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Ontogeny of Functional Design in Tiger Salamanders (*Ambystoma tigrinum*): Are Motor Patterns Conserved During Major Morphological Transformations?

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ABSTRACT The process of metamorphosis in tiger salamanders, *Ambystoma tigrinum,* is used to investigate motor pattern conservatism in vertebrates. Specifically, we examined cranial muscle activity to determine if changes in the motor pattern are correlated with the morphological or environmental changes that occur at metamorphosis.

Twenty-three variables were measured from electromyographic recordings from six cranial muscles in 13 tiger salamanders. These variables described the configuration of the motor pattern: the peak amplitude of activity, duration, relative onset, and time to peak amplitude were measured for each of the six muscles. Univariate and multivariate statistical analyses showed that there was no change in the mean motor pattern associated with the morphological transformation at metamorphosis: larval and metamorphosed individuals feeding in the water have very similar motor patterns. This was true despite significant morphological changes in the design of the feeding mechanism at metamorphosis and despite a significant decrease in aquatic feeding performance following metamorphosis.

There was a change in the mean motor pattern to jaw muscles when metamorphosed individuals fed in water and on land: metamorphosed terrestrial feedings tend to have longer bursts of muscle activity then do aquatic feedings. The environmental changes in the motor pattern cannot be attributed to effects of differing fluid density or viscosity between water and air and are instead related to the shift to feeding by tongue projection on land.

The decrease in aquatic feeding performance that occurs after metamorphosis is not correlated with changes in the motor pattern. Instead, the results suggest that changes in behavioral performance during ontogeny are associated with the transformation of hydrodynamic design of the feeding mechanism from uni- to bidirectional, and that motor patterns driving complex rapid behaviors may be conserved when behavior is altered by changes in peripheral morphology.

How does the behavior of animals change in ontogeny and phylogeny? One approach to this general problem is to consider the levels of biological design that might be responsible for changes in behavior.

Differences in behavior between two species, for example, might be based only on differences in the structure and topography of their musculoskeletal systems. All other things being equal, differences between species in muscle masses, origins and insertions, physiological properties, and lever arms will result in different behaviors. By behavior we mean the actions or movements of animals (Lauder, '86, '88), and such actions are often quantified by measuring animal performance (e.g., Arnold, '83; Emerson and Diehl, '80; Reilly and Lauder, '88a). Alternatively, differences between two species in the timing and sequence of muscle activity

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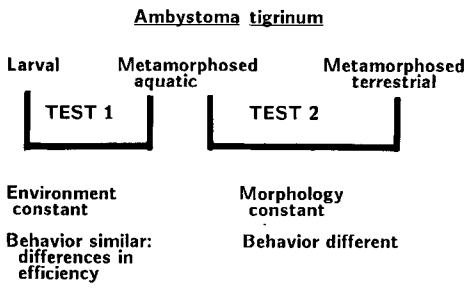

Fig. 1. Schematic diagram of the experimental plan used in this study. *Ambystoma tigrinum* will feed under three conditions: as larvae in the water, as metamorphosed animals in the water, and as metamorphosed animals on land. By comparing the motor pattern between larval and metamorphosed animals feeding in the water (test 1), we test only for the effect of morphological changes occurring at metamorphosis on the motor pattern. As shown by Shaffer and Lauder ('88), the aquatic feeding kinematics used by these two stages are similar, but they differ in efficiency (Lauder and Shaffer, '86). By comparing the motor pattern used by metamorphosed individuals feeding in the water and on land (Test 2), we test only for the effect of the environmental change on the motor pattern. Feeding kinematics are different (Lauder and Shaffer, '86) between these two stages.

(the motor pattern) could produce different behaviors even if the morphology of the musculoskeletal system is identical. Changes at the level of patterned outflow from the central nervous system to the peripheral musculature may in turn be the result of changes in central neuronal interconnections and morphology. Thus, two species may differ in behavior because of changes at any one or more of at least four levels: peripheral morphology, physiological properties of peripheral structures, motor pattern, and central neuronal connections and circuits.

The general research strategy that we have used to pursue the issue of behavioral transformation involves three steps. The first step is the documentation of a change (or lack thereof) in behavior and animal performance. The second step is the examination of the various underlying levels mentioned above for *correlated* changes. Thus, if ontogenetic stages A and B in an animal differ in behavior, we propose to test for mean differences in musculoskeletal morphology and motor pattern between stages A and B. If any significant differences are found, then they can only be said to be correlated with the documented change in behavior. Third, if correlated changes are found, then it is reasonable to conduct *manipulative* experiments to test the hypothesis that the correlated changes between behavior and motor patterns are in fact causally related. Any observed changes in organismal morphology or physiology must experimentally be shown to *account for* changes observed in behavior.

Previous research on lower vertebrate motor patterns (Bemis and Lauder, '86; Lauder, '80, '83; Lauder and Shaffer, '85) has examined one of the three steps, has not used this overall research strategy, and instead has focused on a comparative, phylogenetic analysis of motor patterns in relation to modifications in feeding behavior. This work has indicated that motor patterns may be conservative across a wide phylogenetic range. For example, Lauder ('80; also see '85) has emphasized that the feeding system of ray-finned fishes as phylogenetically diverse as bichirs (*Polypterus*), gars (*Lepisosteus*), and bowfins (*Amia*) shows considerable similarities in the motor patterns to the jaw muscles, despite major morphological changes in the structure of the muscles and bones of the feeding mechanism.

The conclusions derived from these comparative studies of fish feeding systems (Lauder, '80, '85) have been reached by making comparisons *within* a single environment. Thus, the motor pattern to the jaw muscles in fishes may be conservative because the density and viscosity of water may limit the range of possible functional solutions to the problem of prey capture. Similarly, the lack of motor pattern and kinematic diversity in terrestrial feeding systems (Bramble and Wake, '85) may be due to the constraints of feeding in air.

The goal of this paper is to describe the ontogeny of motor patterns in the tiger salamander, *Ambystoma tigrinum,* and to interpret the results in the framework of the three-part research strategy outlined above. We have selected the tiger salamander for several reasons. First, *A. tigrinum* undergoes a dramatic morphological reorganization in the skull and hyobranchium at metamorphosis. Larval life in the water typically lasts from 3 to 4 months, whereas adult terrestrial life may extend for more than 10 years. This metamorphosis provides a major change in morphology across which to examine motor patterns. By using ontogenetic alterations in behavior, it is possible to avoid many of the problems inherent in a comparative analysis of different species. Second, the process of metamorphosis in urodeles, although representing a considerable

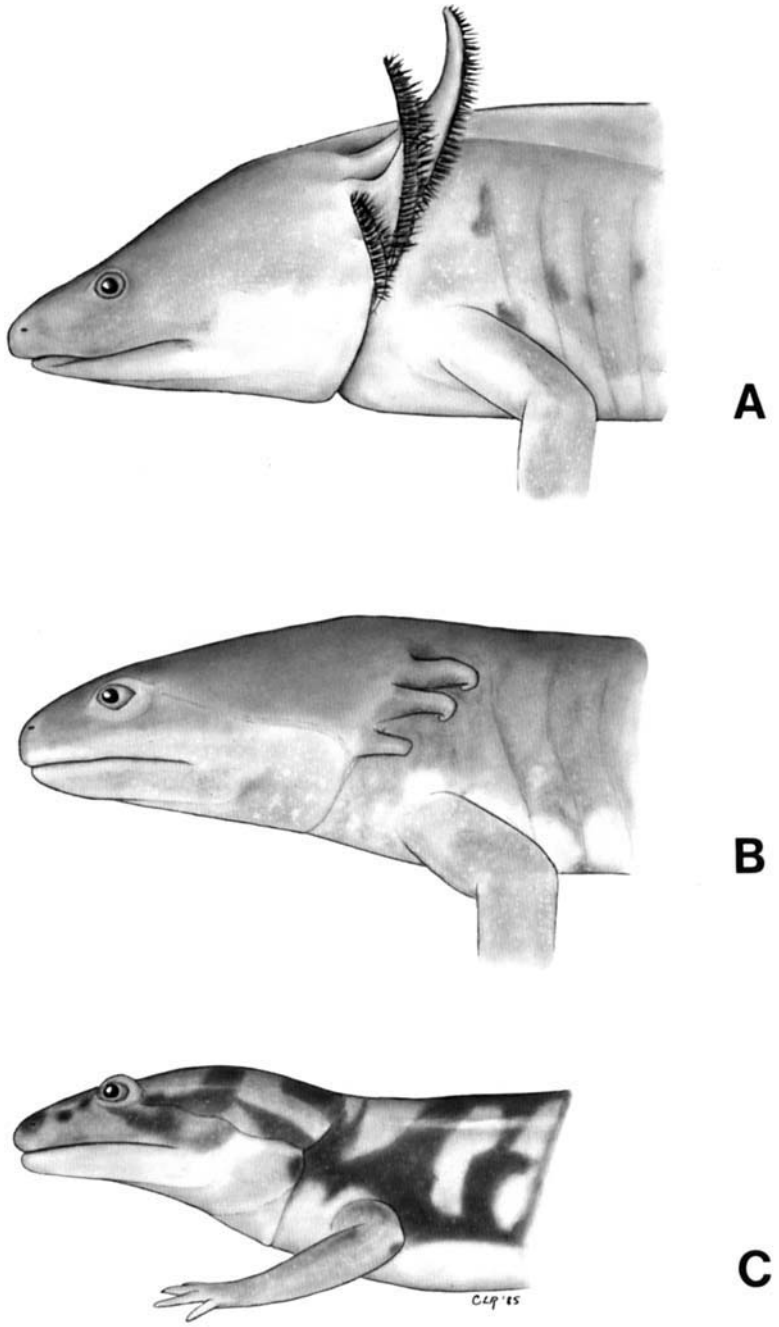

Fig. 2. Lateral views showing the external morphology of the head in *Ambystoma tigrinum* at three times during ontogeny. **A:** Larval. **B:** Mid-metamorphic. **C:** Postmetamorphic (adult). Note the reduction and loss of the external gills during metamorphosis, the reduction in head depth, and the change in location of the eyes. The gular membrane, which in larvae provides a posterior exit of water from the buccal cavity, is sealed to the body wall in adults. Scale bar = 2.0 cm.

