine Ecosystems” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2012 at Charleston, South Carolina.

¹E-mail: babonis@hawaii.edu

Synopsis Since their discovery in 1958, the function of specialized salt-secreting glands in tetrapods has been studied in great detail, and such studies continue to contribute to a general understanding of transport mechanisms of epithelial water and ions. Interestingly, during that same time period, there have been only few attempts to understand the convergent evolution of this tissue, likely as a result of the paucity of taxonomic, embryological, and molecular data available. In this review, we synthesize the available data regarding the distribution of salt glands across extant and extinct tetrapod lineages and the anatomical position of the salt gland in each taxon. Further, we use these data to develop hypotheses about the various factors that have influenced the convergent evolution of salt glands across taxa with special focus on the variation in the anatomical position of the glands and on the molecular mechanisms that may have facilitated the development of a salt gland by co-option of a nonsalt-secreting ancestral gland. It is our hope that this review will stimulate renewed interest in the topic of the convergent evolution of salt glands and inspire future empirical studies aimed at evaluating the hypotheses we lay out herein.

Introduction

Discovered by Schmidt-Nielsen et al. (1958), the physiology of tetrapod salt glands has been studied in great detail. Over the past several decades, much has been learned about the basic mechanisms by which these cephalic glands facilitate the net secretion of concentrated NaCl (or KCl, in some herbivorous taxa), and there have been several thorough reviews summarizing these data (Peaker and Linzell 1975; Gerstberger and Gray 1993; Shuttleworth and Hildebrandt 1999; Hildebrandt 2001; Dantzler and Bradshaw 2009; Holmgren and Olsson 2011). Building on this foundation, recent studies of tetrapods' salt glands have taken the form of comparisons among closely related marine and freshwater species (Bennett and Hughes 2003; Babonis and Evans 2011), the role of water-regulatory proteins in modulating the secretory output of the glands (Muller et al. 2006; Babonis and Evans 2011), variation in the composition of the secretion (Butler 2002), the modulation of secretion by various endocrine and neurological agents (Reina et al. 2002; Krohn and Hildebrandt 2004; Franklin et al. 2005; Hughes

et al. 2006; Butler 2007; Cramp et al. 2007; Hughes et al. 2007; Cramp et al. 2010), phenotypic plasticity of the form and function of salt glands under various environmental conditions (Cramp et al. 2008; Babonis et al. 2009; Gutierrez et al. 2011), the combined osmoregulatory function of salt glands and other organs (Hughes 2003; Laverty and Skadhauge 2008; Babonis et al. 2011), and several recent reports of bacterial infections of salt glands (Klopfleisch et al. 2005; Brito-Echeverria et al. 2009; Suepaul et al. 2010; Oros et al. 2011). Interestingly, although the basic physiology of these glands has been quite well characterized, there have been relatively few hypotheses about the convergent evolution of this specialized tissue across taxa (but see Peaker and Linzell 1975).

The ability of salt glands to secrete concentrated salt solution and the taxonomically wide-spread association between the use of desiccating habitats and the possession of functional salt glands in tetrapods suggest that this tissue may have been critical in facilitating the invasion (or re-invasion) of desiccating environments during the evolution of tetrapods

(Brischoux et al. 2012). Despite this, the past 50 years of research have seen only few hypotheses regarding the potential mechanisms that may have led to the convergent evolution of this gland across diverse taxa (Dunson and Dunson 1973; Peaker and Linzell 1975; Taplin et al. 1982; Babonis and Evans 2011). This paucity of hypotheses regarding the convergent evolution of tetrapod salt glands undoubtedly lies in the lack of several important types of data, notably (1) a thorough catalog of the presence/absence of salt glands from extinct and extant taxa (from which to infer the number of times salt glands have originated), (2) information about the homology of salt glands alternatively named “pre-orbital,” “supraorbital,” and “nasal” (see Technau 1936), as evidenced through the embryological origin of these glands, and (3) a mechanism by which a gland with a salt-secreting function may have evolved by co-option from an ancestral gland with another function. In this review, we attempt an initial remedy to this situation by (1) providing an exhaustive list of the extinct and extant tetrapod taxa currently known to have salt glands (as well as information about the anatomical position of the salt gland in these taxa), (2) summarizing the known embryology of glands from representative taxa, and (3) synthesizing the literature regarding the molecular development of cephalic glands from model systems. We then use these combined results to propose mechanisms by which salt glands may have evolved, independently, across diverse tetrapod taxa and present a call for future empirical studies aimed at testing the hypotheses we lay out herein. Since this review is largely speculative, we believe it is important to start by clearly laying out our assumptions about tetrapod salt glands.

