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Aquatic Prey Capture in Ambystomatid Salamanders: Patterns of Variation in Muscle Activity

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ABSTRACT Functional morphologists commonly study feeding behavior in vertebrates by recording electrical activity from head muscles during unrestrained prey capture. Rarely are experiments designed to permit a partitioning of variation in muscle electrical activity patterns. Analysis of muscle activity during aquatic prey capture in two morphologically distinct species of salamanders, *Ambystoma dumerilii* and *A. mexicanum*, is conducted to assess variation at four levels: between species, among individuals within species, among experiments conducted on different days, and among feedings. The results show that 1) mean correlations among the 11 electromyographic variables measured for each feeding are low and vary considerably among individuals, 2) many of the variables show significant differences among experimental days, 3) only one variable, the difference in timing between the depressor mandibulae and sternohyoideus muscles, showed significant variation between species, and 4) seven of the 11 variables showed significant variation among individuals within species. Overall, the variation between feedings (trials) was high, and there was some variation between days on which the experiments were conducted. Neither electrode position within the muscle nor satiation contributed to the high trial variance. The results suggest that functional analyses of feeding behavior should include an assessment of variation due to individuals, days, and trials, because the amount of variation at these levels may render differences between species nonsignificant.

Research on the functional morphology of vertebrates over the last 15 years has increasingly utilized electromyography as a tool to study muscle function. Many investigators have measured the durations of muscle electrical activity to discover how animals function and to clarify the relationship between form and function in the vertebrate body (e.g., Jenkins and Weijs, '79; Gorniak et al., '82; Gans, '74; Gans et al., '78; Herring, '80; Liem, '73). Electromyography has proved particularly useful in testing hypotheses of muscle function and in comparing neuromuscular patterns associated with bone movement in different vertebrates (e.g., Jenkins and Goslow, '83; Weijs and Dantuma, '75). While much of this research both on feeding mechanisms and on locomotion has emphasized how a particular species functions, researchers have also investigated differences between species in functional design and have based conclusions regarding evolutionary patterns and processes on differences in muscle activity patterns between species. Liem ('78, '80), for example, has proposed that taxa believed to be trophic specialists on the basis of morphological studies, may in fact have extremely diverse feeding repertoires—a hypothesis based in part on the discovery of considerable variation within certain cichlid fish species in muscle activity patterns.

Central to any interpretation of experimental data is an assessment of the amount of variation associated with making the measurements (e.g., the experimental error) and the day-to-day differences in data from the same experimental animal. In addition, for studies in which differences between species are of interest, it is necessary to assess the amount of variation in the variables of inter-

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est due to differences between individuals of the *same* species. For the most part, vertebrate functional morphologists have not attempted to determine the patterns of variation associated with their experimental techniques (for an exception, see Gans and Gorniak, '80, on electromyography), and have commonly lumped data from different individuals into a single mean, without reporting the variation due to individuals within the species.

On a more general level, data on interindividual variation in behavioral and functional characteristics of animals are scare, and yet intrapopulational variation is the basic unit of the evolutionary process. If we are to understand how variation within populations relates to supraspecific (phylogenetic) variation in structure and function, a key problem for morphologists, then an essential first step is to determine if individuals differ in their behavior. Variation among species may then be compared using the level of intraspecific variation as an appropriate yardstick of comparison. Previous work by ethologists on intraspecific variation in behavior has generally focused on the degree of stereotypy in "fixed action patterns" (see Dane et al., '59; Barlow, '77; Schleidt, '74), and has not included quantitative analyses of intraspecific and experimental variation or used components of behavior of interest to functional morphologists.

This paper builds on our analysis of prey capture kinematics in ambystomatid salamanders (Shaffer and Lauder, '85). Our goals here are 1) to provide an analysis of patterns of variation in muscle electrical activity patterns during prey capture in aquatic ambystomatid salamanders; 2) to partition statistically the variance in muscle electrical activity patterns into four levels: variation between species, variation among individuals within species, variation among experimental days on the same individuals, and variation among prey capture events (trials); 3) to assess the significance of the results for both experimental and conceptual issues in vertebrate functional morphology; and 4) to consider the evolutionary significance of these patterns of muscle variation in the light of previous data on skull bone movement and evolutionary patterns in lower vertebrates.

MATERIALS AND METHODS

Specimens

Two species were chosen for the analysis of intra- and interspecific variation in muscle activity patterns during feeding: *Ambystoma mexicanum* (eight individuals) and *A. dumerilii* (two individuals). *A. mexicanum* has an intermediate sized head compared to other nontransforming ambystomatids and in general represents an extension of the normal larval growth allometries for closely related Mexican ambystomatids (Shaffer, '84b). *A. dumerilii* is morphologically and genetically distinct from *A. mexicanum* and has the largest and broadest head of any nontransforming ambystomatid (Shaffer, '84a,b). *A. dumerilii* was chosen to maximize among-species differences in head morphology. Because of the morphological differences between these two species, they were formerly placed in two genera.