TABLE 1. Experimental design for longitudinal and cross-sectional electromyographic data on prey capture in Ambystoma tigrinum[1]

Metamorphic stage	Individual												
	Longitudinal		Cross-sectional										
	4	7	5	9	12	13	8	11	17	6	10	14	23
Larval	x	x	x	x	x	x							
Metamorphosed aquatic	x	x					x	x	x				
Metamorphosed terrestrial	x	x								x	x	x	x

[1]Salamander identification No. is shown at the top of each column. An "x" indicates that data were obtained for that individual.

TABLE 2. Summary statistics for 23 electromyographic (EMG) variables measured from prey capture events (captures only) in Ambystoma tigrinum: *Mean, sd (N)*[1]

EMG variable	Metamorphic stage		
	Larval	Metamorphosed aquatic	Metamorphosed terrestrial
DMMAX	.45, .13 (43)	.55, .12 (16)	.34, .15 (31)
	.37, .15 (22)	.50, .11 (13)	.53, .11 (21)
DMTMAX	17, 16 (43)	24, 13 (16)	25, 19 (31)
	10, 12 (22)	23, 15 (13)	43, 33 (20)
DMDUR	87, 36 (43)	123, 55 (16)	81, 18 (31)
	62, 24 (22)	102, 30 (13)	124, 38 (20)
EPON	−1, 3 (43)	2, 10 (16)	−2, 8 (31)
	−1, 2 (22)	−7, 6 (13)	4, 5 (21)
EPMAX	.36, .07 (43)	.40, .11 (16)	.37, .08 (31)
	.37, .08 (22)	.46, .04 (13)	.35, .09 (21)
EPTMAX	11, 9 (43)	36, 21 (16)	44, 29 (31)
	17, 16 (22)	26, 22 (13)	41, 31 (21)
EPDUR	68, 29 (43)	82, 25 (16)	102, 35 (31)
	65, 26 (22)	91, 46 (13)	111, 39 (21)
RCON	3, 3 (43)	9, 9 (16)	−1, 7 (31)
	0, 3 (22)	2, 9 (13)	0, 6 (21)
RCMAX	.32, .18 (43)	.37, .18 (16)	.36, .15 (31)
	.55, .13 (22)	.21, .15 (13)	.31, .14 (21)
RCTMAX	14, 10 (43)	26, 16 (16)	37, 46 (31)
	11, 8 (22)	15, 5 (13)	26, 29 (21)
RCDUR	48, 20 (43)	69, 15 (16)	112, 56 (31)
	57, 18 (22)	56, 30 (13)	101, 32 (21)
AMEON	0, 3 (43)	14, 5 (5)	0, 4 (30)
	4, 13 (22)	2, 8 (13)	1, 6 (21)
AMEMAX	.50, .16 (43)	.41, .22 (5)	.34, .18 (26)
	.27, .17 (22)	.45, .16 (13)	.50, .15 (21)
AMETMAX	46, 26 (43)	117, 114 (5)	69, 47 (26)
	54, 29 (22)	79, 57 (13)	173, 207 (21)
AMEDUR	106, 36 (43)	165, 146 (5)	180, 150 (30)
	87, 42 (22)	176, 253 (13)	338, 384 (21)
AMION	1, 2 (43)	4, 16 (14)	−1, 5 (30)
	5, 15 (20)	18, 25 (13)	−1, 6 (21)
AMIMAX	.53, .14 (43)	.32, .17 (14)	.24, .19 (26)
	.40, .21 (20)	.38, .16 (13)	.51, .15 (21)
AMITMAX	46, 21 (43)	85, 84 (14)	83, 47 (26)
	41, 30 (20)	63, 38 (13)	161, 188 (21)
AMIDUR	105, 43 (43)	139, 105 (14)	170, 69 (30)
	77, 39 (20)	163, 181 (13)	434, 298 (21)
BH/SAR1ON	−1, 2 (41)	13, 12 (15)	4, 4 (31)
	−1, 4 (22)	9, 11 (13)	2, 4 (21)
BH/SAR1MAX	.48, .13 (41)	.30, .17 (15)	.16, .09 (30)
	.46, .11 (22)	.20, .14 (13)	.43, .11 (21)
BH/SAR1TMAX	21, 21 (41)	44, 21 (15)	38, 20 (30)
	9, 16 (22)	32, 18 (13)	46, 26 (21)
BH/SAR1DUR	126, 93 (41)	92, 38 (15)	89, 28 (31)
	67, 27 (22)	62, 26 (13)	122, 61 (21)

[1]Statistics for the cross-sectional data set are shown above the corresponding values for the longitudinal data set. Statistics are means for all individuals in each data set. All units are milliseconds except for the six variables that measure EMG amplitude (DMMAX, EPMAX, RCMAX, AMEMAX, AMIMAX, BHMAX), which are in millivolts.

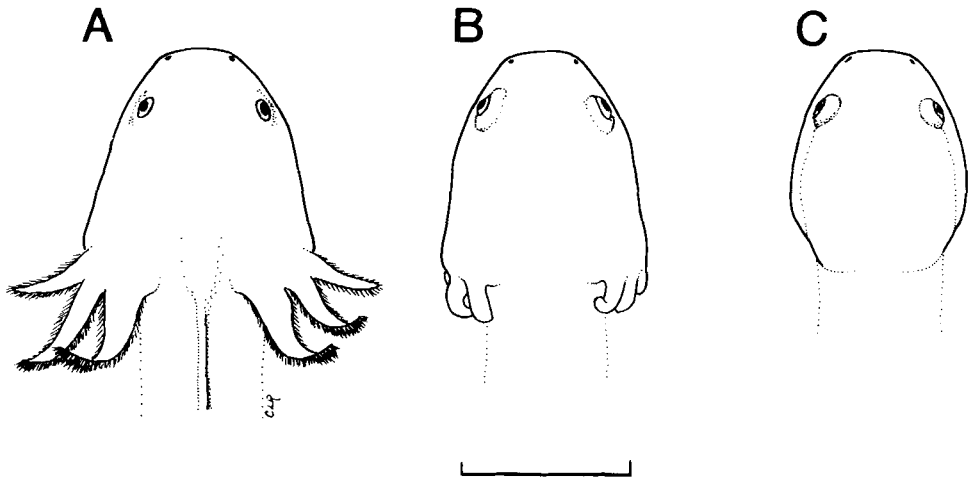

Fig. 3. Dorsal view of the head in *Ambystoma tigrinum* to show the change in external shape at three times during ontogeny. **A:** Larval. **B:** Mid-metamorphic. **C:** Postmetamorphic (adult). Note the reduction in head width and the loss of external gills. Scale bar = 2.0 cm.

modification, is not so great that it is difficult to homologize muscles across the transformation. Thus, one can record from homologous muscles throughout ontogeny. Third, although metamorphosis normally entails an environmental transition from aquatic feeding to terrestrial prey acquisition, it is also possible to study the effect of metamorphosis on the motor pattern in the aquatic environment alone. Metamorphosed individuals will feed both in the water and on land, allowing us to test for mean differences in the motor pattern between aquatic and terrestrial feedings in the same individual (Fig. 1). Fourth, the other analyses required by the research strategy outlined above have been completed (Lauder and Reilly, '88; Lauder and Shaffer, '86; Reilly and Lauder, '88a; Shaffer and Lauder, '88), allowing us to present here a study of the motor patterns that completes the three-step analysis of the mechanisms underlying ontogenetic changes of behavior in *Ambystoma tigrinum*.

