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## Caudal Hedonic Glands in the Dark-sided Salamander, *Eurycea longicauda melanopleura* (Urodela: Plethodontidae)

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Male salamanders within the genus *Eurycea* possess clusters of acinar, exocrine glands (called caudal hedonic glands) embedded in the skin on the middorsal and lateral regions of the tail directly above the vent; they extend posteriorly for several mm as part of an elevated ridge which is quite conspicuous during the breeding season (Noble, 1929; Sever, 1985, 1989). Furthermore, these glands are lacking in females. Sever (1989) provided a historical as well as functional perspective on the nature of caudal hedonic glands. In brief, the glands, first detailed histologically in *Eurycea bislineata* by Noble (1929) and further elucidated in other species of *Eurycea* by Sever (op. cit.), produce secretions during the breeding season that are thought to function as a sexual attractant and play a major role in eliciting the stereotypic "tail-straddling" walk (Arnold, 1977) by females prior to spermatophore deposition by males during courtship behavior in *Eurycea* and possibly in other plethodontid salamanders. (Females place their snouts on the male's rump, and nasolabial grooves transfer chemicals to the nasal cavity.)

Histochemical studies dealing with the secretions of these glands as well as other hedonic glands in *Eurycea* species [e.g., mental hedonic glands (Sever, 1975a, 1976), nasolabial glands, (Sever, 1975b), and cloacal glands (Sever, 1980)] indicate that mucoproteins are released by both caudal hedonic glands and mental hedonic glands (Sever, 1989). A number histological studies yielding information on male reproductive anatomy (Ireland, 1974; Williams et al., 1976; 1984) or female spermathecal (Ireland, 1974) and cloacal anatomy (Sever, 1980) in *Eurycea longicauda* have been conducted; however, no studies have centered on the structure of caudal hedonic glands in this species. The objectives of the present study were to: 1) document the structure of caudal hedonic glands in the dark-sided salamander, *Eurycea longicauda melanopleura*, using light microscopy and 2) compare the morphology and secretions of these glands with similar glands previously reported in other *Eurycea*.

Sixteen male and 12 female adult specimens [males, 42-52 mm in snout-vent length (SVL),  $\bar{x}$  = 47.7; females, 50-56 mm SVL,  $\bar{x}$  = 52.7] of *E. l. melanopleura* were examined histologically during this study. Individuals were collected from caves or springheads in three northeastern Arkansas counties (Fulton, Independence, and

Randolph); nearly all were taken from 15 September to 12 November, 1989, during their peak in reproductive activity (Ireland, 1974; Williams et al., 1984). [In fact, eleven of the 12 females examined were gravid (possessing enlarged vitellogenic ova); of these, eight exhibited spermatozoa within spermathecal tubules.] Salamanders were sacrificed in a dilute chloretone solution, fixed in 10% formalin for at least 48 h, and then stored in 70% ethanol. Tissues samples prepared for light microscopy were removed from the middorsal region of the tail in males and in females (for comparative purposes); in addition, the spermatheca (sperm storage gland of the dorsal cloacal wall) of females and the mental hedonic gland (on the chin) of males were also excised to provide information concerning timing of mating and degree of reproductive readiness (see Sever, 1976), respectively. Histological techniques followed those outlined by Humason (1979). Briefly, the procedures included dehydrating tissues in a graded series of ethanol, clearing in xylene, and embedding in paraffin. Paraffin blocks contained tissue previously oriented so that either sagittal, transverse, or frontal sections were obtained in complete serial sequences (at 8  $\mu$ m in thickness). Three stains [hematoxylin and eosin = H & E (for general cytology); Pollak trichrome = Pollak (for connective tissues and mucosubstances); alcian blue (for sulfated mucosubstances)] were alternately used on sequential groups of three slides. All glands were measured using a calibrated ocular micrometer and are reported in  $\mu$ m as means ( $\pm$  2 SE), followed by ranges in parentheses, and then by the number of glands examined. In caudal hedonic glands, only those exhibiting a bulbous, secretory portion were measured. Prepared slides and voucher specimens are deposited in the Arkansas State University Museum of Zoology (ASUMZ).