All animals were sexually mature adults chosen to minimize within-species variance in overall size (mean snout–vent length of *Ambystoma mexicanum* = 9.2 cm, N = 8; *A. dumerilii* = 13.9 cm, N = 2). *A. dumerilii* were collected in the field (see Shaffer, '84a, for localities), and the *A. mexicanum* were obtained from the Indiana University axolotl colony. Because of the low variance in body size among *A. mexicanum*, we did not statistically remove size from analyses.

Animals were maintained in individual 35-liter aquaria at 17° C and were fed a diet of live earthworms (*Lumbricus*) of varying sizes prior to the recording sessions. During an experiment, feedings were conducted by dropping freshly cut pieces of earthworm (1 cm in length) near the mouth. For several individuals, feedings were conducted on two successive days (3 days for one individual) to assess between-day variance in the 11 EMG variables. All experiments were conducted in the same enclosures.

Electromyography

Six muscles were recorded from each individual using only a slight modification to a previously described technique (Lauder, '83a,b). Bipolar fine wire (steel alloy, 0.051 mm in diameter) electrodes were implanted percutaneously into the center of six cranial muscles while the animal was under anesthesia (tricaine methane sulfonate). Bared electrode tips were 0.5 mm long, and tip length was constant between muscles and experiments. Electrodes from each muscle were glued into a bundle that was sutured to the skin behind the head. Individual pairs of electrodes were often sutured to the skin to stabilize the electrode wire and reduce the chance that electrodes would be pulled out

between experimental days. The six muscles recorded were (abbreviations used in the tables are given in parentheses): (1) the depressor mandibulae (DM), (2) sternohyoideus (SH), (3) adductor mandibulae externus (AMe), (4) adductor mandibulae internus (AMi), (5) the epaxial muscles (EP), and (6) the hypaxial muscles just posterior to the pectoral girdle (HY).

Electromyograms were recorded on a Bell and Howell 4020A tape recorder and played back to a Gould 260 chart recorder at a speed 15 times slower than that used for recording (37.5 cm/sec). The chart speed was 125 mm per second. The amplifier bandwidth (Grass P511J) was 100–3,000 Hz, and electromyograms were amplified 5000 times.

Chart records of electromyograms were placed on a digitizer (50 μm accuracy) and 11 variables digitized for each feeding: the total duration of electrical activity in each muscle (six muscle variables—abbreviations given above), and the onset of activity in five of the muscles relative to the start of activity in the depressor mandibulae (five variables: DM-SH, DM-EP, DM-AMi, DM-HY, DM-AMe). Examples of these two types of measurements are shown in Figure 1A: the relative onset time (RON) of the depressor mandibulae and hypaxial muscles, and the duration of activity (DUR) in the external adductor. The depressor mandibulae was used as the reference muscle because (1) it has a well-defined mechanical action and is a one joint muscle, (2) it is the major mandibular abductor in the head, and (3) use of this muscle allows comparisons between EMG data for salamanders and that for fishes in which the dominant mouth opening muscle is also commonly used as a reference.

In some cases, decisions about the presence/absence of activity in a muscle were aided by signal averaging of activity from several feedings. For example, the adductor muscles commonly showed a reduced level of activity between two bursts (see Fig. 1). A decision to treat this activity as one burst (for the purposes of measuring the duration variables, not for functional analysis) was made by signal-averaging 20 bursts using the onset of activity in the depressor mandibulae as the trigger. This analyis showed that significant activity was present between the two larger bursts and that no consistent point could be found to divide the activity in the adductor muscles into two bursts. Signal-averaging was accomplished by playing the electromyograms off the tape recorder into a DAS A/D converter (sample rate was 3388 Hz per channel) using an IBM XT computer (768 K memory). These data were then averaged using a variety of bin widths (1–5 ms). Analyses of the spike patterns and spike amplitudes within bursts from 20 feedings, following the method of analysis used by Gorniak et al. ('82), were also averaged.

To evaluate the effect of different electrode positions within a muscle on the muscle activity variables, two individuals were chosen and three electrodes were placed into the internal adductor muscle. This muscle was chosen because of its relatively large size and the relatively large amount of variation in the electromyogram between feedings. Recordings were made from these individuals on 4 days, and the duration of adductor muscle activity as indicated by each electrode was measured, as was the timing of activity in each electrode relative to the depressor mandibulae. The ANOVA design was unbalanced, as not all electrodes remained in the muscle during the 2-week experimental period.

