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Neuromast Topography in *Ambystoma* Larvae

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Intra- and interspecific variation in cephalic stitch number, neuromasts per stitch, and neuromast density were examined in *Ambystoma maculatum* and *Ambystoma tigrinum* typical and cannibal morphs. Stitch number remains constant with growth; neuromasts per stitch increase; neuromast density decreases. Stitch number differs significantly between *A. tigrinum* ($\bar{x}$ = 284) and *A. maculatum* ($\bar{x}$ = 242). Same-sized larvae of both species have similar numbers of neuromasts per stitch and neuromast density.

IT is well known that lateral line neuromast topography may be used to distinguish genera and higher level taxa in fishes (e.g., elasmobranchs, Chu and Wen, 1979; holosteans, Jarvik, 1980 and references therein; teleosts, Parvin and Astakhov, 1982) and amphibians (Kingsbury, 1895; Escher, 1925; Hilton, 1947). However, few studies have examined differences between species within a genus (Jollie, 1984 considers this for *Lepisosteus*). Before such intrageneric comparisons can be made, it is necessary to determine the degree of normal intraspecific variation in neuromast parameters, as well as the nature of ontogenetic differences.

In amphibians neuromasts may be single, or clustered in parallel to form stitches. Neuromasts and stitches, in turn, are organized into groups. I examined cephalic neuromast groups, stitches per group, neuromasts per stitch and neuromast density for complete larval developmental series of *Ambystoma maculatum* and *Ambystoma tigrinum,* including typical and cannibal morphs. The specific questions addressed are: 1) how do these neuromast parameters change with growth within a species; 2) do species differ in these parameters; and 3) can they be used to distinguish trophic morphs within a species?

MATERIALS AND METHODS

General Methods.—Developmental series of *Ambystoma tigrinum* and *Ambystoma maculatum* were obtained by field collecting larvae or by hatching field-collected eggs and raising larvae in the laboratory. Larval *A. tigrinum* were collected in northwestern Iowa (Dickinson Co., 43°23′N, 95°11′W) and included both typical (N = 25, 10–62 mm standard snout vent length [SVL]) and cannibal (N = 2, 61,62 mm SVL) morphs. Animals were immediately killed and preserved in 10% formalin (see Lannoo and Bachmann, 1984 for further details of collecting methods, population parameters and cannibal morphs). Larval *A. maculatum* (N = 17, 8–23 mm SVL) were collected and preserved from Halifax Co., Nova Scotia (44°40′N, 63°40′W).

To see neuromasts, preserved animals were first placed in 0.5% trypsin for 12–24 h. This insured separation of the epidermis from the underlying dermis and later allowed neuromasts to be peeled away from the animal's body with the epidermis. After trypsin treatment, animals were placed in 30–35% hydrogen peroxide until skin pigments were bleached (12–72 h). Bleaching insured that no neuromasts were masked by pigment granules. Bleaching with the epidermis still on the animal avoided tissue curling and subsequent difficulty in tissue mounting. After bleaching, the cephalic epidermis (i.e., tissue immediately anterior to gill rami dorsally and gular fold ventrally) was removed. Mid-dorsal and mid-ventral incisions were made along the entire length of the head, and epidermis removed in left and right sections that included both dorsal and ventral skin (Fig. 1). These incisions insured that no neuromasts or stitches were bisected and yielded two tissues

