

EVOLUTION OF BEHAVIOR AND NEURAL CONTROL OF THE FAST-START ESCAPE RESPONSE

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Abstract.—The fast-start startle behavior is the primary mechanism of rapid escape in fishes and is a model system for examining neural circuit design and musculoskeletal function. To develop a dataset for evolutionary analysis of the startle response, the kinematics and muscle activity patterns of the fast-start were analyzed for four fish species at key branches in the phylogeny of vertebrates. Three of these species (*Polypterus palmas*, *Lepisosteus osseus*, and *Amia calva*) represent the base of the actinopterygian radiation. A fourth species (*Oncorhynchus mykiss*) provided data for a species in the central region of the teleost phylogeny. Using these data, we explored the evolution of this behavior within the phylogeny of vertebrates. To test the hypothesis that startle features are evolutionarily conservative, the variability of motor patterns and kinematics in fast-starts was described. Results show that the evolution of the startle behavior in fishes, and more broadly among vertebrates, is not conservative. The fast-start has undergone substantial change in suites of kinematics and electromyogram features, including the presence of either a one- or a two-stage kinematic response and change in the extent of bilateral muscle activity. Comparative methods were used to test the evolutionary hypothesis that changes in motor control are correlated with key differences in the kinematics and behavior of the fast-start. Significant evolutionary correlations were found between several motor pattern and behavioral characters. These results suggest that the startle neural circuit itself is not conservative. By tracing the evolution of motor pattern and kinematics on a phylogeny, it is shown that major changes in the neural circuit of the startle behavior occur at several levels in the phylogeny of vertebrates.

Key words.—Comparative methods, fast-start, fishes, locomotion, neurobiology, phylogeny, startle behavior.

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The study of evolution in motor patterns and behavior has flourished with the ability to analyze muscle activity data in a phylogenetic context to identify key evolutionary transitions in functional systems (Wainwright et al. 1989; Nishikawa et al. 1992; Smith 1994). However, extending this work to the evolution of neural circuits in vertebrates has been less tractable due to the complexity of those circuits and difficulties in identifying them. Recent advances in the understanding of simple model systems provide exciting opportunities for examining the evolution of neural circuits and behavior. This phylogenetic perspective on motor control and behavior is fundamental to examining patterns of diversification in neuromotor systems.

Vertebrate locomotor circuits are well suited to analysis within an evolutionary framework. A central goal in neurobiology and functional morphology is to understand how locomotor movement is generated. To achieve this goal, vertebrate neuromuscular systems have been studied extensively from the perspective of neural circuit design (e.g., Fetcho and Faber 1988; Eaton et al. 1991; Grillner et al. 1998; Roberts et al. 1998; Hale et al. 2001), motor control (e.g., Johnston and Bekoff 1996; Stein and Smith 1997; Smith et al. 1998), and the role of motor control in biomechanics and functional morphology (e.g., Lauder 1983; Wainwright and Turingan 1993; Westneat and Walker 1997; Westneat et al. 1998).

The goal of this paper is to provide a framework for understanding the evolution of a vertebrate neural circuit by

examining the motor patterns that mediate escape behavior and tracing changes in the startle neural circuit through the evolution of fishes. The startle neural circuit is one of the most important and best understood model systems in vertebrate motor control because it is a simple neural circuit, with large cells, and it generates a discrete behavior (Fetcho and Faber 1988; Fetcho 1990). We provide data on key phylogenetically intermediate species and review data on startle response mechanisms within a phylogenetic context. We test hypotheses of evolutionary conservatism in fast-start motor control and behavior and hypotheses of evolutionary correlation between muscle activity and kinematics.

The Fast-Start Startle Response

In fishes and aquatic amphibians, the startle response is initiated by the paired, commissural, reticulospinal neurons called Mauthner cells, or M-cells (Eaton et al. 1981). The M-cell axon extends the full length of the spinal cord, interacting with spinal interneurons to excite motoneurons on one side of the body and inhibit motoneuron activity on the opposite side (Fetcho and Faber 1988; Fetcho 1991). Because these cells and their axons are large, action potentials are transmitted rapidly down the spinal cord, causing rapid inhibition of conflicting motor commands (Eaton et al. 1995).