Caudal hedonic glands (found only in males) are multicellular acinar glands that show a high degree morphological variability (Fig. 1) ranging from being circular to oblong in the pre-secretory stage (Fig. 1C and D) to mostly flask-like during the secretory stage (Fig. 1E and F). No correlation was found between SVL and the size of these glands ( $P > .05$ ). In addition, the columnar epithelium of caudal hedonic glands is variable in thickness in relation to secretory activity ( $\bar{x}$  = 53.7  $\pm$  6.9; 26.9 - 88.4;  $n$  = 20). These glands can be distinguished from other skin glands

(namely, mucous and granular glands) by their size, configuration, and staining properties of their secretions. For example, caudal hedonic glands are much greater in length ( $\bar{x} = 259.2 \pm 6.6$ ; 190.0 - 330.0;  $n = 67$ ) and width ( $\bar{x} = 192.5 \pm 8.8$ ; 115.0 - 300.0;  $n = 67$ ) than either granular glands (length:  $\bar{x} = 194.9 \pm 16.9$ , 150.0 - 288.4,  $n = 16$ ; width:  $\bar{x} = 162.2 \pm 6.9$ , 138.5 - 192.3,  $n = 16$ ) or mucous glands (length:  $\bar{x} = 74.4 \pm 7.9$ , 61.5 - 92.3,  $n = 7$ ; width:  $\bar{x} = 83.6 \pm 10.6$ , 69.2 - 107.7,  $n = 7$ ). In addition, granular and mucous glands are mostly circular in structure (Fig. 1B), except (as in the case with granular glands) when both are oblong by being squeezed among the large caudal hedonic glands (Fig. 1D and F). The ratio of each

gland by number in a 1.5 mm x 0.5 mm rectangle of tissue (viewed by frontal section) in the dorsal tail region is, however, in favor of granular glands in one male (8 mucous glands - 11%, 14 caudal hedonic glands - 19%, and 52 granular glands - 70%). The greatest concentration of caudal hedonic glands occurs within the anterior glandular fields of the elevated ridge, a region lying dorsal to the 4 to 6 dorsal myotomal muscle bundles which lie posterior to the sacrum (approximately 6 - 8 mm in total length). The number of glands decreased dramatically in regions not subtended by adipose tissue.

The staining properties of the glandular secretions are similar to those previously reported by Sever (1989) for