Assumptions

Salt glands are so-defined because they secrete a product that is more concentrated in inorganic salts (NaCl or KCl) than is the blood plasma. Although there is diversity (and in some cases, plasticity) in the type of inorganic salt secreted by salt glands (particularly among lizards), for the purposes of this review, we do not distinguish among glands of different secretory types and merely refer to all such glands as “salt glands.”

Salt glands have evolved independently, multiple times throughout the evolution of tetrapods. We, parsimoniously, assume that the minimum number of independent origins is represented by the number of unique anatomical positions occupied by salt glands across taxa (e.g., “nasal,” “lachrymal,” and

“sublingual” glands represent a minimum of three origins); however, we acknowledge that the actual number of origins may well have been much greater than this (i.e., gain of a nasal salt gland followed by loss of this gland and another independent gain would be indistinguishable from a single-gain scenario in the absence of robust fossil data).

Salt glands are not unique/novel glands, they simply have a unique/novel form/function when compared with other cephalic glands in the same species. Indeed, although salt glands are present in marine (and some desert) taxa, the homologous gland in the nonmarine sister taxon is present but not specialized for the secretion of salt. Since the homologous position in a nonmarine sister taxon is occupied by a gland with a nonsalt-secreting function, convergent evolution of salt glands has likely resulted from the repeated co-option of various existing (unspecialized) glands rather than *de novo* organogenesis.

Anatomy of salt glands in tetrapods

Across diverse tetrapod taxa (see Supplementary Table S1 for an exhaustive list of the tetrapod taxa that have been reported, thus far, to have salt glands), the anatomy of cephalic salt glands is largely consistent (Babonis et al. 2009). This tissue comprises a mass of secretory tubules that terminate blindly (i.e., without secretory acini); thus, they are called compound tubular glands. The secretory tubules are separated by vascularized connective tissue and are arranged radially around the perimeter of a central duct. Together, these structures constitute an individual lobule of the gland; multiple such lobules in association are joined by the connection of their central ducts to a main duct, the conduit whereby secreted salts exit the body (for illustrations, see Schmidt-Nielsen 1960). Unlike other types of cephalic glands, the secretory epithelium of salt glands is populated almost exclusively by salt-secreting principal cells, as exemplified by marine snakes (Dunson et al. 1971; Dunson and Dunson 1974; Babonis et al. 2009). Where variation does exist (e.g., in the salt glands of some turtles and lizards) (Abel and Ellis 1966; Cowan 1969; Van Lennep and Komnick 1970), the various cell types present in the gland are scattered throughout the secretory epithelium rather than being confined to single-function units like the mucus acini versus the serous acini of some mixed-function salivary glands. Although the size of these glands across taxa has been hypothesized to vary with the degree of marine tendency (i.e., the time spent in a marine

habitat and/or the osmolality of the food items) in birds (Technau 1936; Holmes et al. 1961; Staaland 1967; Ernst and Ellis 1969), lizards (Hazard et al. 1998), turtles (Holmes and McBean 1964; Cowan 1969; Dunson 1970), crocodiles (Taplin 1985; Cramp et al. 2008), and snakes (Dunson and Dunson 1974, 1979), the basic tubular morphology of this tissue appears largely invariant across taxa. Interestingly, not all tetrapods inhabiting desiccating environments have a salt gland (see Supplementary Table S2 for a list of species that have been reported to lack a salt gland) suggesting much remains to be learned about the relationship between environmental constraints and salt-gland function in tetrapods.

Distribution and nomenclature of salt glands in tetrapods

Although there are many glands present in the head of the idealized tetrapod (Fig. 1A), only one (or one pair, for paired glands) is the salt gland in any given taxon (Fig. 1B). The anatomical position of the salt gland(s) in tetrapods varies quite extensively among lineages, and three main cephalic areas are currently recognized (1) nasal glands in extinct archosaurs, extant birds, and lizards, (2) orbital glands in turtles, and (3) oral glands in extant crocodiles and snakes (Supplementary Table S1). Interestingly, those glands typically described as “nasal” can vary in location from the vestibule of the nostril, (Fig. 1C, I) to small preorbital structures, midway between the nostril and the orbit (Fig. 1C, II), to the supraorbital position exemplified by the salt gland in the marine iguana, and many marine birds (Fig. 1C, III). This variation in the anatomical location of the body of the gland has resulted in variation in the nomenclature of the gland (Technau 1936) and has contributed to confusion about the homology of this gland across taxa (see later for more details on the homology of these glands). Interestingly, salt glands housed in the frontal region of the cranium are the most widespread among tetrapod lineages.