The Mauthner cells and startle behavior have been studied in depth in a representative of the most basal extant lineage with these cells, the lamprey (*Petromyzon* and *Ichthyomyzon*; e.g., Rovainen 1978, 1982; McClellan and Grillner 1983;

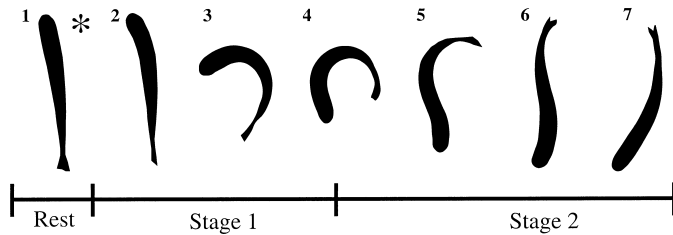


FIG. 1. Movements during a C-start type of fast-start behavior. A startle stimulus (asterisk) applied to the right side of the head of a fish at rest (1) triggers the startle response. In stage 1 (2–4), the fish bends away from the stimulus forming a C-shaped bend with its body (4). In stage 2 (4–7), the fish bends in the opposite direction during its first propulsive tail stroke, and accelerates away from the stimulus.

Currie and Carlsen 1987, 1988; Buchanan 1993), and in the goldfish (*Carassius auratus*; e.g., Furshpan and Furukawa 1962; Furukawa and Furshpan 1963; Faber and Korn 1978; Fetcho and Faber 1988; Eaton et al. 1991), a representative of the modern bony fishes. The comparison between these taxa and their startle response neurobiology and behavior provides a context for examining the evolution of this system in intermediate groups.

Startle responses of larval lamprey and goldfish represent two distinct behavioral patterns, withdrawal and the fast-start, respectively. Larval lampreys live in burrows with their bodies protected and extend their heads into the water column to feed (Hardisty and Potter 1971). When threatened, the anterior end of the body and the head are drawn back in an accordion-like movement (Currie and Carlsen 1985, 1987). Goldfish demonstrate a startle response that is typical of teleost fishes called the fast-start escape response (reviewed by Domenici and Blake 1997). The fast-start (Fig. 1) generally involves two main kinematic stages (Weihs 1973). In stage 1, the fish bends rapidly to one side of the body forming a C shape. In stage 2, the fish bends to the other side of the body with a rostral-to-caudal wave of bending and propels itself away from the stimulus (Fig. 1).

The startle behaviors of lamprey and goldfish involve different patterns of activity of the axial muscles. In lamprey, M-cell stimulation results in strong bilateral muscle activity (Currie and Carlsen 1985) and bending occurs in the direction of pre-existing body bends, causing retraction of the head from the stimulus. If the lamprey is straight when the M-cell is stimulated, the body seizes without bending (Currie and Carlsen 1985). In contrast, during stage 1 of the goldfish fast-start, muscle is active on the opposite side of the body from the stimulus causing the fish to bend and turn away from that direction (Foreman and Eaton 1993). Also unlike the withdrawal response, the Mauthner-mediated fast-start escape response generally involves a rostral-to-caudal wave of muscle contraction on the opposite side of the body from stage 1 muscle contraction (Jayne and Lauder 1993; Westneat et al. 1998).