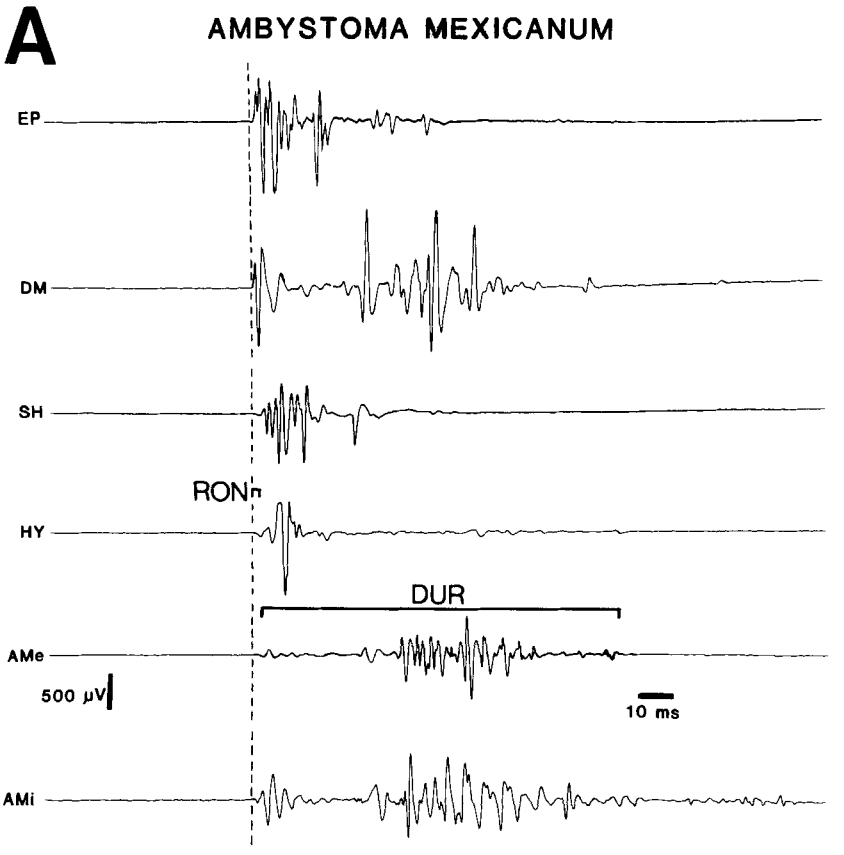
Electrode insertions were judged to be reliable and the signals free of crosstalk (Mangun et al., '81) by (1) direct insertion of the electrodes into the muscle belly through the skin, (2) the different patterns of activity often shown by adjacent muscles, (3) non-overlapping patterns of activity in the muscles during chewing following initial capture, and (4) the consistent differences shown by electrodes in different muscles within an individual in contrast to the extreme similarity in activity patterns shown by multiple electrodes within a muscle.

Pressure recordings

Measurements of the pressure within the buccal cavity of *Ambystoma mexicanum* obtained simultaneously with EMG recordings of head muscle activity were used to ascertain the functional significance of variations in muscle activity (Lauder, '83c). A Millar PC-350 catheter-tipped pressure transducer was inserted inside a short length (5 cm) of flexible polyethylene tubing inserted surgically into the buccal cavity. The effective bandwidth of the recordings was 0–1,000 Hz, although the pressure transducers are capable of accurately recording frequencies up to 10,000 Hz.

Statistical analyses

The experimental design used was a one-way nested analysis of variance (ANOVA) for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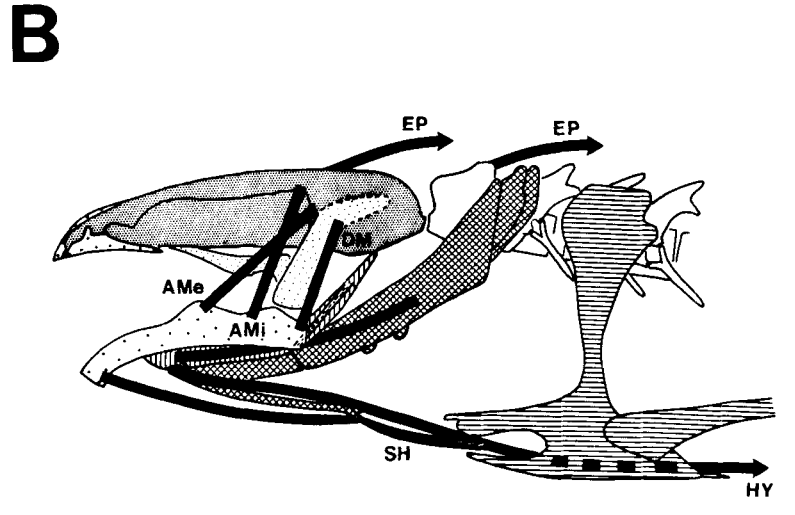
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each variable, where the levels of the analysis are as follows: among species (N = 2), among individuals within species (N = 10), among days within individuals (N = 19), and among trials within days (N = 206). The following statistical model was then used: Y(ijkl) = a(i) + b(ij) + c(ijk) + d(ijkl), where Y(ijkl) is the observed value of each variable, a(i) the difference among species means, b(ij) the difference among individuals within species, c(ijk) the difference among days within individuals, and d(ijkl) the residual error variance. Since *Ambystoma dumerilii* was chosen as a large-headed species to contrast to *A. mexicanum*, the species effect is fixed while the remaining effects are random; this is a mixed-model ANOVA (Sokal and Rohlf, '81). Variance components were estimated using the SAS ('82) Nested procedure. Although the design is somewhat unbalanced at the individuals-within-species level, the F-tests for significant effects were virtually identical to those obtained when the Satterthwaite correction for unbalanced designs (Sokal and Rohlf, '81) was compared with the usual variance ratio for nested designs.