MATERIALS AND METHODS

Specimens and morphology

Electromyographic data were acquired from 13 individual *Ambystoma tigrinum* collected in Lincoln Co., Colorado. All individuals were collected as larvae, transported to the laboratory, and then held individually at 17°C in 40-liter aquaria until metamorphosis occurred naturally. After metamorphosis, individuals were kept in either shallow water (about 4 cm deep) with rocks to allow them access to the air, or in aquaria with moist paper towels. During both the experimental and holding periods salamanders were fed a diet of live earthworms (*Lumbricus*) cut into 1- to 2-cm-long pieces. During the experiments, the pieces of earthworm prey were presented to the animal on the end of a pair of forceps in a similar manner to that used in previous studies (Lauder and Shaffer, '85, '86; Shaffer and Lauder, '88). Nine of the animals used for the electromyographic experiments were the same individuals used for a study of the metamorphosis of feeding kinematics (Shaffer and Lauder, '88) to allow direct individual comparisons of kinematics and motor patterns. Animals of similar size were chosen to minimize overall size effects: morphological measurements (in cm) of the 13 animals during the experimental period were (mean, sd) snout-vent length, 9.7, 0.56; head width, 2.45, 0.33.

Gross morphological features of the cranial musculoskeletal system were studied by dissection of larval and metamorphosed *Ambystoma tigrinum* from the same population as the experimental animals. A Zeiss IVB dissecting microscope was used with a camera lucida to illustrate musculoskeletal anatomy. Several specimens at larval, mid-metamorphic, and adult ontogenetic stages were cleared and double stained for bone and cartilage following the procedures of Dingerkus and Uhler ('77).

Osteological and myological nomenclature follows that used by Drüner ('02), Duellman and Trueb ('86), Edgeworth ('35), Francis ('34), Luther ('14), Piatt ('38, '39, '40), Reilly and Lauder ('88b), and Wilder ('25). In addition, we consider (following Smith, '20) that the metamorphosed subarcualis rectus one muscle (Fig. 7: SAR1) is homologous to the muscle of that name in the larva

(Fig. 6: SAR1), and not to the muscle that occupies a similar anatomical position in the larva, the branchiohyoideus (Fig. 6: BH; Lauder and Shaffer, '85; = ceratohyoideus externus of Drüner, '02). Because of the similarities in line of action and function of the larval branchiohyoideus and the adult subarcualis rectus one and because of the difficulty of reliably recording from the larval SAR1, we present electromyographic data from both the larval BH and the adult SAR1. This allows us to determine if nonhomologous muscles with similar functions possess similar activity patterns.

Experimental design and statistics

The goal of our experimental design was to test for mean differences in cranial muscle activity patterns during 1) the environmental change from feeding in the water to feeding on land, and 2) the morphological changes occurring at metamorphosis. In order to do this, we used the general plan of comparisons outlined in Figure 1. The crucial aspect of this experimental design is the ability of metamorphosed *Ambystoma tigrinum* to feed underwater, as this allows us to test for differences in the motor pattern with morphological and environmental shifts separately (Fig. 1). Without this component of our experimental design, we would not be able to separate changes in the motor pattern at metamorphosis from those that occur with the environmental transition from water to land.

In this paper we will refer to three ontogenetic stages. We are not strictly referring to three distinct morphological units but rather to levels of comparison in the experimental design. The three stages are 1) larval animals, which feed in the water, 2) metamorphosed animals feeding in the water, termed metamorphosed aquatic, and 3) metamorphosed animals feeding on land, termed metamorphosed terrestrial (Fig. 1).

When we compare feedings between larval and metamorphosed aquatic animals, the feeding behavior in both cases is aquatic suction feeding (Lauder, '85; Lauder and Shaffer, '86). These behaviors are similar in kinematic pattern (Shaffer and Lauder, '88) but do differ in performance as documented by Lauder and Shaffer ('86). Test 1 in Figure 1 thus tests for differences in the mean motor pattern before and after metamorphosis. Comparing the metamorphosed aquatic stage to the metamorphosed terrestrial stage (Fig. 1, Test 2) tests for differences in the mean motor pattern between the two environments.

Two statistical experimental designs were used to implement the conceptual plan outlined in Figure 1, and both univariate and multivariate analyses were conducted. First, a *longitudinal* design was used for the two individuals for whom we had electromyographic data at all three stages (Table 1). These data were analyzed with a two-way (univariate) factorial analysis of variance (ANOVA) (Sokal and Rohlf, '81) with metamorphic stage as a fixed effect and individual variance as a random effect. Second, a *cross-sectional* design was used for the 11 individuals that were studied at one stage each (Table 1). These data were analyzed using a three-level (univariate) nested ANOVA (Sokal and Rohlf, '81). Variance in each motor pattern variable is partitioned into components attributable to metamorphic stages, to individuals within a metamorphic stage, and to error variance as in previous research (Lauder and Shaffer, '85; Shaffer and Lauder, '85). We adopted the $P \leq .01$ level of significance rather than the normal $P \leq .05$ to minimize the problem of finding significant results only because of the fact that many comparisons were being made simultaneously.

Approximately ten feedings were available for each individual, although sample sizes varied among individuals: actual sample sizes are given with the summary statistics for both the longitudinal and cross-sectional data sets (Table 2). A total of 146 feedings were analyzed for this paper.

Although correlations among the variables were low (for example, with the cross-sectional data set and a 19-by-19 matrix, only 13 correlations out of 171 comparisons were above 0.40), univariate analyses still may provide an unreliable overall guide to multivariate effects (Bray and Maxwell, '85; Dunteman, '84; Harris, '75; Willig et al., '86). Therefore, we used the largest data set (the cross-sectional one) to conduct a series of multivariate analyses. Because of the many variables used in our univariate analyses, we first chose a biologically meaningful subset of eight variables, then used a principal components analysis to reduce further the dimensionality of the data set to four factors. The large number of variables and the relatively small number of observations for each individual (about ten) precluded a direct a priori multivariate analysis of variance (MANOVA). The principal components

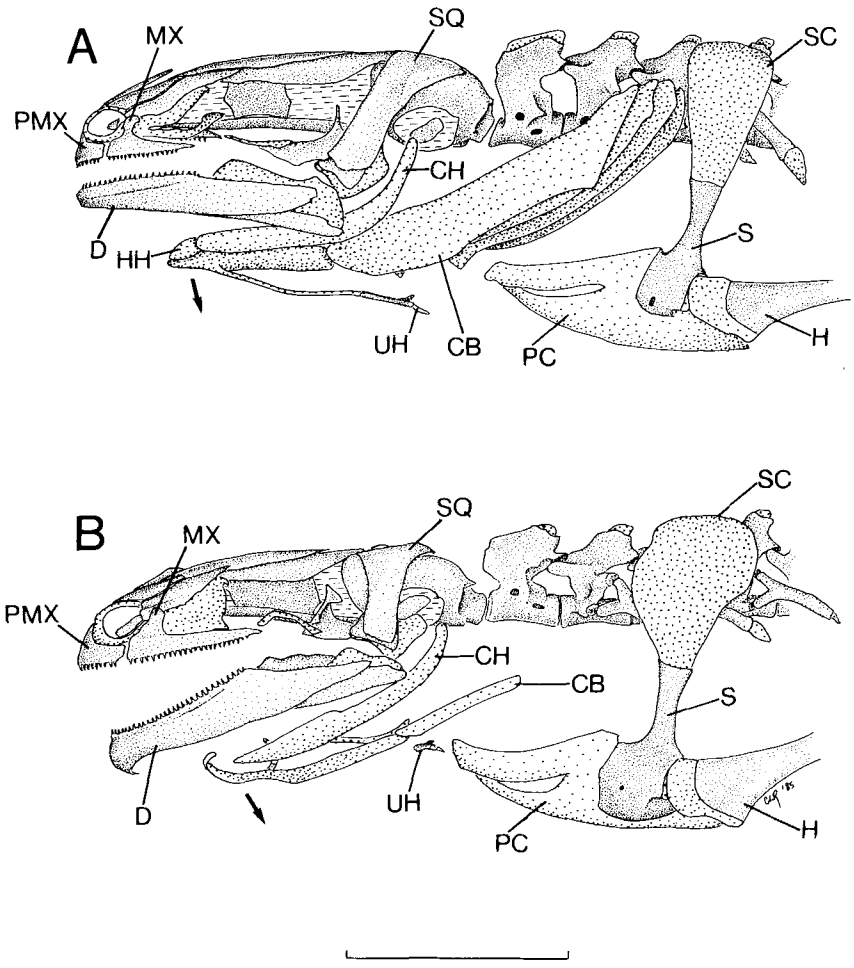

Fig. 4. *Ambystoma tigrinum.* Lateral view of cranial osteology in a larval individual (**A**) and a metamorphosed individual (**B**). Arrows indicate the direction of hyoid depression during feeding. Scale bar = 1.0 cm. Abbreviations for this and subsequent figures: AME, adductor mandibulae externus muscle; AMI, adductor mandibular internus muscle; BH, branchiohyoideus muscle; BHY, Basihyal cartilage; CB_{1-4}, ceratobranchial bones 1 through 4; CH, Ceratohyal cartilage; GH, geniohyoideus muscle; D, dentary bone; DM, depressor mandibulae muscle; EP, epaxial muscles; H, humerus; HB, hypobranchial cartilage; HH, hypohyal cartilage; IH, interhyoideus muscle; IMP, intermandibularis posterior muscle; MX, maxillary bone; PC, procoracoid cartilage; PE, pectoralis muscle; PMX, premaxillary bone; RC, rectus cervicis muscle; S, scapula; SAR1, subarcualis rectus 1 muscle; SC, scapular cartilage; SQ, squamosal bone; UH, urohyal.

analysis factored the correlation matrix of eight variables (peak activity and duration for the depressor mandibulae, epaxial, rectus cervicis, and adductor mandibulae externus muscles) for 90 individual feeding trials. Four principal components were extracted, and plots of the factor scores were examined for patterns of dispersion among stages.