A wealth of recent research (summarized by Domenici and Blake 1997; Westneat et al. 1998) has demonstrated a diversity of behavioral and muscular motor patterns associated with escape behavior among fishes, suggesting the need for an evolutionary approach to fast-start biology. In the context of the differences in startle mechanism of lampreys and goldfish, we

address several hypotheses regarding the startle behavior and its motor pattern across the phylogeny of fishes. First, we ask the question: Are startle motor patterns conservative? The evolution of motor control patterns has often been found to be conservative across species and phylogenetic groups (Wainwright et al. 1989; Lauder and Shaffer 1993), although recent data show that motor patterns are often variable at lower phylogenetic levels (Friel and Wainwright 1998; Alfaro and Westneat 1999). Using comparisons among both distantly and closely related taxa, we test the hypothesis of motor pattern conservatism (little or no change across taxa) against its alternative, that neural control of the startle response shows major evolutionary changes. Second, previous work on lamprey and goldfish suggests the hypothesis that changes in motor control will be associated with differences in the behavior of the fast-start. Specifically, phylogenetic change in motor control is predicted to be correlated with kinematic parameters such as body curvature, rapidity of the response, and escape velocity. To test these predictions, we search for phylogenetic correlations between motor control features and the kinematics of the startle response across lineages at the base of the actinopterygian phylogeny and among teleosts.

MATERIALS AND METHODS

Data on the fast-start were analyzed from *Polypterus palmas buettikoferi* (bichir), *Lepisosteus osseus* (longnose gar), *Amia calva* (bowfin), and *Oncorhynchus mykiss* (rainbow trout). For *P. palmas* and *A. calva* we examine new variables in addition to ones presented previously (Westneat et al. 1998). *Polypterus palmas*, *L. osseus*, and *A. calva* are basal actinopterygians representing three of the four extant non-teleostean actinopterygian lineages. *Oncorhynchus mykiss* is a teleost that has been a model species in which to study fast-start behavior (Webb 1975, 1976; Williams et al. 1989; Harper and Blake 1990). To test correlations of behavior with muscle activation and muscle function, we recorded kinematics, electromyograms (EMGs), and intramuscular pressure.

Study Specimens

Polypterus palmas ($n = 3$; 23.2–24.7 cm total length [TL]) were purchased from fish wholesalers in Chicago, Illinois, *Amia calva* ($n = 4$; 17.8–22.0 cm TL) were collected by seine and hook and line from the Big Muddy River in southern Illinois. *Lepisosteus osseus* ($n = 4$; 50.0–57.6 cm TL) were caught by gill nets in Sweet Water Creek in eastern North Carolina. *Oncorhynchus mykiss* ($n = 4$; 31.5–37.3 cm TL) specimens were obtained from a trout farm in North Carolina. *Polypterus palmas* and *A. calva* were kept in 100-L aquaria at 20°C. *Lepisosteus osseus* were kept in cattle tanks at 20°C \pm 1°C. *Oncorhynchus mykiss* were also kept in cattle tanks but in slightly cooler water (18°C \pm 1°C) because the species is less tolerant of high temperatures. All procedures for animal care and research were approved by Field Museum of Natural History Animal Care and Use Committee (ACUP FMNH 92–3).

Electromyography and Muscle Pressure

Methods follow those of Westneat et al. (1998) but are summarized briefly here. Fish were anesthetized with dilute tricaine methane sulfonate solution (MS222, Argent Chemicals, Redmond, WA). The center of mass in the anterior-posterior direction of each fish was determined. The center of mass was 0.4 body lengths (bl) from the snout in *P. palmas* and 0.5, 0.4, and 0.4 bl from the snout for *L. osseus*, *A. calva*, and *O. mykiss*, respectively. An oil-filled pressure cannula (polyethylene tubing, 1.1 mm ID, 1.6 mm OD; Intramedic, Franklin, NJ) was implanted at that position from the lateral surface on the left side of the body. We targeted a depth of 1.5 cm from the lateral surface in the epaxial muscle to record pressures from the large, anterior pointing muscle cone.