To examine correlations among characters for *Ambystoma mexicanum*, we computed a Pearson product-moment correlation matrix of all 11 variables for each salamander (data from different days were pooled). These matrices were then averaged, cell by cell, for a mean correlation across individuals. Within-cell homogeneity of correlation coefficients was tested by using the z-transformation and comparing the weighted mean value to chi-square with seven degrees of freedom.

Multiple regressions were conducted to determine which of the electromyographic variables were the best predictors of buccal cavity pressure. The magnitude of the negative buccal pressure and the time to peak negative pressure were used as dependent variables in separate analyses. The eleven EMG variables served as independent variables.

RESULTS

Anatomy

Figure 1B shows a schematic diagram of the head of *Ambystoma mexicanum* and the lines of action and relationships of the muscles studied. The morphology of the head and the mechanics of feeding in aquatic ambystomatids will be considered in detail elsewhere, and a description of these head muscles and their development is given by Piatt ('38). The two adductor mandibulae muscles function to adduct the lower jaw and both insert on the mandible. Both muscles originate on the dorsal and lateral surfaces of the skull. The adductor mandibulae internus (Fig. 1B: AMi) possesses a more vertical fiber orientation and inserts nearer the jaw joint than the external adductor. The adductor mandibulae externus has obliquely inclined fibers and lies superficial to the internal adductor.

The depressor mandibulae and sternohyoideus act, respectively, to open the mouth and expand the volume inside the mouth cavity by depressing the hyoid. The depressor mandibulae arises from the quadrate and fascia and skin posterior to the quadrate and inserts posteroventral to the quadratomandibular articulation. Because of the location of its insertion on the mandible, contraction of the depressor mandibulae will act to depress the lower jaw. The sternohyoideus or rectus cervicis also mediates mandibular depression through its attachment to the mandible by the mandibulohyoid ligament (Fig. 1B). This ligament transmits posteroventral hyoid movement to the mandible. Since the tensile forces exerted by this ligament on the mandible are applied in a posterodorsal direction to the posterior aspect of the mandible, the tip of the lower jaw will move ventrally when the hyoid is depressed.

The epaxial muscles elevate the head on the vertebral column, and have a broad insertion on the dorsal aspect of the skull and anterior vertebrae (Fig. 1B: EP). The hypaxial musculature serves to stabilize the pectoral girdle. The division of the hypaxialis of interest to this investigation passes dorsal to the pectoral girdle (Fig. 1B: HY) and extends anteriorly to join the sternohyoideus (rectus cervicis).

Fig. 1. A. Pattern of muscle activity during feeding on a piece of earthworm by *Ambystoma mexicanum*. The six muscles shown were recorded simultaneously; the dashed line represents onset of activity in the depressor mandibulae muscle. B. Schematic diagram of the major functional units in the head of *Ambystoma* used for these experiments, and the lines of action of the head muscles used for electromyographic recording. Code: white, vertebral column; black, muscle; short dashes, ligaments; horizontal lines, pectoral apparatus; cross-hatching, hyoid apparatus; large stipple, jaw apparatus; dense, fine stipple, cranium; fine stipple, suspensory apparatus. AMe, adductor mandibulae externus; AMi, adductor mandibulae internus; DM, depressor mandibulae; DUR, duration of muscle activity; EP, epaxial muscles, HY, hypaxial muscles; RON, relative onset of muscle activity; SH, sternohyoideus muscle (rectus cervicis).

Day-to-day variation

There is considerable heterogeneity among variables in day-to-day variation in activity patterns. Table 1 shows the results of comparisons of the 11 electromyographic variables between experimental days. Of 82 possible comparisons, 15, about a fifth, showed statistically significant variation between days at the $P < .01$ level. (Because of the multiple comparisons performed, we used the .01 level for significance). This variation is not a function of individual differences since all individuals showed day-to-day variation in some characters. However, certain variables vary more than others and show relatively greater variation between experimental days. This overall pattern is clear from the among-days component of the nested ANOVA (Table 2), which summarizes the proportion of total variance in each variable attributable to between-day differences in individuals. Five variables (EP, AMi, HY, DM, and DM-AMe) have relatively large fractions of their total variance attributable to differences among days, while the remaining variables show little day-to-day variation.

Character correlations

The mean Pearson product-moment correlations and their ranges for eight individual *Ambystoma mexicanum* are shown in Table 3. There are no large positive or negative mean correlations among characters. Of 55 pairwise correlations, the extreme mean values are .48 (AMe and AMi) and -.31 (HY, DM-HY), with 80% of the correlations below .30. However the *range* of correlations among the eight individuals is extreme, frequently

TABLE 1. One-way ANOVA comparing 11 electromyographic variables of the feeding mechanism for different individuals between experimental days in Ambystoma mexicanum *and* A. dumerilii[1]