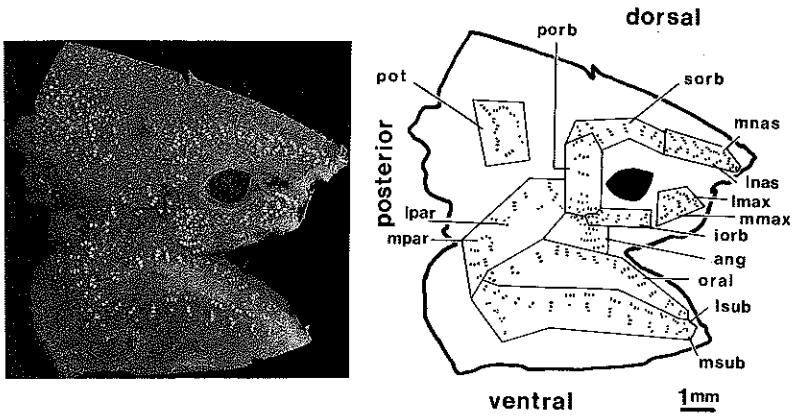

Fig. 1. A darkfield photograph and tracing showing neuromast location and group organization in an *Ambystoma maculatum* larva (SVL 23 mm). The upper border of the tissue is the dorsal midline, the lower border the ventral midline, the dark oval the eye and the anterior notch the mouth. Postotic neuromasts are difficult to discern in the photograph. Group abbreviations: ang = angular; iorb = infraorbital; lmax = lateral maxillary; lnas = lateral nasal; lpar = lateral parietal; lsub = lateral submandibular; mmax = medial maxillary; mnas = medial nasal; mpar = medial parietal; msub = medial submandibular; oral = oral; porb = postorbital; pot = postotic; sorb = supraorbital.

that could be flattened easily for microscopic examination. Tissues were placed in water between two glass microscopic slides and viewed with a dissecting microscope at 10–40× under darkfield illumination.

Stitches per group and neuromasts per stitch were counted. Only neuromasts in well-defined stitches, and stitches in well-defined groups were considered, thereby avoiding confusion with ampullary lateral line organs (Fritzsch and Wahnschaffe, 1983; Münz et al., 1982, 1984) and/or skin glands (Hetherington and Wake, 1979). Stitches were assigned to groups based on criteria discussed in the following section.

Surface area of each tissue was determined using a Zeiss IBAS Image Analyzer. For each tissue three values were obtained and averaged. Average values for right and left tissues of the same animal were then summed.

For all tests, data were normally distributed and parametric statistics were used. Linear regressions and pooled t-tests were calculated using MINITAB (Ryan et al., 1976).

Neuromast Group Definitions.—Ambystoma neuromasts, stitches and stitch groupings are illustrated in Fig. 1. In Fig. 1 (left), neuromasts appear against darkfield illumination as light ovals. Neuromasts are grouped into clearly defined stitches, composed predominantly of three neuromasts per stitch. Stitch orientation differs. These differences are critical to the functioning of neuromasts as the maximum sensitivity of each neuromast is perpendicular to the long axis of its stitch (Flock, 1967).

Fig. 1 (right) illustrates stitch groupings, which I base on stitch orientation. These divisions differ from those used by previous authors (Kingsbury, 1895; Escher, 1925; Hilton, 1947), who based their nomenclature on common nerve pathways. In most cases my groupings simply subdivide traditional groups. The groups identified here are:

Supraorbital group—single row of stitches; long axis of each stitch oriented approximately transverse to long axis of body, radially with respect to eye.

Nasal group—anterior extension of supraorbital group; two stitch rows, one medial and one lateral; medial row of stitches oriented anterolaterally to posteromedially, lateral row of stitches oriented anteromedially to posterolaterally; adjacent medial and lateral stitches perpendicular, as illustrated in Escher (1925: fig. 1e).

Postorbital group—single row of stitches; long axis of each stitch approximately parallel to long axis of body and radial to eye.

Infraorbital group—single stitch row; each stitch with long axis approximately transverse to long axis of body and radial to eye; an anterior extension of postorbital stitches.

Maxillary group—anterior extension of infraorbital group; two stitch rows, one medial and one lateral; medial row of stitches oriented anterolaterally to posteromedially, lateral row of stitches anteromedially to posterolaterally; adjacent medial and lateral stitches perpendicular.

Parietal group—stitches arc back from postorbital group to submandibular group; two stitch rows, one medial and one lateral; anterior portion of medial row oriented anterolaterally to posteromedially, posterior portion of medial row oriented anterior to posterior; anterior portion of lateral row oriented anteromedially to posterolaterally, posterior portion of lateral row oriented dorsoventrally; adjacent medial-lateral stitches perpendicular.