To measure muscle activity, bipolar electrodes constructed from 0.05-mm diameter insulated, stainless steel wire were inserted into the epaxial muscle. Care was taken to standardize electrode construction to minimize signal variation due to electrodes. Electrodes were implanted at four positions in the white epaxial muscle along the fishes length: at the site of the cannula (the midbody electrode), eight to 10 body segments anterior and posterior to the cannula site (called the anterior and posterior electrodes, respectively), and at the level of the cannula on the opposite side of the body (the opposite electrode). Anterior and midbody electrodes were in trunk (precaudal) muscle, and posterior electrodes were in myomeres of the caudal region.

The pressure cannula was connected to a physiological pressure transducer (Gould/Statham, Costa Mesa, CA, model T-P23ID) that was amplified with a 40-kHz amplifier (Omega, Knightdale, NC, model DMD-520). Pressure signals were simultaneously recorded to the high-speed video display (Kodak Systems, Rochester, NY) and an eight-channel DAT tape recorder (model RD-130TE, TEAC, Tokyo, Japan). EMG signals were amplified by a factor of 5000–10,000 by AM Systems, Sequim, WA, model 1700 amplifiers, and recorded on the DAT tape recorder.

EMGs were digitized by an NB-MIO-16 analog-to-digital converter driven by LabVIEW virtual instrument software (National Instruments Corp., Austin, TX). The sample rate was 5000 points per second per channel. The digital record was then analyzed using a six-channel analysis algorithm custom designed using the LabVIEW virtual instrument library. Only fast-starts with simultaneous muscle activation during stage 1 were selected for analysis.

The muscular motor pattern of each fast-start was characterized by 12 EMG variables of three types: (1) duration (msec) of muscle activity; (2) onset time (msec) of myomeres relative to the time of first head motion; and (3) mean amplitude (mV) of the rectified bursts of activity. Because EMGs from the opposite side electrode during fast-starts to that side did not differ significantly from cannula position electrode EMGs during bends to that side of the body, only data from the cannula position electrode is presented. However, data from the cannula and opposite electrodes were compared to determine the extent of bilateral muscle activity in stage 1.

Kinematics

After recovery, fish were startled by a sudden movement in the tank near the head. A high-speed video camera (Kodak

Ektapro equipped with an image intensifier) recorded the motion of the fish at 1000 images per second. Kinematic data were taken from points digitized along the midline at 4-msec intervals for *P. palmas*, *L. osseus*, and *O. mykiss* and at 2-msec intervals for *A. calva*. *Amia calva* fast-starts were digitized at a higher frequency because, in general, they were faster than the other species—more than double the velocity of either *P. palmas* or *L. osseus* fast-starts and approximately one and a half times the velocity of *O. mykiss* fast-starts.

Stage 1 and stage 2 durations were determined directly from the video. The initiation of stage 1 was determined as the first head movement of the fish. We marked the transition between the two stages as the change in turning angle of the head, as has been done in previous studies (Domenici and Blake 1991, 1993). The end of stage 2 was defined as the end of the first tail stroke after the stage 1 body bend. Stage 1 and stage 2 angles of head movement were analyzed by determining the angular movement of the midline digitized from the rostral tip to the base of the head.

Distance traveled was calculated from digitized points as the overall distance moved by the center of mass in stage 2. Maximum angular velocity and maximum velocity of the center of mass were determined from velocity calculations from the digitized data and frame-by-frame calculations of head angle using a spline function using the generalized cross validation method implemented in QuickSAND 5.0 (Walker 1998).

Curvature (κ) was calculated at the center of mass as described by Westneat et al. (1998). κ is a dimensionless index of body flexion that is scaled by body size and ranges from zero (straight body) up to values of 10 or higher (tightly coiled, head touching tail). A fifth-order polynomial equation was fitted to nine points digitized along the body axis consisting of the center of mass and the four points anterior and posterior to it. The first and second derivatives of the polynomial (y) were taken, and curvature was calculated about the center of mass point as:

$$\kappa = \frac{|y''|}{(1 + y'^2)^{3/2}} \quad (1)$$

By calculating κ as the inverse radius of curvature, increased flexion is proportional to increased curvature. For comparative purposes, κ was then multiplied by body length to arrive at nondimensional curvature.