	A. mexicanum (individuals)							*A. dumerilii*
Variable	1	2	3	4[2]	5	6	7	1
DM-SH	NS	.01	NS	.01	NS	NS	---	---
DM-EP	NS	NS	.003	NS	NS	.002	NS	NS
DM-AMi	.0002	NS	NS	NS	NS	.0001	.001	---
DM-HY	NS	NS	NS	NS	NS	NS	NS	NS
DM-AMe	NS	.0001	NS	NS	NS	.003	NS	NS
DM	NS	NS	NS	NS	.007	NS	NS	NS
SH	NS	NS	NS	NS	NS	NS	---	---
AMe	NS	NS	NS	NS	NS	NS	NS	NS
EP	NS	.003	NS	NS	NS	NS	NS	.01
AMi	NS	.003	NS	.0001	NS	NS	NS	---
HY	NS	NS	NS	.0002	NS	NS	NS	NS

[1]A significant difference means that the variable shows day-by-day variance in an individual.
NS, not significant at 0.01 level; ---, no between-day data for this variable.
[2]Comparison among three experimental days.

TABLE 2. Variance components for the 11 electromyographic variables from the feeding mechanism of A. mexicanum *and* A. dumerilii[1]

	Variance component (%)			
Electromyographic variable	Among species	Among individuals within species	Among days within individuals	Among trials within days (error)
DM-SH	51**	13	6**	30
DM-EP	9	23**	5	63
DM-AMi	7	4	2	87
DM-HY	0	30**	1	69
DM-AMe	12	19**	12**	57
DM	0	40*	15**	45
SH	25	33**	2	40
AMe	0	65**	2	33
EP	0	12	24**	64
AMi	0	27	24**	49
HY	0	29*	14**	57

[1]No symbol, not significant at 0.05 level.
*Significant at 0.05 level.
**Significant at 0.01 level.

TABLE 3. Mean correlations (r) of 11 electromyographic variables for eight Ambystoma mexicanum[1]

	DM-SH	DM-EP	DM-AMi	DM-HY	DM-AMe	DM	SH	AMe	EP	AMi	HY
DM-SH	1	.19	.37	.21	.12	.13**	−.04	.08	.21	.06	.18
DM-EP	.54, −.38	1	.37**	.37***	.33*	.05*	.19	−.16*	.02***	.11	−.03*
DM-AMi	.61, −.08	.74, −.29	1	.06*	.42***	.07*	−.04**	−.11	.14	−.07	.05
DM-HY	.62, −.37	.98, −.12	.53, −.38	1	.12***	.06	−.01*	−.05	.08***	.02	−.31
DM-AMe	.65, −.15	.80, −.10	.79, .04	.93, −.30	1	−.05	−.07	−.15**	−.10	.07	−.11**
DM	.46, −.40	.39, −.38	.62, −.32	.46, −.20	.15, −.23	1	.28	.31	.31	.28	.14
SH	.20, −.27	.70, −.23	.54, −.48	.73, −.45	.49, −.36	.54, −.01	1	.13*	.20	.27	.27*
AMe	.31, −.20	.36, −.54	.31, −.54	.40, −.47	.27, −.75	.58, −.18	.63, −.24	1	.32***	.48***	.29
EP	.53, −.12	.51, −.55	.53, −.19	.71, −.34	.14, −.36	.49, −.19	.77, −.14	.88, −.03	1	.23***	.23
AMi	.28, −.20	.40, −.19	.24, −.37	.30, −.12	.47, −.24	.52, −.19	.68, −.12	.90, .08	.82, −.15	1	.22**
HY	.55, −.10	.41, −.59	.29, −.32	.05, −.59	.45, −.57	.49, −.22	.58, −.35	.64, −.07	.73, −.16	.71, −.30	1

[1]Average correlations (mean of eight) are presented above the diagonal, while the range for the eight individuals is presented below the diagonal. Significance tests for heterogeneity of correlation coefficients within each cell are based on seven degrees of freedom.
*Significant heterogeneity at the .05 level.
**Significant heterogeneity at the .01 level.
***Significant heterogeneity at the .001 level.

spanning a range of one or more (mean range = .81, SD = .20). This results in about half (23 of 55) of the pairwise comparisons showing significant heterogeneity, even though each is based on a relatively small (13–36) number of trials. This variation is not associated with individuals. That is, certain individuals do not have consistently high or low correlations among different muscles. Rather, a given individual may have a large correlation between one pair of variables and a small one between another pair.

Although there is enormous variation among individuals, certain patterns of mean correlation are apparent. In general, the onset time variables and the muscle duration variables are relatively highly correlated among themselves (mean correlation for onset variables = .26, mean for duration = .26), while correlations between onset and durations are low (mean = .01). Also, negative mean correlations only occur between onset and duration variables, although for a given individual virtually all characters occasionally show a negative correlation (Table 3).

Levels of variance in activity pattern

For each of the 11 onset and duration variables we have partitioned the total variation (represented by 207 feeding trials for each variable) into components attributable to differences among species means, differences among individuals within species, differences among days within individuals, and the residual variance among trials (feedings) within days. These variance components are presented in Table 2.