Two nested MANOVAs were then conducted on the factor scores for components 1 and 2 (Chatfield and Collins, '80). The MANOVA analyses corresponded directly to tests 1 and 2 of Figure 1 and utilize the same experimental design and F-ratio construction as the univariate ANOVAs described above.

Data analysis: techniques and variables

The motor pattern to the jaw muscles was quantified by measuring the pattern of electrical activity produced by six individual muscles during prey capture. Electromyographic (EMG) data were gathered from

TABLE 3. Two-way analysis of variance for 23 electromyographic (EMG) variables (longitudinal data set) measured from prey capture events (captures only) in Ambystoma tigrinum[1]

EMG variable	Metamorphic stage (2, 2)	Individual (1, 50)	Interaction (2, 50)
DMMAX	1.0	0.8	8.4**
DMTMAX	4.66	8.2*	4.4
DMDUR	6.3	2.7	4.7
EPON	5.0	0.1	4.0
EPMAX	1.6	1.4	4.3
EPTMAX	2.0	0.2	4.1
EPDUR	1.3	4.2	10.7**
RCON	0.1	2.8	19.7**
RCMAX	5.1	2.0	6.2*
RCTMAX	2.0	10.5*	4.9
RCDUR	2.0	0.1	15.4**
AMEON	0.3	0.6	4.4
AMEMAX	3.6	2.6	4.6
AMETMAX	1.1	20.8**	22.2**
AMEDUR	1.2	19.6**	17.0**
AMION	0.1	62.7**	19.8**
AMIMAX	0.1	1.7	5.6*
AMITMAX	30.3	1.0	0.2
AMIDUR	15.1	0.2	1.1
BH/SAR1ON	44.5	1.9	0.2
BH/SAR1MAX	5.3	1.4	5.1*
BH/SAR1TMAX	67.5	0.2	0.2
BH/SAR1DUR	2.0	6.7	15.7**

[1]Entries in the table are F-values: The degrees of freedom for each test are given below the effect. Variable descriptions are given in the text.
*P ≤ .01.
**P ≤ .001.

TABLE 4. Three-level nested analysis of variance for 23 electromyographic (EMG) variables (cross-sectional data set) measured from prey capture events (captures only) in Ambystoma tigrinum[1]

EMG variable	Among metamorphic stages (2, 8)	Among individuals within a metamorphic stage (8, 79)	Among feedings within individuals
DMMAX	18	40**	42
DMTMAX	3	6	91
DMDUR	15	22**	63
EPON	0	27**	73
EPMAX	0	29**	71
EPTMAX	44*	13*	43
EPDUR	21	22**	57
RCON	33	11	56
RCMAX	0	52**	48
RCTMAX	8	26**	66
RCDUR	41	33**	26
AMEON	59*	16**	25
AMEMAX	14	33**	53
AMETMAX	20	13	67
AMEDUR	9	26**	65
AMION	0	51**	49
AMIMAX	49*	12*	39
AMITMAX	13	23*	64
AMIDUR	21	5	74
BH/SAR1ON	51	21**	28
BH/SAR1MAX	62	23**	15
BH/SAR1TMAX	25*	0	75
BH/SAR1DUR	1	24*	75

[1]Values represent variance components (in %); Degrees of freedom for stage and individual effects are shown. Variable descriptions are given in the text.
*P = .01.
**P = .001.

each individual using techniques similar to those practiced previously (Lauder and Shaffer, '85; Wainwright and Lauder, '86). Bipolar stainless steel electrodes (0.051 mm in diameter) were implanted percutaneously into six muscles of the head. Bared electrode tips were approximately 0.5 mm long, and the electrode tips were about 1 mm apart in the muscle. Electrode pairs were glued together and were often sutured to the skin to minimize displacement. All six pairs of electrodes were glued together and sutured to the back of the animal.

EMG signals were amplified 5,000–10,000 times using Grass P511J preamplifiers and a bandpass of 100–3,000 Hz. The signals were recorded on a Bell and Howell 4020A FM tape recorder for future analysis.

Quantitative analyses of variation in muscle activity among individuals and among electrode placements within a muscle have been presented elsewhere (Lauder and Shaffer, '85; Shaffer and Lauder, '85): we have not found differences in electrode placement to be a significant source of variation in our data.

The motor pattern to six muscles was recorded simultaneously: the depressor mandibulae (DM), the epaxial muscles (EP), the rectus cervicis (RC), the adductor mandibulae internus and externus (AMI and AME), and the branchiohyoideus (BH, in larvae) and the subarcualis rectus one (SAR1, in metamorphosed animals). These muscles were chosen because 1) they cover all major functional components of the head (mouth opening, mouth closing, head elevation, hyoid depression, hyoid elevation); 2) they can be homologised across metamorphosis; and 3) they are present through ontogeny. Two other possible sets of muscles that might have been studied are the intrinsic tongue muscles and the muscles of the gill arches. We did not record from these muscles because the tongue muscles are acquired at metamorphosis and the gill arch muscles are lost at metamorphosis. These muscle groups are thus inappropriate for the study of motor pattern conservatism across a major morphological change.

Electromyographic data from each feeding were played from the FM tape into a 12-bit analog-to-digital converter sampling each channel at 1,274 Hz. These data were then stored on a hard disc for subsequent analysis. Each feeding resulted in a digital data file (data matrix) consisting of six columns, one for each muscle, and 5,096 rows corresponding to the 4 seconds that each analog feeding was sampled. This digital file was then run through a program that measured a total of 23 variables from the muscle activity pattern. Uniform settings for baseline noise and the detection of activity in a muscle were used for all analyses, thus avoiding any subjective component in the determination of muscle onset and offset times. This program used the onset of activity in the depressor mandibulae muscle as a reference from which to determine when other muscles started their electrical activity. As in previous research (Lauder and Shaffer, '85), the depressor mandibulae was chosen as a reference because of its consistent and high level of activity and its role as a major mouth opening muscle.

Twenty-three variables were measured by the computer program from the digital file of each feeding (variable abbreviations given here correspond to those used in the tables and throughout this paper). The onset of activity in each muscle (in ms) was measured relative to the onset of activity in the depressor mandibulae (five variables total): EPON, the onset of activity in the epaxial muscles; RCON, the onset of activity in the rectus cervicis muscle; AMEON, the onset of activity in the adductor mandibulae externus muscle; AMION, the onset of activity in the adductor mandibulae internus muscle; BH/SAR1ON, the onset of activity in the branchiohyoideus (in larvae) or the subarcualis rectus one muscle (in adults).

The duration of electrical activity in each muscle (in ms), the maximum (rectified) amplitude of activity in each muscle (in volts), and the time from the onset of activity to peak amplitude (in ms) were also measured for a total of three variables for each muscle: respectively, depressor mandibulae (DMDUR, DMMAX, DMTMAX); epaxial muscles (EPDUR, EPMAX, EPTMAX); rectus cervicis (RCDUR, RCMAX, RCTMAX); adductor mandibulae externus (AMEDUR, AMEMAX, AMETMAX); adductor mandibulae internus (AMIDUR, AMIMAX, AMITMAX); branchiohyoideus/subarcualis rectus one (BH/SAR1DUR, BH/SAR1MAX, BH/SAR1TMAX).