Statistical Analysis

We tested for differences among the four taxa for kinematic and EMG variables. We first performed a multivariate analysis of variance (MANOVA) to test for the effect of species, fast-start direction (ipsilateral or contralateral to the side with pressure cannula), and the interaction term between species and direction. We found no significant effect of direction (left vs. right) on kinematics or muscle pressure (Wilks' λ , $P = 0.09$) and no significant interaction term between direction and species (Wilks' λ , $P = 0.38$). Thus, the data from ipsilaterally and contralaterally directed fast-starts were combined for kinematic analysis. For kinematic data, nested analyses of variance (ANOVAs), with two replicate trials for each individual

nested within species, were performed to determine species that differed significantly in kinematics.

A potential bias in the kinematic data is the difference in size among the species. Several studies have shown that kinematic variables scale with body size (e.g., Webb 1976; Domenici and Blake 1993; Hale 1999). To examine size effects it would be necessary to have greater intraspecific variation in size and overlapping size ranges among the species. In the absence of those data, we are conservative in our interpretations of species differences in kinematic variables.

Patterns of variation in EMG data were analyzed in several ways. First, two chi-square tests were performed. We tested the association of species with bilateral EMG activity in stage 1 by constructing a contingency table of frequency of occurrence for the four species. Similarly, we tested the association of species with stage 2 EMG activity by constructing a contingency table of frequency of occurrence for the four species.

Interspecific differences in stage 1 motor patterns were tested by ANOVA for the anterior, cannula, and posterior electrodes for ipsilateral fast-starts and for the opposite electrode for contralateral starts (individual was not a nested effect because only one trial per individual was used). For stage 2, the A, C, and P electrodes were tested for a species effect with ANOVA, using only contralateral fast-starts (in which stage 1 was away from and stage 2 back toward the cannula). For all sets of ANOVA tests, a sequential Bonferroni criterion (Rice 1989) was used to determine significance levels. Statistics were performed using JMP 3.0 (SAS Institute, Cary, NC).

Evolutionary Analysis of Fast-Start Characters

The phylogenetic relationships among major vertebrate groups and among basal ray-finned fishes are resolved with broad agreement among biologists (Patterson 1982; Lauder and Liem 1983; Forey and Janvier 1993; Bemis et al. 1997; Coates 1999). We examined fast-start behavior and neural control features within a four-taxon phylogeny of basal actinopterygian fishes and a broader phylogeny that included cephalochordates (the sister-group to vertebrates), hagfishes and lampreys, several sarcopterygians (including frogs), and the major lineages of ray-finned fishes.

Characters for phylogenetic analysis were features of the fast-start mechanism derived from our data or the literature. Some features for our four-taxon analysis are quantitative characters, rather than discrete categories. Discrete character states were derived from continuous quantitative characters by gap coding (Archie 1985; Goldman 1988). Using species means for variable traits, a division was performed at the largest gap between taxa to produce alternative states 0 and 1. The method has been shown to be a useful way to analyze numerical data in both taxonomic (Warheit 1992) and phylogenetic (Westneat 1995) studies. All characters optimized on the vertebrate tree were discrete features that did not require this step. Character mapping was performed using MacClade 4.0 (Maddison and Maddison 2000).

Phylogenetic analysis of fast-start characters was also performed using several comparative methods to reveal patterns of evolution in escape behavior and neurobiology

and to test correlations between characters. Using a four-taxon phylogeny for *P. palmis*, *L. osseus*, *A. calva*, and *O. mykiss* with all branch lengths set equal to 1.0, we reconstructed ancestral states for several kinematic and EMG variables using Compare 4.4 (Martins 2001). Ancestral states were computed using the phylogenetic generalized least squares (PGLS) ancestor method (Martins and Hansen 1997; Martins 1999). This approach computes ancestor values similar or identical to those calculated using squared-change parsimony (Maddison 1991) or maximum-likelihood (Schluter et al. 1997) and provides standard errors of the ancestor estimates.