Orbital salt glands are found only in chelonians and occur in two phylogenetically divergent lineages: the sea turtles (Cheloniidae and Dermochelyidae) (Schmidt-Nielsen and Fänge 1958; Hudson and Lutz 1986) and the diamondback terrapin, *Malaclemys terrapin* (Emydidae) (Schmidt-Nielsen and Fänge 1958). Although the morphology and the function of the lachrymal glands (and their ducts) have been well characterized for turtles (Ellis and Abel 1964; Abel and Ellis 1966; Cowan 1969; Marshall 1989; Marshall and Saddler 1989), the

identity of the chelonian salt gland has been an intense subject of debate. Historically, this gland has been dubbed the nasal gland (Benson et al. 1964; Holmes and McBean 1964), the lachrymal gland (Abel and Ellis 1966) and the Harderian gland (Dunson and Taub 1967; Dunson 1969; Chieffi-Baccari et al. 1992, 1993). Although some debate still exists regarding the nomenclature of the salt-secreting glands in chelonians (Chieffi-Baccari et al. 1992, 1993), most researchers in this field still consider them to be modified lachrymal glands (Belfry and Cowan 1995; Lutz and Musick 1997; Hirayama 1998; Reina and Cooper 2000; Oros et al. 2011), and we will refer to them here as such. Although salt glands have not been reported officially in either flatback sea turtles (*Natator depressus*) or Kemp's Ridley sea turtle (*Lepidochelys kempii*), the presence of osteological characteristics consistent with large lachrymal glands in extinct chelonian sea turtles (Hirayama 1998), combined with a recent study of the phylogenetics of sea turtles (Naro-Maciel et al. 2008), suggests that salt glands are ancestral in this group.

Oral salt glands have evolved independently in at least two lineages of tetrapods: extant crocodylians and snakes. Among crocodylians, lingual salt-secreting glands were originally identified by Taplin and Grigg (1981) in the tongue epithelium from *Crocodylus porosus* and have since been identified in all species of the Crocodylidae that have been studied (Supplementary Table S1), including the freshwater species (Taplin et al. 1985). Interestingly, the other two lineages of extant crocodylians (alligatorids and gavialids) appear to have (presumably homologous) lingual glands that lack the capacity to produce a hypertonic salt secretion (Taplin et al. 1985). These observations suggest that either lingual salt glands evolved in the ancestor to all modern crocodylians, but the concentrating capacity was lost in modern alligatorid and gavialid lineages or that functional salt glands evolved by modification of unspecialized lingual glands after the crocodylids split from the alligatorid and gavialid lineages (crocodylian relationships after Man et al. 2011).

Among snakes, salt glands have evolved at least four times in lineages that have independently undergone an evolutionary transition to marine life: the file snakes (Acrochordidae) (Dunson and Dunson 1973), rear-fanged water snakes (Homalospidae) (Dunson and Dunson 1979), and, within the Elapidae, two lineages of sea snakes (Laticaudinae and Hydrophiinae) (Dunson et al. 1971). Similar to the crocodylians, all these lineages