Oral group—single row of stitches; stitches follow rim of mandible from midline anteriorly to junction of parietal and submandibular groups posteriorly; stitches approximately transverse to body axis.

Submandibular group—two stitch rows, one lateral and one medial; anterior extensions of lateral and medial parietal rows; lateral submandibular stitches parallel to long axis of body, medial stitches transverse.

Angular group—single diffuse row; located posterior to jaw angle; stitches oriented parallel to body axis.

Postotic group—stitches loosely organized; located caudally and dorsally; extension of dorsal (and medial?) body groups; develop from at least one postotic placode; the postotic group is innervated by branch of posterior lateral line nerve.

All cephalic stitches other than postotic develop from preotic placodes and are innervated by anterior lateral line nerve branches. Supraorbital and nasal stitches are innervated by the supraorbital nerve; postorbital, infraorbital and maxillary groups are innervated by the infraorbital nerve; and parietal, oral, submandibular and angular stitches are innervated by the postorbital nerve (Escher, 1925).

Results

Stitch number remains constant with growth for larval *Ambystoma* (slopes of regressions not significantly different from 0; $P = 0.25$ for *A. tigrinum*; $P = 0.10$ for *A. maculatum*). In *A. tigrinum* there are significantly more total cephalic stitches ($\bar{x} = 284.1$; range 221–344) than in *A. maculatum* ($\bar{x} = 242.1$; range 189–288) (t-test, $P < 0.001$; Table 1); thus, this character can be used to distinguish populations of these species. However, intraspecific variation is too great to assign unidentified individuals to species based on this character alone. In seven of the ten neuromast groups: supraorbital, postorbital, parietal, oral, angular, submandibular and postotic (Table 1), *A. tigrinum* has significantly more stitches than *A. maculatum*. Within individuals, contralateral stitch counts vary. The greatest variation observed in *A. maculatum* was 9.2% (129 vs 137 stitches) and in *A. tigrinum* 11.5% (139 vs 155 stitches). There were no differences in stitch number between cannibal and typical morph *A. tigrinum* (cannibal morph stitch numbers were 275 and 319).

Table 1. A Comparison of Mean Number of Cephalic Neuromast Stitches by Group in 27 *Ambystoma tigrinum* and 17 *Ambystoma maculatum* Larvae. Asterisks indicate significant differences between species (t-test). See Fig. 1 for group location and orientation. Contralateral values were summed. Totals do not add precisely due to rounding during data compilation.

	A. tigrinum no. stitches		*A. maculatum* no. stitches			
Stitch groups	$\bar{x}$	SE	$\bar{x}$	SE	t	*P*
Nasal	35.2	2.0	36.4	1.8	0.41	0.68
Maxillary	23.0	1.0	22.2	0.8	0.57	0.57
Supraorbital	23.4	0.6	19.8	0.8	3.52	0.01*
Postorbital	15.2	0.4	9.0	0.4	3.91	0.01*
Infraorbital	9.1	0.4	8.4	0.4	0.95	0.35
Angular	12.4	0.6	10.0	0.4	3.28	0.01*
Parietal	41.0	1.4	31.4	1.8	4.00	0.01*
Submandibular	56.7	1.3	46.2	1.4	4.89	0.01*
Oral	41.4	1.4	35.4	1.6	2.81	0.01*
Postotic	27.8	1.6	20.6	1.8	2.90	0.01*
Total	284.1	6.7	242.1	7.0	3.64	0.01*

Average number of neuromasts per stitch increases with growth from one to three in *A. maculatum* and one to seven in *A. tigrinum*. For *A. tigrinum* neuromasts per stitch = 0.4 + 0.1 SVL ($P < 0.001$; $r^2 = 0.87$). For *A. maculatum* neuromasts per stitch = 0.2 + 0.1 SVL ($P < 0.001$; $r^2 = 0.82$). Same-sized larvae of both species have similar numbers of neuromasts per stitch. Cannibal morphs averaged about seven neuromasts per stitch. Total numbers of neuromasts increased from 258–690 in *A. macula-*

tum and from 283–2,168 in *A. tigrinum*, including cannibal morphs.