The goal of these measurements from each feeding was to characterize completely and quantitatively the motor pattern in these six

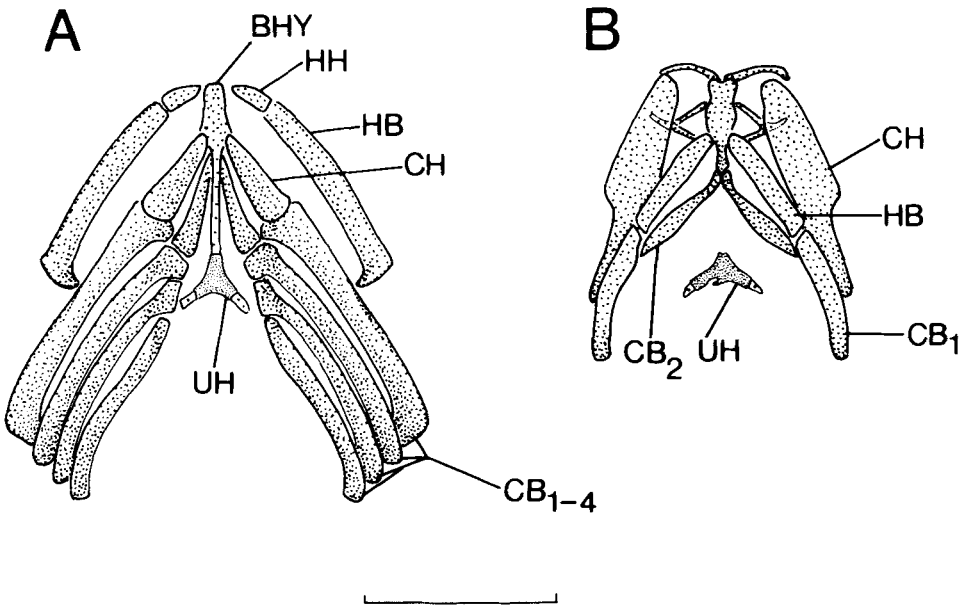

Fig. 5. *Ambystoma tigrinum*. Ventral views of the hyobranchial apparatus in a larval individual (**A**) and a metamorphosed individual (**B**). Note the loss of ceratobranchials 3 and 4 and the alterations in hyoid and basibranchial shape at metamorphosis. Scale bar = 1.0 cm. For abbreviations, see legend to Figure 4.

jaw muscles to allow us to determine precisely which aspects (if any) of the pattern change during ontogeny.

RESULTS

Morphology

General

The morphology of both larval and adult ambystomatid salamanders has been described in some detail in the literature (Bonebrake and Brandon, '71; Carroll and Holmes, '80; Edgeworth, '35; Jarvik, '63; Krogh and Tanner, '72; Larsen and Guthrie, '75; Latimer and Roofe, '64; Lauder and Shaffer, '85, '86; Luther, '14; Piatt, '38, '39, '40; Regal, '66; Reilly, '87). However, these works do not provide a complete description of ambystomatid morphology in the context of metamorphosis and functional analysis, and we present additional information here to aid in the interpretation of changes in the motor pattern during ontogeny.

Basic design features

As metamorphosis proceeds in *Ambystoma tigrinum*, striking changes occur in the external morphology of the head (Figs. 2, 3). The head becomes shallower and narrower in lateral view, and the external gills are reabsorbed. The eyes change from an anterolateral orientation located deeply in eye sockets in the skull to a more anterior orientation raised up off the surface of the head (Figs. 2, 3). As the external gills are being reabsorbed, the gular membrane, which has a free posterior margin in larvae, attaches and fuses to the skin of the body wall. In metamorphosed individuals there is no connection posteriorly from the buccal cavity to the exterior. In terms of feeding mechanisms, the process of aquatic feeding has been converted from a unidirectional flow system in which water enters the mouth and leaves via the gill slits and gular membrane to a bidirectional system in which water must both enter and leave the buccal cavity via the mouth (Lauder and Shaffer, '86).

Osteology and myology

Equally striking modifications occur in the musculoskeletal system of the head. Ceratobranchials 3 and 4 are lost completely at metamorphosis (Figs. 4, 5: CB) as is the median cartilaginous connection of the urohyal to the basihyal. Major changes in hyoid and branchial arch shape occur. The large lateral mass of ceratobranchials visible between the pectoral girdle and squamosal in

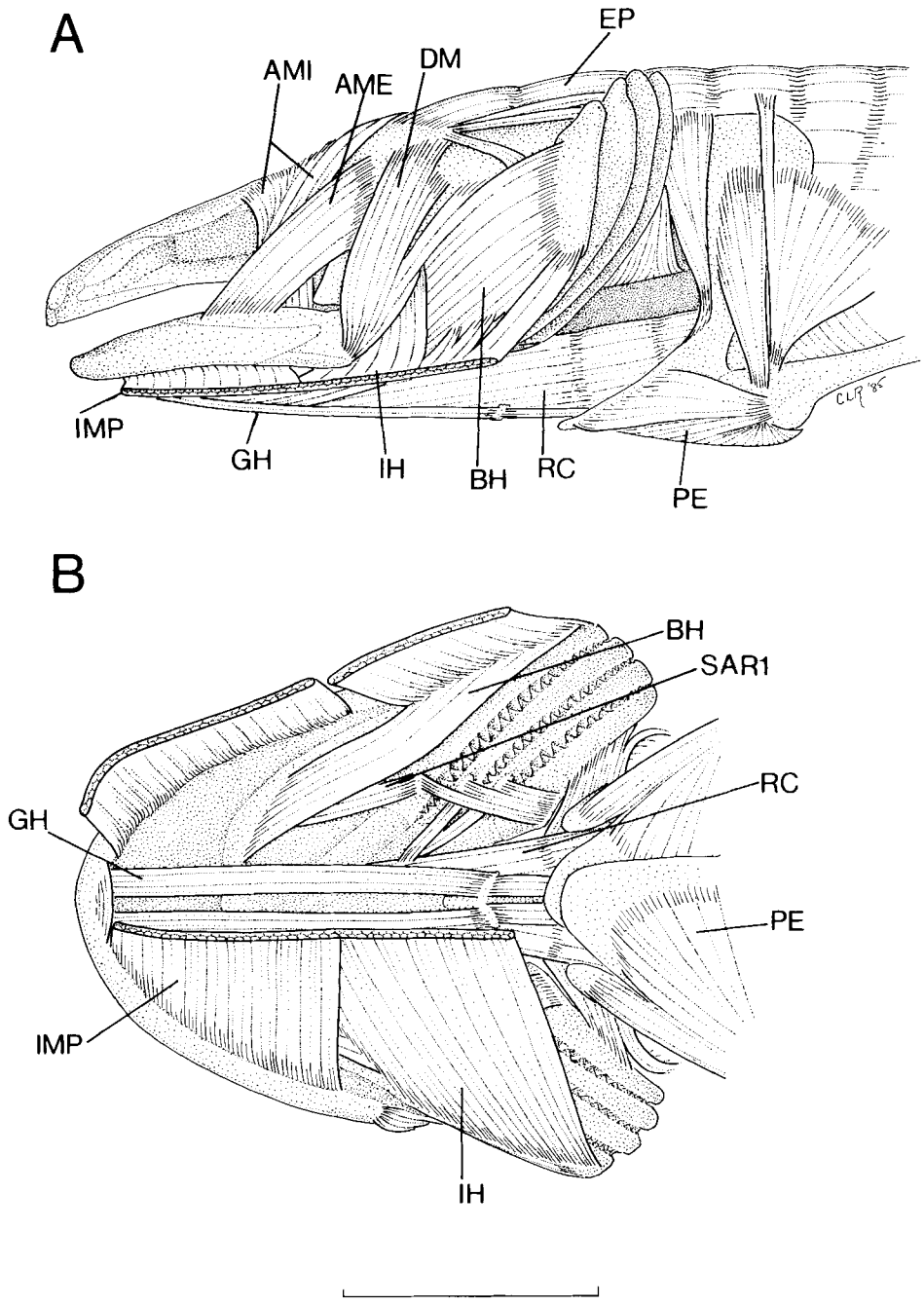

Fig. 6. *Ambystoma tigrinum*. Lateral (**A**) and ventral (**B**) views of the cranial musculature in a larval individual. The ventral throat muscles (interhyoideus and intermandibularis posterior) have been reflected on the left side to show the deeper musculature. Scale bar = 1.0 cm. For abbreviations, see legend to Figure 4.

TABLE 5. Loadings of eight variables on principal components 1–4 (PC1-4)[1]

EMG variable	Component loadings			
	PC1	PC2	PC3	PC4
DMMAX	−.76	−.05	.09	−.05
DMDUR	−.75	.38	.10	.09
AMEMAX	−.58	−.35	.12	.52
RCMAX	−.50	.38	−.39	−.34
EPDUR	.06	.70	.45	.04
RCDUR	.06	.65	−.57	−.05
AMEDUR	.26	.57	.06	.63
EPMAX	.01	.20	.75	−.38
Variance explained	22.6%	21.2%	16.3%	11.8%

[1]Factor scores for prey captures on components one and two are plotted in Figure 10. The variance explained by each of the first four components is given (in %) below each column. Variable descriptions are given in the text.

larvae (Fig. 4A) is greatly reduced in size after metamorphosis (Fig. 4B). The toothed margin of the gape increases, and a fleshy tongue pad forms in the buccal cavity dorsal to the basihyal.

The lines of action and major muscle masses change relatively little at metamorphosis (Figs. 6, 7). The muscles spanning the mandibular rami ventrally are the intermandibularis posterior and interhyoideus. This latter muscle lies in the posterior margin of the gular flap in larvae (Fig. 6: IH). The geniohyoideus connects the urohyal and mandibular symphysis. Both jaw adductor muscles maintain their relative positions through ontogeny, although the adductor mandibulae externus has a slightly more extensive insertion on the dentary after metamorphosis (Figs. 6, 7: AME).

Following metamorphosis the depressor mandibulae acquires a fan-shaped origin from the skull and looses the fibers that intermingle with the fibers of the branchiohyoideus. After metamorphosis the subarcualis rectus one muscle extends from the first ceratobranchial to the ceratohyal, and the larval branchiohyoideus muscle is lost.