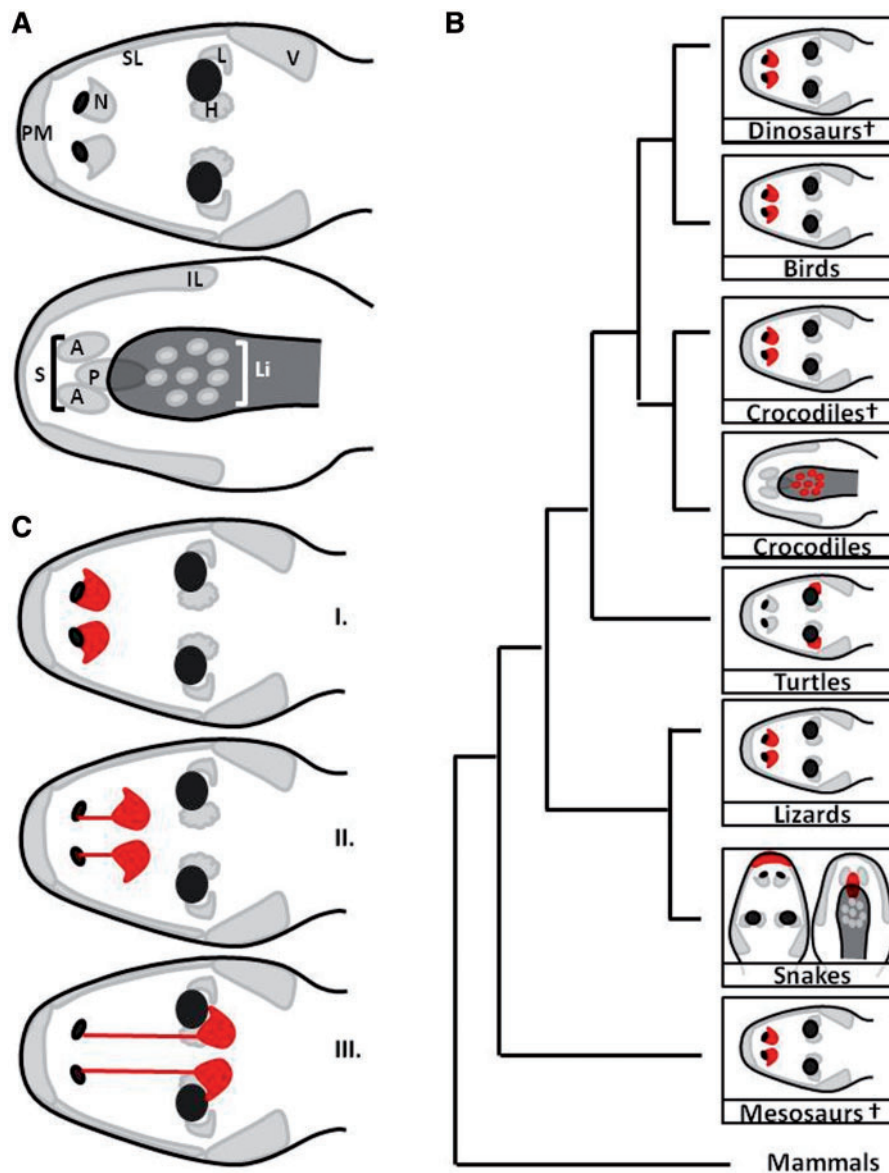


Fig. 1 Cephalic glands in the tetrapod lineages listed in Supplementary Table S1. **(A)** An idealized tetrapod exhibiting all possible cephalic glands. Top-down view with anterior to the left and posterior to the right. The cranium/maxilla is pictured on the top, and the mandible/lower jaw is pictured on the bottom. Small black ovals are nostrils, and large black ovals are eyes; glands are outlined in dark grey and filled with light grey. **(B)** Salt glands are present in representatives of each of the pictured lineages and occupy the gland in each lineage highlighted in red. Among snakes, salt glands have been identified in two different locations; however, each species of snake with a salt gland has only one of these. **(C)** An evolutionary scenario to illustrate how traditionally defined “nasal” glands (highlighted in grey) might have migrated from a position near/in the nostril (I) to either a “preorbital” (II) or “supraorbital” (III) position. The length of the duct differs in each of these scenarios, resulting in a different cranial location of the body of the gland. A, anterior (sublingual glands); H, harderian gland; IL, infralabial gland; L, lachrymal gland; Li, lingual glands; N, nasal gland; P, posterior (sublingual gland); PM, pre-maxillary gland; S, sublingual gland(s); SL, supralabial gland; T, tongue; V, venom gland. †An extinct lineage.

evolved oral salt glands: acrochordids, laticaudines, and hydrophines have a posterior sublingual salt gland located in the lower jaw beneath the tongue casing, whereas the homalopsids have a pre-maxillary salt gland. It is noteworthy that despite their relatively close ancestry with lizards, snakes followed independent evolutionary pathways leading to their

convergence on salt glands; no snakes studied thus far have a salt gland that is homologous with the nasal gland of lizards.

Embryology and homology

Glands occupying distinct cephalic positions (e.g., the lachrymal salt glands of turtles, lingual

glands in extant crocodiles, and sublingual and pre-maxillary glands in snakes) are clearly not homologous with salt glands in any other taxon. Thus, these examples represent a minimum of four independent convergent evolutionary events. The case is not as clear for the “nasal” salt glands of extinct archosaurs, modern birds, and modern lizards. Indeed, the homology of the “nasal,” “pre-orbital,” and “supraorbital” glands has been questioned (Dunson 1969), likely because of the dramatic variation in the position of the body of the gland in the cranium. From embryological studies of various bird taxa, it is known that nasal glands develop initially as an outgrowth of the nasal epithelium (Marples 1932). This primordial bud develops into the distal-most portion of the duct and grows posteriorly to the position where the body of the gland is to develop. From there, the body of the gland expands from the posterior end of the duct. If this developmental scheme is also true of nasal-gland development in extinct archosaurs (as proposed by Fernandez and Gasparini 2000; Gandola et al. 2006) and modern lizards (as yet, unstudied), it can be assumed that all glands (independent of where the mature body of the gland lies) that develop from an outgrowth of the nasal epithelium are homologous. Following these assumptions, it is reasonable to assume that the diversity of modern “nasal” glands (this term now includes those glands alternatively labeled “preorbital” and “supraorbital”) is simply a result of variation in the length of the duct of the nasal gland, resulting in a gland body that may be housed anywhere from the nostril to the supraorbital position. Although it is possible that the “nasal” salt glands of lizards are not homologous with the “supraorbital” glands of birds, we find this to be unlikely. Only detailed embryological studies of cephalic glands in lizards and birds will resolve this issue. Considering that salt glands have been positively identified in representatives of at least 8 of the 26 currently recognized families of lizards (Vidal and Hedges 2009), in at least 40 families of birds (Supplementary Table S1) representing nearly all orders of birds except the Passeriformes (Hackett et al. 2008), and several lineages of extinct crocodylians and dinosaurs, nasal salt glands may indeed be an ancestral characteristic in the diapsids.