By far the largest proportion of the variance is contained in the among-trial component. This accounts for 30–87% of the total, depending on the variable. Because the among-trial component is the lowest level of our nested design, it includes measurement error, stochastic variations in motor output, as well as biologically significant differences between feeding trials. One possible source of such "error" is our determination of the end of muscle activity. However, if this source of error were large, one would expect the relative onset variables (the first five in Table 2) to have much smaller among-trial components than the duration variables. This is not the case.

The among-individual variance component is generally the next largest (Table 2), although again there is considerable variation from muscle to muscle. Since we tested eight

Ambystoma mexicanum and only two *A. dumerilii*, this variance is primarily an indication of variance within *A. mexicanum*. These generally large, statistically significant values suggest that consistent differences in activity patterns exist among individuals even given the large amount of variation among trials and days.

The differences between the two species are generally low, often accounting for virtually none of the overall variance. The single exception is the relative onset time of the sternohyoideus (DM-SH) where differences between species account for over half of the total variance. The duration of activity in the sternohyoideus muscle (SH) had a large among-species variance component, but this value was not significant. (With more degrees of freedom, this variable may be significantly different between the species). Species means for all of the onset times are quite similar (Table 4); for example, the difference in onset time between the depressor mandibulae (a mouth-opening muscle) and the adductor mandibulae externus (a jaw adductor) is less than a millisecond (mean = -.76 ms; N = 215 feedings). Differences in mean durations are much larger (Table 4), but when compared to the total variation in muscle activity durations found at other levels of the analysis, they account for very little of the total variance.

TABLE 4. Mean (± one standard error), N = 215, for 11 electromyographic variables of the feeding mechanism compared between A. mexicanum *and* A. dumerilii[1]

Variable	Mean (in milliseconds) ± 1 SE	
	A. mexicanum	*A. dumerilii*
DM-SH	2.80 ± 0.23	−2.11 ± 0.53
DM-EP	−0.25 ± 0.28	−2.63 ± 0.39
DM-AMi	0.45 ± 0.35	−1.94 ± 0.78
DM-HY	1.57 ± 0.40	0.08 ± 0.83
DM-AMe	−0.76 ± 0.23	−3.01 ± 0.53
DM	67.7 ± 2.14	59.0 ± 4.76
SH	39.1 ± 1.83	66.1 ± 6.82
AMe	129.9 ± 3.65	116.9 ± 10.1
EP	50.9 ± 3.18	36.9 ± 3.43
AMi	116.5 ± 3.08	98.1 ± 3.35
HY	39.1 ± 1.74	47.6 ± 5.14

[1]See text for discussion of these data.

Between electrode variance

There was no significant variation between electrodes within a muscle (Table 5). In both of the analyses conducted for differences in activity pattern within a muscle (for the AMi and DM-AMi variables), electrode variance was 0% of the total variation and was the smallest variance component.

DISCUSSION

The most important result of this study is the ubiquitous variation in our electromyographic (EMG) data. This variation resulted in significant variance, for most characters, between individuals within species and between experimental days. In addition, the variation between individual feeding trials was high (Table 2), always accounting for at least 30% of the total variance for a given variable. The data emphasize the importance of (1) keeping factors contributing to the total variance separate rather than subsuming variance components into a single summary statistic and (2) collecting EMG data from many individuals over a reasonable time span. Simple tests of significance (e.g., t-tests) conducted on data in which these different factors have not been accounted for may provide grossly inaccurate results as discussed below.

TABLE 5. Nested analysis of variance showing the patterns of variation resulting from experiments in which three electrodes were placed in the same muscle (AMi) in two individuals[1]

Source of variation	Degrees of freedom	Sum of squares	Mean square	F (significance)
Among individuals	1	218.6	218.6	2.39 (NS)
Among days within individuals	4	541.3	135.3	1.00 (NS)
Among electrodes within days	8	813.6	101.8	0.23 (NS)
Trials	159	74,509.9	468.6	
Total	172	76,083.4		

[1]Data are for the AMi duration. Recordings were made on four different days.
Variance component (%): among individuals, 0.31; among days, 0.01; among electrodes, 0.00; among trails, 99.68.

Character correlations

An important consideration in any functional analysis is the correlation of different, supposedly "independent" variables. If two characters are tightly coupled, this will be reflected in a high correlation; if this correlation is genetically based, then it represents a functional constraint that cannot be avoided without altering the underlying genetic covariance structure.

A striking result of our analysis is the high degree of variation among individuals in character correlations (Table 3). The correlations for each individual in Table 3 are generally based on over 20 observations (each cell above the diagonal is the mean correlation of eight individuals), a sample size that yields statistical significance for values of r greater than about 0.4. While the mean correlations for most variables were relatively low in this study, virtually all individuals had significant correlations for some pair of variables, and over half the pairwise correlations show heterogeneity among the eight individuals (Table 3). We cannot say whether the variation in correlations represents repeatable differences between individuals or simply day-to-day variation within individuals. Measurements of the same individual on many days would be needed to provide the statistical power to evaluate variation in correlation coefficients between days and to provide an indication of differences in the correlation structure between individuals. However, it is clear that very different conclusions would be drawn regarding the extent of character interdependencies if only one or a few individuals were used to represent the species.