The motor pattern

Summary statistics for the 23 variables in each of the two data sets are presented in Table 2. Representative myograms from one individual *Ambystoma tigrinum* at three ontogenetic stages are shown in Figure 8.

In the longitudinal data set none of the 23 variables show a significant effect of metamorphic stage (Table 3). In this experimental design our samples sizes are low, and the high F-values for four of the variables (AMITMAX, AMIDUR, BH/SAR1ON, and BH/SAR1TMAX) suggest that larger sample sizes might reveal these to be significant differences among metamorphic stages. Of these variables with large F-values, the means for the two adductor mandibulae internus variables are most different for the terrestrial stage (Table 2), indicating that no change in the motor pattern accompanied the morphological changes at metamorphosis in these variables. Terrestrial feedings exhibited substantially longer duration adductor mandibulae internus activity than did either of the two aquatic stages. The mean for the variable with the highest F-value, BH/SAR1TMAX (Table 2) is most different for larvae, indicating that the SAR1 muscle in metamorphosed individuals has a different time to maximum activity than the branchiohyoideus (BH) muscle in larvae. The two individuals studied proved to be different primarily in the adductor muscle variables, as shown by the significant individual effects in Table 3.

The cross-sectional data set (Table 4) has much greater statistical power to test for an effect of metamorphic stage. In the univariate analyses, four variables were found to have a significant proportion of variance associated with stage (Table 4). Three of these (EPTMAX, AMIMAX, and BH/SAR1TMAX) involve maximum voltages. Comparisons among the nonhomologous BH and SAR1 muscle variables showed that larvae are significantly different from other stages in the time to maximum activity (Table 4: BH/SAR1TMAX). Similarly, larvae are most different from other stages in the maximum amplitude of electrical activity (Table 4: BH/SAR1MAX), a variable having a high (but not significant) among-stage variance com-

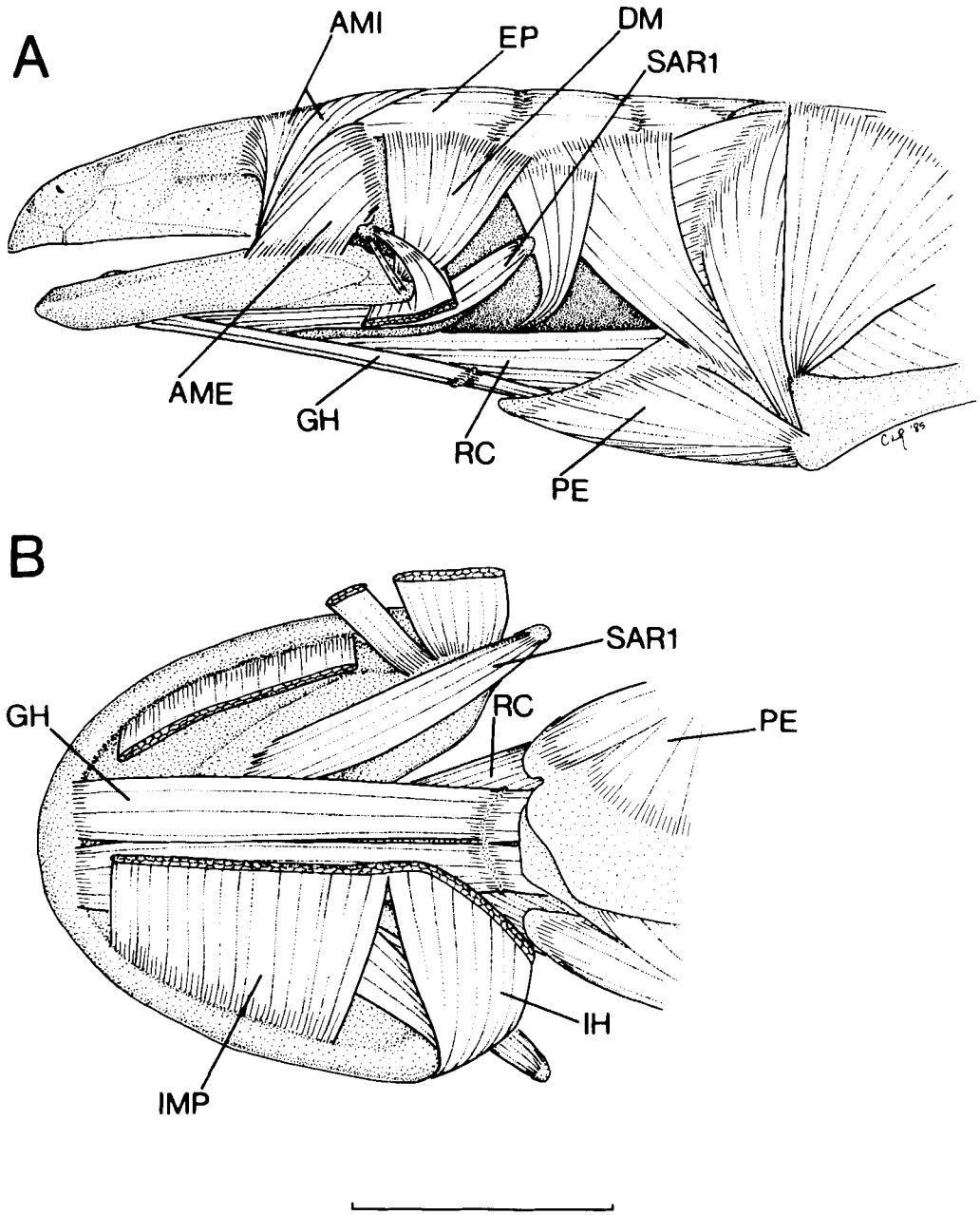

Fig. 7. *Ambystoma tigrinum*. Lateral (**A**) and ventral (**B**) views of the cranial musculature in a metamorphosed individual. The ventral throat muscles (interhyoideus and intermandibularis posterior) have been reflected on the left side to show the deeper musculature. Scale bar = 1.0 cm. For abbreviations, see legend to Figure 4.

ponent. These variables reflect changes in function between the nonhomologous branchiohyoideus and subarcualis rectus muscles during ontogeny. Two of the significant variables were most different in larvae, indicating a change in motor pattern in the time to maximal epaxial muscle activity and the amplitude of activity in the adductor

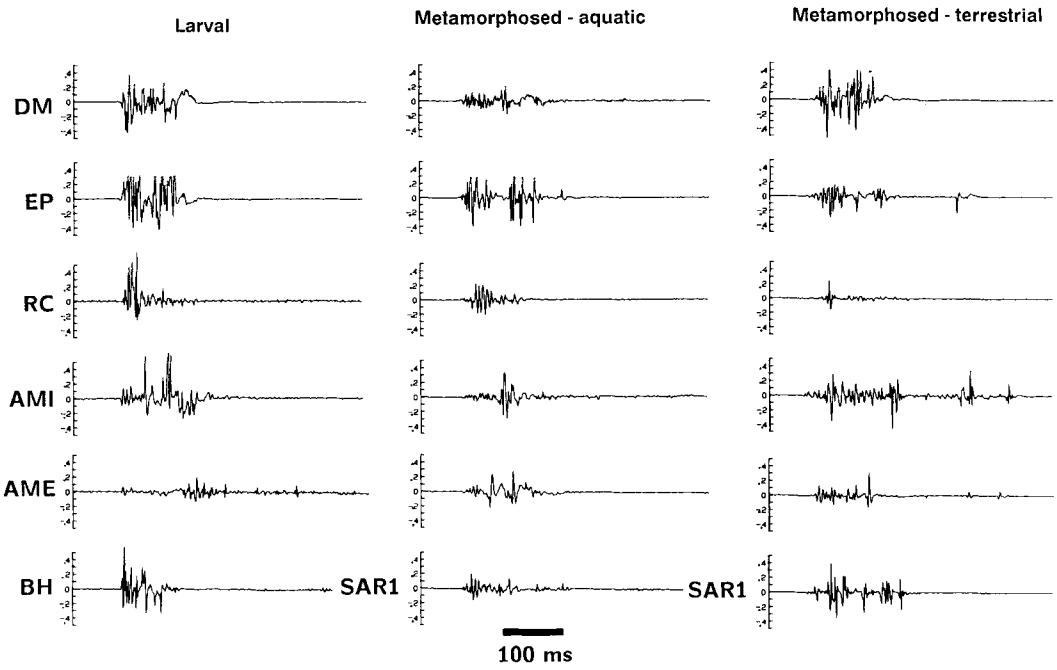

Fig. 8. Representative electromyographic (EMG) traces from one individual *Ambystoma tigrinum* to show the motor pattern at three ontogenetic stages. These data were converted from analog to digital as discussed in "Materials and Methods," and then this digital file was plotted. The vertical scale is in millivolts. The motor pattern was found not to be significantly different between the larval and the metamorphosed aquatic stages. For abbreviations, see legend to Figure 4.

mandibulae internus (Table 4: EPTMAX, AMIMAX). Among individuals, variance was significant in 18 out of the 23 total variables, indicating a high degree of heterogeneity among individuals within stages.