There have been several detailed embryological studies of turtles (Ewert 1985, and references therein) including marine turtles (Miller 1985, and references therein), yet the embryology of the lachrymal gland does not appear to have been described. Despite this, all lineages (extinct and extant) of turtles have evolved salt glands in the position of the lachrymal

gland (but see Chieffi-Baccari et al. 1993). Considering that the ducts of the lachrymal glands in those turtles that have been studied all open in the same location (onto the lateral portion of the nictitating membrane) (Cowan 1973), all the glands identified as “lachrymal” among turtles are, indeed, likely homologous. The phylogenetic distance between modern lineages exhibiting salt glands (sea turtles and terrapins) makes it difficult to assess whether salt glands evolved twice among turtles (both times in the position of the lachrymal gland) or whether the lack of salt-secreting abilities of this gland among other turtles represents loss of the lachrymal salt gland subsequent to its origin in the ancestor to all turtles. Evidence of large interorbital foramina (Hirayama 1998) in the skulls of fossil emydine turtles would be suggestive of the presence of salt glands in these taxa and provide more support for a single origin of salt glands among turtles.

Embryological studies of species with oral salt glands are also lacking. The lingual salt glands of crocodylians are reported to develop from the dorsal epithelium of the tongue (Ferguson 1985), but no other data on the generation of the secretory tubules or the onset of secretory-cell identity are available. Comparative studies of lingual-gland development in alligators (or gavials) and crocodiles, with special focus on the acquisition of a salt-secreting function, would be particularly useful for understanding the molecular mechanisms that underlie convergence. Similarly, among snakes, there have been no developmental studies of either the sublingual or pre-maxillary glands. As such, we cannot distinguish between two possible scenarios among snakes that salt glands evolved multiple times (once as the sublingual gland in the file snakes, at least once [and probably twice] as the sublingual gland of laticaudine and hydrophine sea snakes, and once as the pre-maxillary gland of water snakes) or that salt glands evolved only twice, represented by the two unique anatomical positions, and that salt glands were lost in the intervening taxa. Considering, again, the phylogenetic distance between file snakes and sea snakes (or, indeed, between laticaudine and hydrophine sea snakes), we think it is more likely that salt glands evolved at least three (and potentially four) times in snakes.

Toward a coherent evolutionary hypothesis on the diversity of salt glands

The diversity in the location of modern salt glands alone suggests that this structure has evolved multiple times, independently, among modern tetrapod

taxa; however, similarities in the location of the gland and, importantly, the position of the duct, combined with the presumed embryological origin of the nasal salt glands in both extant (e.g., birds and lizards; see earlier) and extinct lineages (e.g., birds, dinosaurs, mesosaurs, and metriorhynchid crocodiles) (Supplementary Table S1), are consistent with the hypothesis that nasal salt glands were also present in the ancestor of all diapsids (Fernandez and Gasparini 2000). Interestingly, from this putative starting point, deviations are observed in turtles, one of the first groups to diverge from the ancestral diapsid form, extant crocodylians, which likely evolved from an ancestor that had lost the original nasal salt glands, and snakes, which, as a group, have likely experienced several origins of salt glands. The various origins of salt glands in nonhomologous positions may suggest that constraints specific to each of these lineages led to the development of a salt gland in these novel locations.