Variance components of EMG variables

Our experimental design allows the partitioning of variance in each EMG variable into four parts: differences between the two species, differences among individuals within each species, differences between days for each individual, and differences among feeding trials within each day. Functional analyses have usually dealt only with the first level, and no quantitative data have been presented on variation at the other three levels.

Our data indicate that by far the greatest proportion of the variance for most variables is that among trials, accounting for between 30 and 87% of the total (Table 2). This suggests that muscle activity patterns during a successful high-speed feeding event (40–90 milliseconds) can show considerable variability. At least two biologically important factors could contribute to this variation. It may be that the feeding mechanism is extremely sensitive to small differences in prey orientation or size. Alternatively, there may be a range of muscle activity patterns, any combination of which is sufficient to produce a successful capture by suction feeding. Given the extent of control exercised during these experiments (standardizing prey size, type, and orientation as much as possible), the latter explanation appears more likely. In either case, because of the extensive variation in muscle activity patterns between prey captures, many feedings are necessary to summarize the feeding repertoire of an individual.

Variation between days was generally low, accounting for 1 to 24% of the total variance. Of those variables that do show significant among-day variance (DM-SH, DM-AMe, DM, EP, AMi, and HY), several individuals are consistently variable between days, while others never are. *Ambystoma mexicanum* individual number 2 showed significant among-day variability for five of six muscle activity variables, individual 4 for four variables, individuals 5 and 6 and *A. dumerilii* were variable for two muscles, and individual 1 for one variable (Table 1). This suggests that both different muscles *and* different individuals show variable responses to experimentation on different days, again emphasizing the need to examine a range of individuals over time.

The variability among individuals is further indicated by the differences among individual means within species, representing the second largest overall component of variance (Table 2). We have found a similar result for kinematic data from the feeding mechanism (Shaffer and Lauder, in press). For almost all variables, this component of the total variance is large and statistically significant. Other research on muscle activity patterns during chewing in lungfishes (Bemis and Lauder, unpublished observations) shows very similar patterns of variation in muscle activity parameters. This suggests that the large amount of among-individual variation and day-to-day variation in EMG variables is not peculiar to the aquatic feeding mechanism of salamanders.

It is important to emphasize that neither differences between electrodes nor changes in muscle activity patterns with number of

prey eaten appear to be important components of variation. As indicated in Table 5, the between-electrode variance within a muscle is effectively zero, and thus differences between individuals cannot be attributed to the fact that the electrodes were located in different parts of the muscle. In addition, one possible source of variation within an individual is satiation: As an experiment progresses, muscle activity variables may change as the animal becomes satiated and motivation alters. This did not occur in these experiments, as indicated by analyses of the slope of least-square regressions of muscle variables against feeding number.

The two species chosen for comparison were picked to represent extremes in head size and shape within aquatic ambystomatids. Even so, differences between *Ambystoma mexicanum* and *A. dumerilii* were generally small: Only one variable (DM-SH, Table 2) showed a significant difference between species, although if more individuals were used, the SH variable might be significant with the increased degrees of freedom. The differences between the mean values for the electromyographic variables in the two species (chosen to maximize differences in head morphology) are often less than differences among randomly chosen individuals of *A. mexicanum*. Again, this emphasizes that several individuals within a species should be examined, as individual and species effects are confounded with very small sample sizes.

Experimental design in functional analysis

While we certainly have not examined all possible sources of variation in this study, we feel that certain recommendations for functional analyses are warranted. First, the data presented in this paper indicate that it is necessary to conduct electromyographic analyses with a particular experimental design in mind. The patterns of variation revealed in electromyographic variables indicate that, if comparisons among species or individuals are of interest, it is not experimentally sound to leave among-individual or among-day sources of variation unaccounted for. The data provide no support for the notion that differences between species will "overwhelm" variation within a species in EMG patterns. If small numbers of individuals are used or if data collected on different days are not assessed for patterns of variation, then levels of variation in the experimental design are confounded. A well-designed experiment is particularly important in functional morphology, because collecting large amounts of data is often expensive and time consuming. There will frequently be a limited number of experiments one can carry out, making the decision of how to distribute sampling effort (i.e., whether to add more individuals or species at the expense of experimental days or feedings) critical. Our data indicate that *individual variation may well be very large and particular attention should be paid to it.* The results also provide a preliminary indication that differences among experimental days are less important contributors to total variance than variation within an individual, and that the number of experimental days on which an individual is recorded could be sacrificed for an increased sample of trials and individuals if the total number of experiments is limited.

Two final points regarding experimental design deserve emphasis. First, we have shown that individual salamanders exhibit extensive variation in muscle activity patterns and in the correlation among variables. However, because much of the total experimental variation occurs between feedings and experimental days (i.e., within individuals) in the nested ANOVA (Table 2), adding a few more individuals to an experimental design may not significantly increase the probability of detecting differences between species in the mean value of a variable. It will provide a more precise estimate of the among-individual variance component, but if this component is large, this added precision still may be insufficient to detect small differences among species.