Character correlations were evaluated using independent contrasts (Felsenstein 1985) in the context of the four-taxon phylogeny. We used PDAP (phenotypic diversity analysis program, ver. 6; Garland et al. 1993, 1999) to test the hypothesis that the evolution of motor pattern was correlated with evolutionary change in escape behavior. Specifically, we calculated correlations between stage 1 EMG duration and stage 1 curvature, duration, angle of rotation, angular velocity and between stage 1 amplitude and stage 1 curvature, angle of rotation, and angular velocity. We also examined the correlations of stage 1 EMG duration and amplitude with distance traveled and maximum escape velocity in stage 2.

RESULTS

Kinematics and Intramuscular Pressure

All four species we examined had a typical fast-start startle behavior with an initial rapid bend away from the stimulus around the center of mass (stage 1) usually followed by a propulsive tail stroke (stage 2). MANOVA showed that there were significant differences in kinematic variables among species (Wilks' λ , $P < 0.0001$; Table 1). A major difference was the amount of bending that occurs during stage 1. By the end of stage 1, *L. osseus*, *A. calva*, and *O. mykiss* formed a C shape with their bodies, typical of the fast-starts of mature fishes (Fig. 1). Maximum curvature at the center of mass, which occurred near the end of stage 1, did not differ among these three species ($P = 0.12$) with average κ -values scaled to body length ranging from 4.9 to 6.5 (Table 1). In contrast, *P. palmis* generally turned its body into a complete circle, or O shape, with its head and tail crossing. Maximum bending at the center of mass was significantly higher ($P < 0.0001$) in *P. palmis* than in the other three species (κ scaled to body length = 11.2; Table 1).

As indicated by stage 1 duration, *P. palmis* took significantly longer to initiate propulsive movement away from the stimulus (91 msec) than the other three species and, correspondingly, had a significantly larger angle of head movement (145 degrees; Table 1). *Lepisosteus osseus* took a significantly shorter time than the other species (29 msec) and had a smaller angle of turning (22 degrees; Table 1). *Amia calva* and *O. mykiss* fell between the other two species with durations of, respectively, 38 and 40 msec and turn angles of 102 and 73 degrees. Maximum angular velocity in stage 1 (Table 1), in contrast, was greatest for *A. calva* (3.5 degrees msec⁻¹). Velocities were not significantly different between *P. palmis* (2.5 degrees per msec) and *O. mykiss* (2.4 degrees

TABLE 1. Kinematic data and results of nested ANOVA testing species and individual effects for fast-start escape timing, performance, body curvature, and intramuscular pressure for *Polypterus palmas*, *Lepisosteus osseus*, *Amia calva*, and *Oncorhynchus mykiss*. Data are species means (standard deviation) for fast-starts to both directions. Sample size is four individuals, eight fast-starts, for all species except *P. palmas*, for which sample size is three individuals, six fast-starts. Significant differences are in bold.

Variable	<i>P. palmas</i>	<i>L. osseus</i>	<i>A. calva</i>	<i>O. mykiss</i>	Species	Indiv
Maximum curvature	11.2 (1.4)	4.9 (1.0)	6.5 (1.5)	6.3 (2.0)	28.1*	1.7
Stage 1 duration (msec)	91 (11)	29 (5)	38 (6)	40 (7)	87.9*	0.6
Stage 1 angle (degrees)	145 (30)	22.4 (5.6)	101.7 (14.7)	73.3 (21.5)	67.3*	1.7
Stage 1 maximum angular velocity (degrees/msec)	2.5 (0.64)	1.3 (0.35)	3.5 (0.39)	2.4 (0.37)	49.7*	1.6
Stage 2 duration (msec)	332 (112)	164 (66)	79 (20)	244 (78)	13.5*	0.8
Stage 2 angle (degrees)	64 (26)	16 (10)	37 (27)	24 (20)	4.0**	0.6
Distance stage 2 (body length)	0.61 (0.12)	0.24 (0.08)	0.43 (0.14)	0.46 (0.11)	21.2*	4.2*
Stage 2 maximum center of mass velocity (body length/sec)	3.57 (0.40)	2.21 (0.88)	7.50 (2.19)	4.96 (2.52)	22.3*	3.2**
Maximum pressure (kPa)	33.7 (26.7)	46.3 (12.2)	45.8 (22.6)	45.1 (10.7)	0.58	0.8