Gasparini et al. (2006) and Pierce et al. (2009) suggested that skull morphology among extinct crocodylians may have been influenced by a shift toward a more highly aquatic lifestyle, including changes in feeding strategy (e.g., a shift toward ambush predation) (Seymour et al. 2004) and increases in the mechanical resistance of the snout. They used these ideas to propose that the evolution of new feeding habits was likely the driving force separating the skull morphologies across species. In this light, it is possible that the shape of the snout imposed constraints in relationship to the capture of prey and that ambush predation limited the capacity of the skull to house a salt gland, leading to a second origin of salt glands among crocodylians in the soft tissue of the tongue's epithelium. Recent phylogenetic studies suggest that turtles are sister to the archosaurian lineage (Shen et al. 2011; Voronov et al. 2011) and, thus, should be placed within the Diapsida. This suggests, then, that the anapsid turtle skull is derived from a diapsid ancestor and that turtles may, therefore, have evolved from a lineage that possessed nasal salt glands (Fernandez and Gasparini 2000). Considering that the chelonian anapsid skull constitutes a major modification from the ancestral diapsid form, it is not unreasonable to hypothesize that the lachrymal position of the salt gland in turtles may have resulted from functional constraints associated with this extensive cranial remodeling. Similarly, among the four lineages of snake that evolved salt glands, it is possible that deviation from the putative ancestral nasal gland is a result of the relatively recent evolution of modern snake taxa from burrowing or aquatic ancestors

with reduced ocular structures (Walls 1940; Heise et al. 1995; Caprette et al. 2004). For example, the covering of the eye of snakes by a scale fused with the scales of the body would preclude egress of secretions to the external environment from an orbital salt gland. Functional constraints linked to ancestral ecology in this group (e.g., loss of lachrymal glands) (Taub 1966), reliance of this group on vomerolfaction, or indeed a combination thereof might well have played a significant role in the modification of oral glands.

An evo/devo approach to the study of convergent evolution in salt glands

To develop useful hypotheses about the mechanisms that may have supported the convergent evolution of salt glands across taxa, it is necessary to first define the features that must have appeared during the evolution of a salt-secreting gland. As aforementioned, all salt glands identified thus far have a compound tubular shape with extensive secretory epithelium that is populated in large part by principal secretory cells at the expense of the mucous cells or other cell types that typify this epithelium in unspecialized glands. To our knowledge, there have been only few studies aimed specifically at the development of cephalic glands in nonmammalian tetrapods (e.g., Marples 1932; Ellis et al. 1963; Kochva 1965; Nogawa 1978; Ovadia 1984; Chieffi Baccari et al. 1995, 1996; Rehorek et al. 2005), and all these studies are limited to morphological/histochemical surveys and lack molecular data. In contrast, the development and regeneration of salivary glands (particularly the submandibular glands, sublingual glands, and parotid glands) in mammalian models are active areas of research extending well beyond descriptive embryology to include vast details regarding the molecular regulation of gland shape and cellular identity (recently reviewed by Tucker 2007; Larsen et al. 2010; Harunaga et al. 2011; Lombaert et al. 2011). From these mammalian studies, it is possible to develop hypotheses about the molecular regulation of compound tubular shape and salt-secreting versus mucus-secreting cellular identity and, therefore, to postulate about the mechanism by which salt glands were co-opted from unspecialized glands.

Glandular organogenesis

The organogenesis of salivary glands is a well-conserved process in mammals (Tucker 2007), and Supplementary Table S3 summarizes some of the signaling molecules involved in each stage. In brief, the earliest stages of glandular development

(stage 1: pre-bud; Supplementary Table S3) involve thickening of the oral epithelium and proliferation of the gland primordium to form the initial bud (stage 2; Supplementary Table S3). Continued cell proliferation in the gland primordium leads to further outgrowth and invasion of the surrounding mesenchyme (stage 3: pseudoglandular; Supplementary Table S3), a process that relies on signaling molecules from both the epithelium of the developing gland and the surrounding mesenchyme. At the same time, the earliest rudiments of a lumen begin to form through the directed expression of apoptotic signals (in those cells destined to form the cavity of the lumen) or the expression of anti-apoptotic signals (in those cells destined to become the epithelium lining the lumen). Cells destined to become the epithelium lining the lumen begin to express polarizing signals (as apical/basal polarity is a defining feature of epithelia) by this stage, and, furthermore, some evidence suggests that cells in this stage (stage 4: cannalicular; Supplementary Table S3) are already fated to become either duct cells or acinar cells (Walker et al. 2008). Extensive branching morphogenesis follows initial formation of the lumen, ultimately giving rise to the gross architecture of the gland (stage 5: terminal bud; Supplementary Table S3). This process is, again, regulated by opposing signals from the growing epithelium and the surrounding mesenchyme. Although we believe that studies of *de novo* glandular organogenesis in marine and nonmarine tetrapods will represent a new and important contribution to this field, studies of this type are unlikely to reveal the evolutionary mechanism resulting in the possession of a specialized salt-secreting gland in a marine taxon or in the possession of an unspecialized homologous gland in its nonmarine sister taxon. Thus, we use the remainder of this discussion to develop hypotheses about the co-option of an unspecialized gland that was already in place.