A summary bar diagram of the pattern of electrical activity in the six muscle groups for the longitudinal data set is shown in Figure 9. Variation in the offset of muscle activity is generally greater than in the onset, and onset times are very similar to each other. There is little consistent variation in the pattern of muscle duration with ontogenetic stage.

Multivariate dispersion of the motor pattern among the three ontogenetic stages is illustrated in Figure 10, and the loadings on components 1–4 are given in Table 5. Aquatic feedings (a comparison of larval and metamorphosed aquatic feedings) are quite similar to each other with broadly overlapping distributions on the first two principal components (which together account for 43.8% of the variance). Terrestrial feedings, however, have distinctly high values on both principal components 1 and 2 (Table 5: PC1 and PC2). An examination of the component loadings shows that high PC1 scores are determined by negative loadings of depressor mandibulae amplitude and duration and by negative loadings of the amplitude of activity in the rectus cervicis and adductor mandibulae externus. Principal component two reflects positive loadings of the duration of activity in the epaxial muscles, rectus cervicis, and the adductor mandibulae externus (Table 5).

The multivariate analysis of variance (MANOVA) comparing the mean factor scores of larval and metamorphosed aquatic feedings (test 1 in Fig. 1) on the first two principal components showed no significant differences ($P = .136$, DF = 2, 4). In contrast, the MANOVA comparing metamorphosed aquatic and terrestrial feedings (test 2, Fig. 1) was significant at $P = .014$ (DF = 2, 4). Discriminant function analysis correctly classified 84% of the larval feedings and only 62% of the metamorphosed aquatic feedings in the test 1 comparison, while 92% of both

metamorphosed aquatic and terrestrial feedings were correctly classified in the test 2 comparison.

DISCUSSION

Are motor patterns conserved during ontogeny? Our data show clearly that the mean motor pattern to the six jaw and hyoid muscles does not change with metamorphosis. Thus, the motor pattern is conserved across this major morphological transformation.

Four aspects of our data and analysis support this interpretation. (It is important to remember that comparing mean motor patterns across a morphological transformation is test 1 in the experimental plan of Fig. 1.) First, very few of the 23 variables in the univariate analyses (Tables 3, 4) show a statistically significant stage effect. Second, there is virtually no congruence between the longitudinal and cross-sectional analyses in the few variables that are significant. As the statistical power of our two-way ANOVA was limited by the fact that we were able to obtain longitudinal recordings from only two individuals, one might wish to examine all variables having large F-values, regardless of whether these are statistically significant or not. If there is congruence among the two analyses in variables with high F-values or variance components, that would suggest that there really is a change in the motor pattern that our statistical analysis failed to detect as significant. This is not the case for any of the homologous muscles (Tables 3, 4). Third, the level of intercorrelation among the variables studied was low, and there was little congruence among the two data sets in the structure of the correlation matrices. No variables emerged as being consistently highly correlated with each other. Fourth, the principal components analysis (Fig. 10) shows considerable overlap between larval and metamorphosed aquatic stages, and the MANOVA comparing these feedings was not significant. The MANOVA effectively tests the centroids of the two aquatic polygons in Figure 10 to determine if they are significantly different. As we have examined muscles that control all major aspects of head function that are present throughout ontogeny, we conclude that there is no evidence that the mean motor pattern changes at metamorphosis when the environment is held constant.

However, there is a multivariate *environmental* effect on the motor pattern (Fig. 10). Although univariate analyses did not show a modification of the motor pattern with the transition from water to land (test 2 in Fig. 1; Tables 3, 4), the multivariate analysis (of the cross-sectional data set) revealed that metamorphosed terrestrial individuals do have distinctive muscle activity patterns. Thus, the MANOVA shows that the centroids for the metamorphosed aquatic and terrestrial polygons in Figure 10 are significantly different. Terrestrial feedings tended to have longer activity durations in the jaw muscles and lower amplitude activity in the rectus cervicis and adductor mandibulae externus.

The differences between aquatic and terrestrial feedings could be due to changes in the hydrodynamic nature of the environment alone. Because air is less viscous and dense than water, one might expect that, *ceteris paribus,* feedings in air should be more rapid than in water because of the reduced resistance, and that peak bone excursions should be reached more quickly. Kinematic data indicate that in fact the converse is true (Shaffer and Lauder, '88), and the electromyographic data support this result. Terrestrial feedings involve longer-duration muscle activities (Tables 2, 5; Fig. 10) and there is no evidence of a purely physical effect on the motor pattern.

However, the change from aquatic to terrestrial motor patterns might well be due to sensory feedback. The change in motor pattern between metamorphosed aquatic and terrestrial individuals need not involve the use of different feeding circuits, but could represent modulation of a single feeding circuit based on environment. Terrestrial feeding involves tongue projection toward the prey (Shaffer and Lauder, '88), which is achieved with muscles acquired at metamorphosis. The tongue projection feeding behavior with the concomitant longer duration muscle activities could well be activated by sensory inputs from skin mechanoreceptors or by the sensation of gravity.

The lack of significant mean differences in the motor pattern between pre- and postmetamorphic *Ambystoma tigrinum* bears on previous hypotheses of motor pattern conservatism in vertebrates (e.g., Bramble and Wake, '85; Byrd, '85; Goslow, '85). There is no a priori reason why motor patterns should be conserved across major environmental and morphological changes in ontogeny and phylogeny. It is possible to adduce evidence both

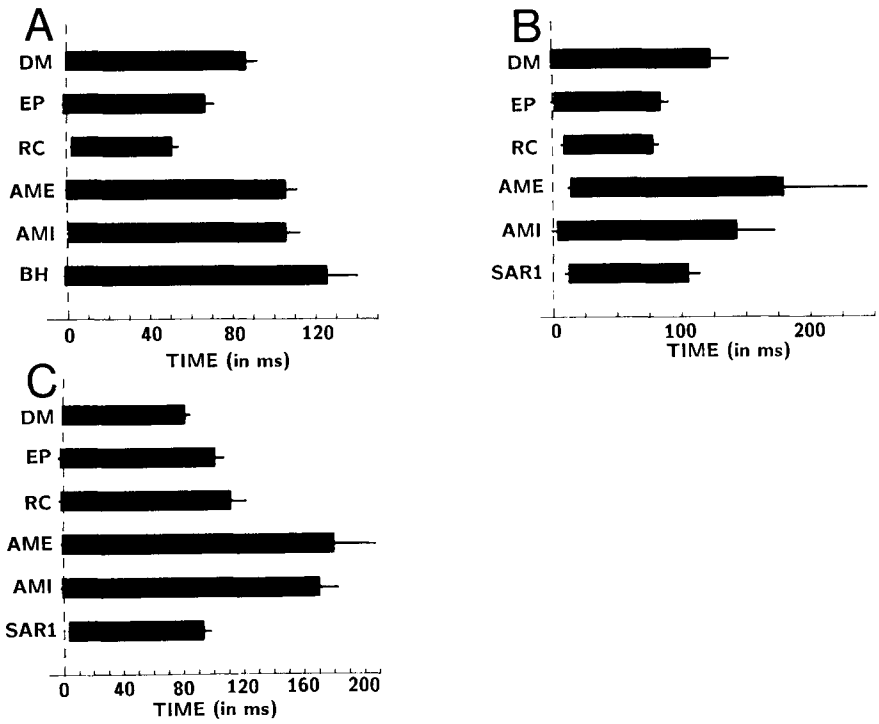

Fig. 9. Bar diagrams summarizing the EMG pattern in larval (**A**), metamorphosed aquatic (**B**), and terrestrial (**C**) stages. The start and end of each bar indicate the mean onset and offset of activity for each muscle. The thin lines extending horizontally from the bars represent 1 standard deviation of the mean onset and offset times. Note that this figure presents only an overall summary and confounds variation due to individuals and feedings. This figure is thus useful for an overall view of the pattern of muscle duration during ontogeny but cannot be used for statistical comparisons of the ontogenetic stages (see Tables 3 and 4 for these results). For abbreviations, see legend to Figure 4.

in support of the view that the motor pattern should not change as well as in support of the idea that morphological and environmental shifts should be accompanied by significant changes in the average motor pattern. In support of conservatism, many aspects of central nervous system structure are similar across vertebrate lineages (e.g., Northcutt, '77, '78; Sarnat and Netsky, '74), and such similarities may reflect a corresponding conservatism of motor output to peripheral musculature. In invertebrates, neuronal circuitry may be conserved across species, even when peripheral muscles have been lost (Arbas, '83; Dumont and Robertson, '86). In vertebrates, key proposed examples of motor pattern conservatism have included analyses of limb muscle motor patterns in chicks (Bekoff, '76; Bekoff and Kauer, '84) and the development of mastication from suckling behavior in mammals (Byrd, '85; Herring, '85; Moyers, '73; Sessle, '76). Analyses of trigeminal motor neuron populations in two vertebrates, frogs and lampreys (Alley and Barnes, '83; Barnes and Alley, '83; Homma, '78), have shown that motoneuron populations tend to be conserved across morphological changes occurring in metamorphosis. (Note that these last studies actually show that motoneuron morphology and relative position tends to be conserved, not the motor *output* from the neural circuits.)