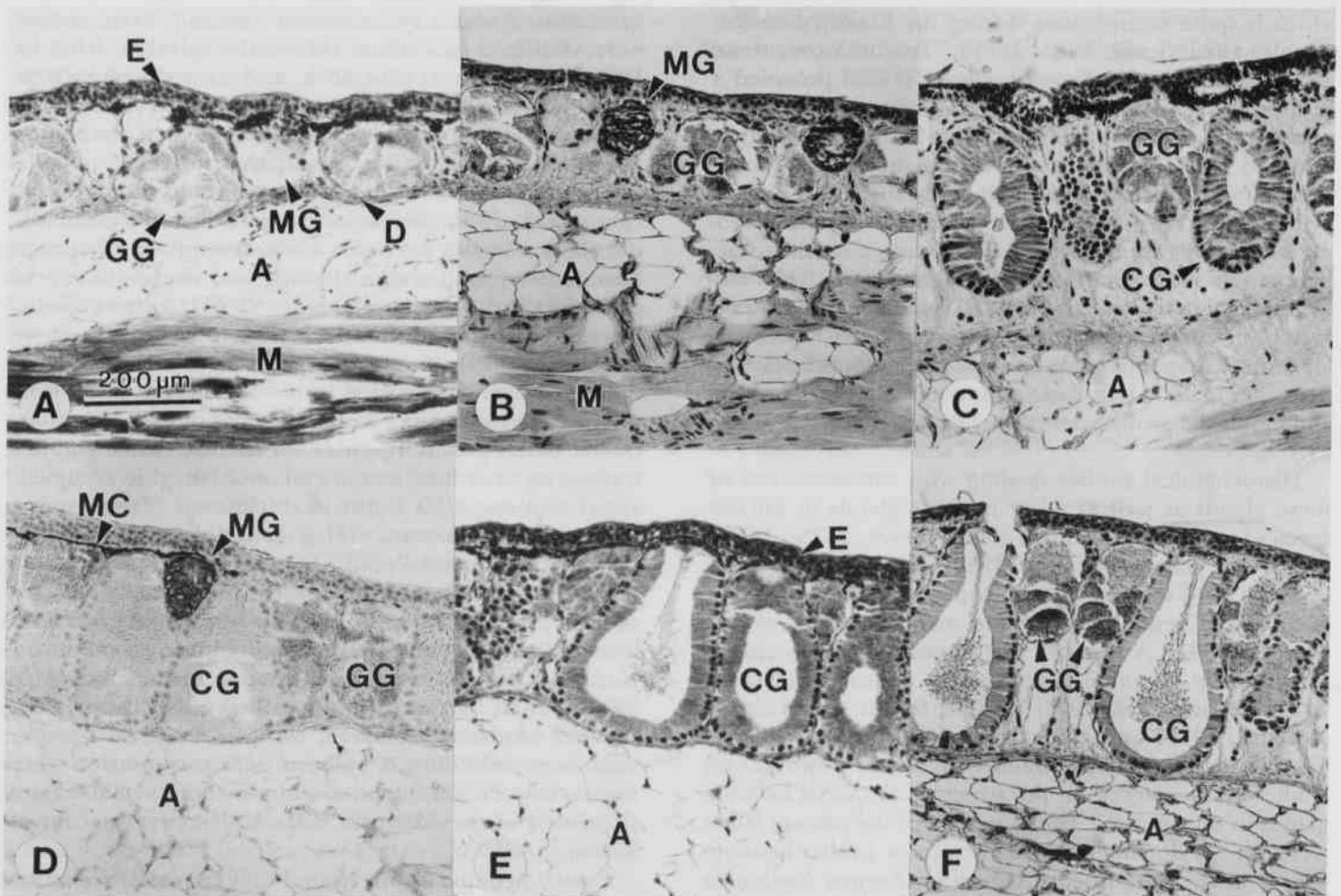


Fig. 1. Sagittal sections through the middorsal region of the tail directly above and posterior to the vent in *Eurycea longicauda melanopleura* illustrating the caudal hedonic glands and their relationship to other skin glands. Line in A (= 200  $\mu$ m) is the same for B - F. A. Section of female skin (ASUMZ 13938) stained with Pollak trichrome showing mucous glands (MG), granular glands (GG), a thin epidermis (E), the lower layer of the dermis (D) lying above adipose tissue (A), and the dorsal musculature (M). Notice that cells and secretions of the mucous glands fail to stain; granular glands are slightly acidophilic. B. Skin of male (ASUMZ 14258) stained with H & E illustrating an epidermal region (caudal portion of tail) devoid of caudal hedonic glands. The mucous glands exhibit a basophilic coloration; abbreviations are the same as those in A. C. Skin of male (ASUMZ 14250) stained with H & E showing the presence of early-developing, caudal hedonic glands (CG). D. Male (ASUMZ 13931) stained with alcian blue; both the caudal hedonic glands and the granular glands show no staining reaction compared to the brilliant blue of mucous glands. MC = layer of melanocyte cells. E and F. Caudal hedonic glands of male in D in region of maximum development (Pollak stain). The epithelial lining of the caudal hedonic gland appears light purple; the secretory column is also light purple, except for the portion near the pore of the gland which stains light-to-dark brown.

other *Eurycea*. In the following, we briefly summarize the reactions observed in the present study. Mucous glands liberate fibrous secretions that are weakly basiphilic using H & E and Pollak but are strongly positive (dark blue) with alcian blue, whereas glandular gland secretions are generally eosinophilic using H & E and Pollak (actually brown in color) but show no reaction to alcian blue. As mentioned above, Sever (1989) identified secretions from caudal hedonic glands as being comprised of mucoproteins. In *E. l. melanopleura*, these secretions were eosinophilic using H & E and showed no affinity for alcian blue; however, with Pollak, the reactions were mixed. In many cases, more than one coloration was evident within the secretory substance. For instance, in the narrowed, dorsal tubular neck of an individual gland (Fig. 1E and F), a secretory column possessing a cap (a secretory plug?) attached to a stalk stained light-to-moderately dark brown. The bulk of loosely-organized secretory material making up a central luminal mass and appearing light blue or purple gradually merged with or into this stalk. The above description was typical of a majority of the most well-developed caudal hedonic glands. Interestingly, nearly the same staining characteristic was evident in mental hedonic glands examined during this study (not pictured). The fact that both secretions are mucoproteins may partially explain the similarity in the nature of the secretory product.

The caudal hedonic glands of *E. l. melanopleura* differ in several respects from those of other species of *Eurycea* (*E. bislineata*; *E. cirrigera*; *E. junaluska*; *E. nana*; *E. wilderae*) as illustrated by Sever (1985, 1989). For one, the flask-like structure of these glands in the hypertrophied stage (as in *E. l. melanopleura*) was not demonstrated for these other species. Furthermore, the size of caudal hedonic glands in *E. l. melanopleura* is over twice as large as other species. The disparity in gland size can be attributed to differences in adult body size (*E. l. melanopleura* averaging around 10 mm greater in SVL compared of the other species). A study comparing the caudal hedonic glands of *E. lucifuga*, a species of comparable body size to *E. l. melanopleura*, is warranted and would help clarify species-specific differences within the genus.

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