Co-option of an existing gland

The complete set of cephalic glands in tetrapods (Fig. 1A) includes both compound tubular and compound acinar glands of mucous, serous, and mixed function (Tucker 1958). Assuming a similar complement of shapes and functions of glands in the ancestor of modern marine taxa, two scenarios are likely for the evolution of salt glands: co-option of an existing tubular gland or co-option of an existing acinar gland. To keep these comparisons simple, this review will focus on the evolution of salt-secreting glands from ancestral glands with

a mucus-secreting or mixed (mucoserous) function. Since many cephalic glands have a mucus-secreting component (e.g., wholly mucous acini, mixed mucous, and serous acini, or mucus-secreting cells lining the ducts) (Babonis and Evans 2011), we find the hypothesis that salt glands evolved from mucous glands to be most plausible; however, the approach we apply in this section could be applied with equal validity to hypotheses invoking co-option from another ancestral type of gland.

Co-option of an existing (unspecialized or mucus-secreting) compound tubular gland likely involves a change in cellular identity without a concomitant change in glandular morphology. This process may have been gradual, whereby portions of the gland adopted a salt-secreting function simply through a gradual change in the domain of expression of signals regulating the acquisition of salt-secreting cellular identity (see Fig. 2A for an example). In contrast, co-option of an existing (unspecialized or mucus-secreting) compound acinar gland invokes a change both in the cell's identity and in the shape of the gland (Fig. 2B and C). This would involve a shift from mucus-secreting to salt-secreting cellular identity and a shift from acinar to duct/tubule cellular identity and likely resulted from either (1) loss of the acinar component of the ancestral gland by re-specification of these cells as duct/tubule cells (Fig. 2B) or (2) actual loss of the presumptive acinar epithelium and compensatory growth of the portion of the gland already specified as duct to form ductal/tubular termini (Fig. 2C). Since the acinar component of a typical mammalian salivary gland is specified early (Walker et al. 2008), evaluation of this hypothesis will require careful studies of the timing and location of expression of cell-identity markers (pre-acinar versus pre-ductal markers) (Supplementary Table S3) during early glandular development (Fig. 2D). Evidence of apoptotic signals in the pre-acinar component of salt glands and a lack of these signals in the early development of nonsalt-secreting salivary glands might suggest that the homogeneous makeup of salt glands is a result of actual loss of other cell types. In contrast, a lack of pre-acinar markers in the absence of apoptotic signals early in glandular development may support the hypothesis that these cells have undergone early re-specification as duct cells. Although Supplementary Table S3 is far from an exhaustive list of molecular components of salivary-gland development, this summary should provide a solid starting point from which to test specific hypotheses about changes in the timing or distribution/range of expression of various cell-identity markers in specialized and unspecialized glands across tetrapods.