Secondly, if significant variation is present among and within individuals, then it is *not* appropriate to lump data for each species and conduct t-tests for the differences between species. For example, Table 4 shows a summary of the means and standard errors for the 11 EMG variables analyzed in this study with standard errors based on 215 feeding trials. If a t-test is conducted for the difference between *A. mexicanum* and *A. dumerilii* using these data for the SH variable (duration of electrical activity in the sternohyoideus muscle), the two species are found to differ significantly at the 0.0001 level. However, reference to Table 2 shows that whenever the variation due to individuals, days, and trials is partitioned from the total variance, *no significant difference between the species is found* in the SH variable. A similar result is found if t-tests are conducted for the data in Table 4 for the following variables:

AMi, DM-AMe, DM-AMi, and DM-EP. In every one of these cases, a t-test would give a significant difference between the two species at the 0.05 level. Again, reference to Table 2 shows that *in no cases* does a significant difference remain in these patterns of muscle activity whenever the variation in the variables due to individuals, days, and trials is taken into account. The reason is simply that in the t-test approach, all observations are considered to contribute independent information, and all differences are attributed to between-species causes. Neither of these assumptions is met with these or similar data. This clearly emphasizes the critical importance of designing EMG recording and analysis to take account of sources of variation below the level of interest to the experimenter. Even for the relatively modest sample sizes used for this study, an experimental design that allows the partitioning of variance components provides considerable insight into the biology of feeding behavior.

Functional and evolutionary aspects of muscle activity variation

We have emphasized that variation abounds in these electromyographic data, and we stress the care that must be exercised in interpreting the patterns of variation. However, the discovery of extensive variation among individuals within a species in (1) patterns of jaw movement, (2) patterns of muscle activity, and (3) the correlation structure among functional variables is encouraging for microevolutionary applications of functional analysis: The results clearly indicate that variability exists for selection to act upon. Whether this variation will result in evolutionary shifts in functional traits depends on its genetic basis (Lande and Arnold, '83) and represents an important avenue for future research.

The only variable of the 11 measured muscle activity patterns that differs significantly among species is the difference in onset time between the sternohyoideus and depressor mandibulae. This finding is significant in the light of previous research on the functional morphology of feeding in lower vertebrates (Lauder, '80, '85). Of all features of their feeding mechanism, the role of the hyoid apparatus has been phylogenetically and functionally the most conservative. In sharks, rayfinned fishes, coelacanths, lungfishes, and aquatic salamanders, the hyoid possesses a strong ligamentous connection to the mandible and is instrumental in opening the mouth and developing negative pressures in the buccal cavity. The key muscle involved in mediating mouth opening via the hyoid apparatus is the sternohyoideus. However, unlike rayfinned fishes, salamanders lack an operculum and significant movement of the jaw suspension that could increase the buccal volume during feeding. Whereas other muscles may show fairly high levels of variation between feedings on the same prey type in the same location, the data show that the onset of activity in the sternohyoideus muscle is relatively stereotyped within species, implying that little variation occurs in this critical muscle. The existence of differences between the two species studied argues that differences in suction feeding performance may be related to functional divergence in the role of the hyoid in the feeding mechanism.

An importance final question concerns the functional significance of the variations in muscle activity patterns demonstrated here. It is possible that the variation among muscles (which is generally small, .25 to 2.8 milliseconds for the relative onset variables; Table 4) is largely stochastic and represents "noise" in the feeding system. In this view, trial variance would be high due to essentially random variations in the motor output to the jaw muscles from the nervous system. Whereas there is little doubt that variation exists in the precise pattern of nervous stimulation reaching muscles producing a movement, and that much of this variation (trial variance here) will not significantly alter the movement profile, it is of interest to consider the possibility that the variation has functional significance.

We have used the pattern of pressure change within the buccal cavity during suction feeding as a measure of feeding performance and quantified the relationship between variation in muscle activity patterns and pressure change by using multiple regression models. The aim of the analysis is to ascertain if variation among feedings in muscle activity variables can be used to predict variation in buccal cavity pressure change. If the variation in muscle activity patterns reported here is stochastic, then none of the EMG variables will explain a significant proportion of the variance in the dependent (pressure) variables. On the other hand, if some of the muscle activity variations do bear a relationship to feeding performance, then these variables will enter the regression model with significant coefficients. The results of these multiple regressions show that significant models can be

constructed and that the hyoid muscle variables (DM-SH and SH) explain the greatest variance in buccal pressure. Thus, for a statistical model in which muscle activity patterns are used to predict the magnitude of negative pressure in the buccal cavity, the SH variable is the only one with a significant coefficient. This model explains 50% of the variation in pressure. Similarly, in a model predicting the time to the peak negative pressure, the duration of activity in the depressor mandibulae (DM) and the onset of the sternohyoideus activity (DM-SH) are both significant variables. This analysis indicates that, although some of the trial variance may be due to stochastic variations in motor output, the variations in muscle activity (and especially the hyoid muscles) can be used to understand variation in a measure of feeding performance. It is especially interesting that those muscles that have retained the most conservative role phylogenetically are those in which we find significant variations among species and those which explain most of the variance in feeding performance.

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