On the other hand, motor patterns are the product of neural *circuitry* that may change both during ontogeny and between species (Bush and Clarac, '85; Ewert, '76; Laming, '81; Levine and Truman, '82; Technau and Heisenberg, '82). Even quite similar simple behaviors may be produced by very different neural circuits (Dumont and Robertson, '86;

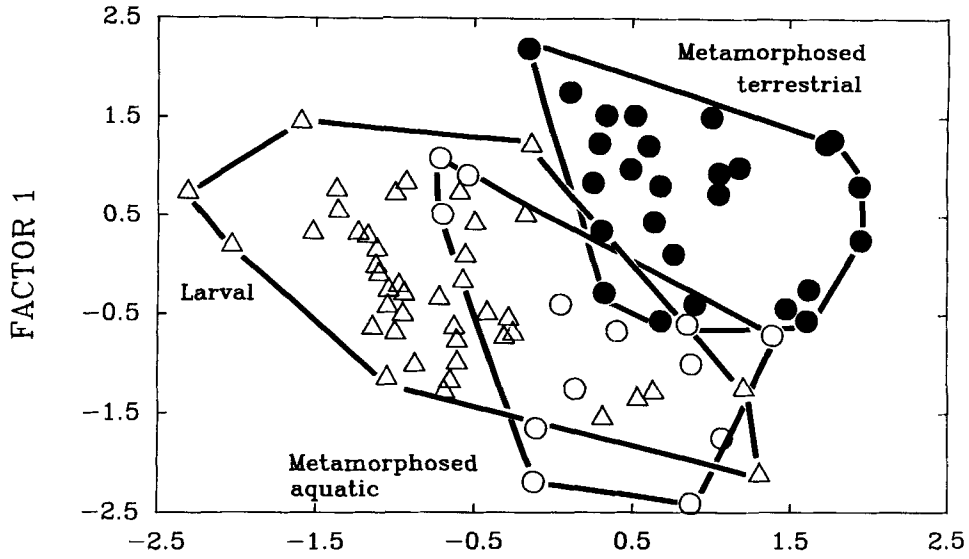

Fig. 10. Principal components analysis on 90 feedings and eight variables (see text for details) to illustrate the multivariate relationship among the motor patterns for the three ontogenetic stages. Each point represents one feeding. Larval and metamorphosed aquatic feedings overlap broadly on principal components one and two (and the polygon centroids are not significantly different, MANOVA $P = .136$), but the polygon centroid for terrestrial feedings is significantly different from that for metamorphosed aquatic prey captures (MANOVA $P = .014$).

Lauder, '86), and it is clear that new functional specializations may indeed be correlated with changes in the motor pattern. The work of Graf and Baker ('83, '85a,b) has shown elegantly that changes in neuronal circuitry accompany metamorphosis in flatfish (*Pseudopleuronectes*). During ontogeny, behavioral changes also may be accompanied by modification of motor patterns (Bentley and Hoy, '70), and the anatomical basis of motor systems may vary considerably from species to species. Also, metamorphosis may involve alterations in neuronal populations and reorganization of dendritic fields (Casaday and Camhi, '76; Taylor and Truman, '74; Truman and Reiss, '76; Truman et al., '85), implying that neuronal circuits have changed. Fetcho ('86a,b) has shown that the organization of motoneuron pools innervating axial musculature in aquatic vertebrates such as fishes and nontransforming salamanders is very different from that of terrestrial vertebrates. Thus, the central neuronal organization containing the circuits that produce motor patterns to the axial muscles in vertebrates is not phylogenetically conservative.

Mechanisms of behavioral transformation

The results presented above on motor patterns in *Ambystoma tigrinum* now need to be considered in light of the three-part research program for the analysis of behavioral transformation outlined in the opening section.

First, what are the behavioral changes (if any) that occur during ontogeny for which we would like to determine a mechanistic basis? Mean changes in feeding behavior or performance have been documented for both test 1 and test 2 comparisons (Fig. 1). Lauder and Shaffer ('86) showed that there was a significant decrease in performance by metamorphosed *Ambystoma tigrinum* feeding in the water when compared with larval performance. Reilly and Lauder ('88a) documented similar significant performance decreases in *Notophthalmus viridescens* at metamorphosis. In addition, Shaffer and Lauder ('88) have documented mean changes in feeding kinematics by *Ambystoma tigrinum* between terrestrial and aquatic metamorphosed individuals. Thus, there are significant behavioral changes for both com-

parisons outlined in Figure 1.

Second, do correlated changes occur in mean morphology or physiology during ontogeny that *might* serve to explain any significant mean behavioral differences? The results of this study show that the lines of action and basic design of the musculoskeletal system remain relatively unchanged throughout ontogeny. However, there is a major change at metamorphosis in several features of the head: the gill slits close, and the head changes from a unidirectional to a bidirectional feeding system (Figs. 2, 3). As documented above, there is no change in the mean motor pattern at metamorphosis (Fig. 1: test 1; Tables 3, 4), but there is a significant change in the mean motor pattern used for feeding in the two environments (Fig. 1: test 2; Tables 3, 4).

Third, is there any evidence that the correlated changes in form or function *account for* the observed changes in behavior in each of the two tests? For test 1, we observed no significant change in the motor pattern nor any major alteration in musculoskeletal design. Both of these factors can thus be eliminated as possible causal bases of ontogenetic alterations in feeding performance. We therefore hypothesize that it is the change in functional design of the head (from unidirectional to bidirectional) that causes the decrease in feeding performance during ontogeny. Lauder and Shaffer ('86) showed that the metamorphosis of the feeding mechanism from unidirectional to bidirectional flow has a significant effect on the pattern of pressure generated in the mouth cavity of tiger salamander. Lauder and Reilly ('88) conducted a series of *manipulative* experiments that examined buccal pressures and feeding performance in axolotls (*Ambystoma mexicanum*) in which the gill openings had been sutured closed. These experiments tested the hypothesis that experimental closure of the gill slits alone (with no change to the musculoskeletal system) would produce a significant decrease in buccal pressure and feeding performance compared to control animals. This indeed was the result. Thus, the hypothesis that the causal basis for ontogenetic changes in behavior/performance is the change in gross design of the skull and not differences in motor pattern is corroborated.

For test 2, there is a documented behavioral transformation (Shaffer and Lauder, '88), no change in morphology (Fig. 1), and a significant change in the motor pattern (Tables 3, 4). Do the changes in the motor pattern account for the alteration in behavior? Although no direct (manipulative) experimental evidence exists on this point, the biomechanics of the feeding system strongly indicate that the observed changes in motor pattern in test 2 do account for the observed behavioral transformation. One of the key distinguishing features of terrestrial feeding kinematics in *Ambystoma tigrinum* is the longer duration in nearly all kinematic variables measured: terrestrial feedings take longer to complete than aquatic feedings (Shaffer and Lauder, '88). The motor pattern shows a similar trend, with terrestrial feedings exhibiting longer-duration muscle activities (Fig. 10; Table 5: high loadings of the duration of activity in the epaxialis, rectus cervicis, and adductor mandibulae externus). Thus, as one might expect biomechanically, longer feedings are associated with longer durations of muscle activity.

CONCLUSIONS

The issue of conservative muscle activity patterns is an important one in functional morphology because it directly affects how one explains behavioral and functional transformations in animals. Is behavioral transformation a function of alterations in the motor pattern, peripheral morphology, or both? The data presented here and previous research on vertebrate feeding systems (Byrd, '85; Herring, '85; Lauder, '83, '85; Wainwright and Lauder, '86) indicate that motor patterns are conserved during the origin, either in ontogeny or phylogeny, of behavioral novelties. How general might these results be? Are novel behaviors generally produced by modifications of the musculoskeletal system or might motor patterns be changed also (Lauder, '88)? There is still far too little quantitative evidence to generalize on this issue, but it is possible to suggest several future research directions that will help resolve the question. First, more case studies explicitly and quantitatively comparing motor patterns across major morphological and environmental changes are needed. Are environmental changes necessarily associated with alterations in the motor pattern? Second, comparative phylogenetic analyses are needed on motor pattern transformation in relation to changes in morphology and behavior. The mapping of behavioral, morphological, and motor pat-

tern characters onto a phylogeny will enable us to ascertain quantitatively the degree of concordance in transformation of these three types of characters. Third, comparative analyses of differing types of behaviors are needed to assess the generality of results on feeding systems. Ballistic behaviors, such as most aquatic feeding by lower vertebrates, may be associated with conserved motor patterns, whereas behaviors requiring significant sensory feedback during their execution may not show conserved motor patterns. By integrating results from ontogenetic and phylogenetic analyses, perhaps a general picture of the relationship between behavior and its morphological and neural bases will emerge.

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