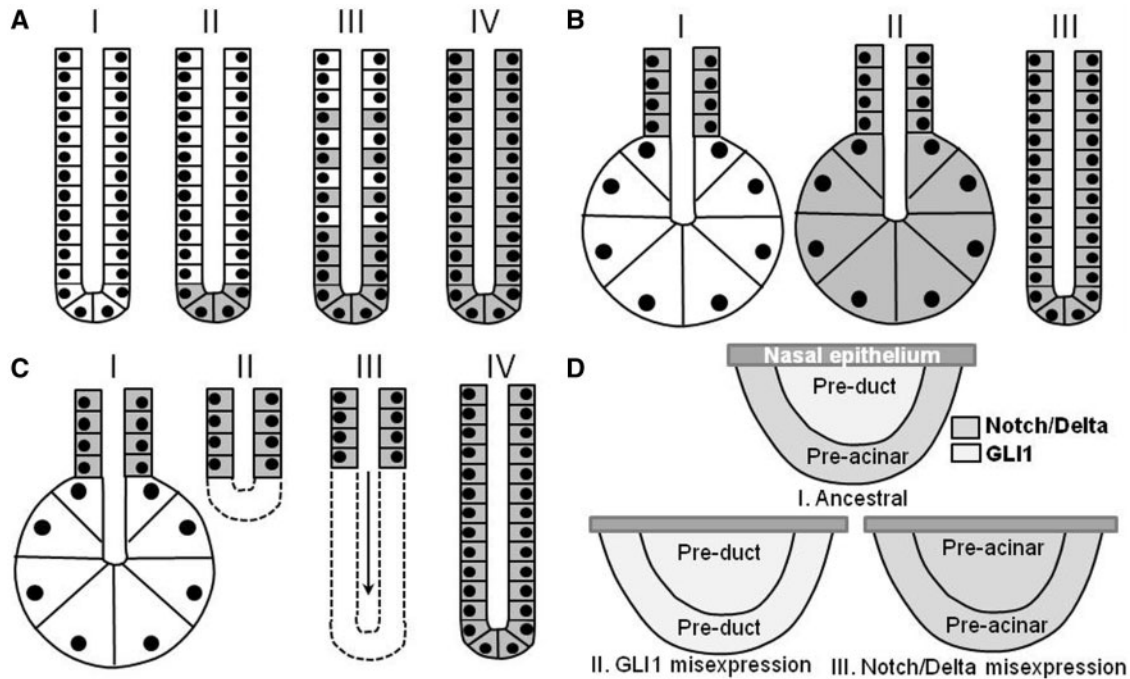


Fig. 2 Hypothetical scenarios for the co-option of an ancestral gland to form a salt gland. (A) The appearance of salt-secreting cells (grey) in the secretory epithelium may have occurred gradually (forms I–IV), first in only a small portion of the gland and later taking on a homogeneous distribution. (B) An ancestral acinar gland exhibiting nonsalt-secreting (white) cells in the acini (I) may have undergone first a transition to become populated by principal cells (grey; II) followed by a change in the shape of the gland from acinar to tubular (III). (C) Alternatively, loss of the acinar component of the ancestral gland (I, II), followed by elongation of the ductal/salt-secreting component (III) may have resulted in a homogeneous tubular secretory epithelium (IV). (D) The ancestral acinar gland may have expressed Notch/Delta (Dang et al. 2009) in the pre-acinar component and the transcription factor GLI1 (Fiaschi et al. 2011) in the pre-ductal component (I). Misexpression of GLI1 (II) in the pre-acinar component may have resulted in a shift in the identity of these cells from pre-acinar to pre-ductal. For comparison, misexpression of Notch/Delta (III) might have resulted in a gland that was homogeneously acinar in cell type.

Future directions for this research

The hypotheses we have developed in this article are speculative and clearly point out the lack of knowledge on the evolution of salt glands in tetrapods. Understanding the evolutionary history of tetrapods' salt glands is an exciting field of investigation, but it will require not only a thorough resolution of the presence and locations of salt glands throughout the evolutionary history of tetrapods (e.g., using reconstruction of ancestral states) (Witmer 1997; Fernandez and Gasparini 2000) but also a precise investigation of the functional constraints of nasal salt glands in lineages that deviate from the putative basal bauplan (nasal salt glands) and detailed molecular studies of glandular development in various taxa. Because of the number of tetrapod lineages that have independently re-invaded marine habitats, there are many examples of closely related marine and nonmarine sister taxa among tetrapods, providing abundant opportunities for comparative studies. Furthermore, there are many species that have salt

glands with mixed function (serous-secreting and mucus-secreting cells) that would also make nice developmental models (e.g., the skink *Tiliqua rugosa*) (Saint Girons et al. 1977). By examining the development of the salt gland in these species, it will be possible to identify the signals leading to the development of salt-secreting and mucus-secreting cells in the same gland at the same time. Finally, recent studies of rectal (salt) gland morphogenesis in lagoon sharks (Fishelson et al. 2004) and orbital-gland morphogenesis in various nonmammalian tetrapods (Chieffi-Baccari 1996; Rehorek et al. 2005, 2007) provide a basis for assessing morphological changes occurring during the development of specialized and unspecialized cephalic glands (e.g., development of the salt-gland capsule and the associated capillaries and amplification of the basolateral membrane of principal cells) but do not provide molecular hypotheses about the signals regulating these various morphological events. These initial comparisons can then be used to (1) evaluate hypotheses about the

mechanisms leading to the acquisition of a specialized salt-secreting gland in any individual marine lineage, (2) make comparisons of developmental mechanisms of salt glands across lineages to understand the processes by which convergent evolution occurs, and (3) to compare the developmental pathways resulting in specialized and unspecialized glands to understand how existing structures may be modified through evolution. It is our hope that this review will provide a starting place for anyone interested in pursuing these ideas further.

Acknowledgments

We wish to recognize numerous colleagues, especially those in attendance at the 2012 Society for Integrative and Comparative Biology (SICB) meeting, for their thoughts and comments during the development of these ideas and Dr. Eric Rottinger (Kewalo Marine Laboratory) for translation and interpretation of German texts cited in this manuscript. This manuscript was submitted as part of a symposium at the 2012 SICB meeting.

Funding

Support for this symposium was provided by National Science Foundation [IOS-1132369 to H.B. Lillywhite], Society for Integrative and Comparative Biology, the University of Florida, Sable Systems International, Vide Preciosa International, Inc. (Dave & Tracy Barker) and The Gourmet Rodent, Inc. F.B. was funded by the National Science Foundation [IOS-0926802 to H.B. Lillywhite (USA) and the CNRS (France)].

Supplementary Data

Supplementary Data are available at *ICB* online.

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