Despite increases in neuromast number, neuromast density decreases with growth from 12.1–2.4 neuromasts per mm² in *A. tigrinum*, and from 13.3–4.8 neuromasts per mm² in *A. maculatum*. The regressions for neuromast density vs SVL are: neuromast density = 12.1–0.2 SVL for *A. tigrinum* and neuromast density = 16.7–0.6 SVL for *A. maculatum*. Same-sized heterospecific larvae have similar neuromast densities. Cannibal morphs had the lowest neuromast densities (2.4 neuromasts per mm²).

Discussion

Neuromast stitch number remains constant with growth and distinguishes larval populations of *Ambystoma tigrinum* from those of *A. maculatum*. It appears, therefore, that stitch number may be a useful taxonomic character for distinguishing other closely-related amphibian species. Stitch number is not useful for distinguishing cannibal morph *A. tigrinum* from typical animals.

Neuromasts per stitch and neuromast density change with growth and are not useful taxonomic characters. Unexpectedly, while neuromasts per stitch increase with growth, neuromast density decreases. Cannibal morphs, being the largest members of a population, usually have the most neuromasts per stitch and lowest neuromast densities. Cannibal morphs do not differ from typical morphs of the same size in these neuromast parameters.

Neuromast number affects the mechanosensory ability of the neuromast system as a whole; the more neuromasts an animal has, the greater will be its ability to perceive water displacements. However, there is no evidence that *A. tigrinum* has better mechanosensory perception than *A. maculatum*. Indeed, it is difficult to assess what the interspecific difference in average total stitches of 14.3% (240 vs 280 stitches) means, given that there can be at least an 11.5% difference between left and right sides of the same animal. Görner et al. (1984) have shown that *Xenopus* adults with all but two (out of approximately 100) stitches ablated on either side still orient toward a stimulus, although their precision is greatly reduced. The large left-right within-individual variance in stitch number certainly argues against finely-tuned functional differences in this neuromast parameter.

Neuromast orientation affects mechanosensory ability (Flock, 1967). In *Ambystoma*, some neuromast groups are composed of parallel stitches, while in other groups, adjacent stitches are perpendicular. Because neuromasts are directionally sensitive, a perpendicular stitch arrangement enables those animals with only two stitches to be sensitive to stimuli through 360°. A series of these perpendicular stitch couplets implies high discriminatory ability. Three of the four groups containing perpendicular stitches (nasal, maxillary and submandibular) are near the snout and are presumably involved in prey detection. Both *A. tigrinum* and *A. maculatum* feed on many of the same prey species (Branch and Altig, 1981). There are no significant interspecific differences in nasal and maxillary stitch number dorsally, and ventrally, there are no differences in perpendicular stitch number as measured by lateral submandibular stitches (Fig. 1; $\bar{x} = 18.6$, SE = 0.6, *A. tigrinum*; $\bar{x} = 17.6$, SE = 0.8, *A. maculatum*; t-test, $P = 0.30$).

Typical *A. tigrinum* morphs tend to be microphagic while cannibal morphs tend to be macrophagic (Collins and Holomuzki, 1984; Lannoo and Bachmann, 1984). I predicted that the gross changes in cannibal head morphology (i.e., broadening of the head and enhanced vomerine teeth development) may correlate with concomitant changes in sensory input mediated by neuromast topography. This prediction, however, was not supported. Cannibal morphs develop from typical-looking larvae, they retain typical morph neuromast topographies.

With the exception of the *Ambystoma* data presented here, quantitative aspects of amphibian neuromast topography have been considered only for *Xenopus laevis* (Shelton, 1970). Neuromast topography may be of systematic use in amphibians, as it has been for fish. Because neuromasts are polarized, some functional information may also be derived from topography. However, because the precise role of neuromasts in the behavioral ecology of an animal is yet unknown, it is difficult, at this time, to assess the functional significance of topography and topographical differences.

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