* $P < 0.001$.

** $P < 0.01$.

msec⁻¹), but *L. osseus* had slower stage 1 movement with significantly lower maximum angular velocity (1.3 degrees msec⁻¹) than the other species.

Stage 1 was followed by a subsequent bend of the body in the opposite direction during stage 2 to generate forward propulsion. Stage 2 duration for *A. calva* and *L. osseus* (79 and 164 msec, respectively) was significantly shorter than for *P. palmas* with an average stage 2 duration of 332 msec (Table 1). *Oncorhynchus mykiss* stage 2 duration (24 msec) was intermediate and did not differ significantly from either *P. palmas* or *L. osseus*. *Amia calva* had a significantly shorter stage 2 duration than the other three species examined. The angle of head movement in stage 2 is lower than in stage 1 (Table 1). It did not differ significantly among *L. osseus*, *A. calva*, and *O. mykiss* (means ranging from 16 to 37 degrees), but, as in stage 1, was significantly greater for *P. palmas* (64 degrees).

We also compared the distance traveled in stage 2 (Table 1) and the maximum center of mass velocity during the stage 2 movement (Table 1). Center of mass distance traveled in stage 2 was significantly greater for *P. palmas* (0.61 bl) than for other species. *Oncorhynchus mykiss* and *A. calva* had intermediate values for stage 2 distance, 0.43 bl and 0.46 bl, respectively, and *L. osseus* had the shortest distance traveled (0.24 bl; Table 1). The maximum velocity of the center of mass in stage 2 was highest for *A. calva* (7.5 bl sec⁻¹), significantly greater than *L. osseus* (2.2 bl sec⁻¹), *P. palmas* (3.6 bl sec⁻¹), and *O. mykiss* (5.0 bl sec⁻¹; Table 1).

Elevated intramuscular pressure occurred during the fast-start in all four species studied (Table 1). No significant effect of species, direction, or interaction term was found for maximum intramuscular pressure ($P = 0.52$) or pressure duration ($P = 0.08$).

Muscle Activity

Profiles of muscle activity for the fast-start of *P. palmas*, *L. osseus*, *A. calva* and *O. mykiss* (Fig. 2) and the timing of mean muscle activation patterns relative to important kinematic events of the fast-start (Figs. 3, 4) illustrate the neuromotor control of the fast-start behavior. Chi-squared tests, used to examine overall differences in EMG activity, demonstrate significant differences in the presence of bilateral

activity in stage 1 (Pearson $\chi^2 = 2.29$, $P < 0.0001$). In stage 1 of *P. palmas* and *A. calva*, muscle is active on both sides of the body simultaneously (see also Westneat et al. 1998; Table 2, Figs. 2–4). In contrast to the bilateral muscle activity of *P. palmas* and *A. calva*, *L. osseus*, and *O. mykiss* have unilateral muscle activity on the side of the body toward which the fish is bending in stage 1 (Table 2, Figs. 2–4). There are also qualitative differences in muscle activity in stage 2 (Pearson $\chi^2 = 15.6$, $P < 0.002$). In stage 2, a wave of posteriorly propagating muscle activity is present in *L. osseus*, *A. calva*, and *O. mykiss* (Table 3, Figs. 3, 4). Even though *P. palmas* consistently performed a stage 2 tail stroke, the species rarely (one trial) exhibited EMG activity during stage 2 (see also Westneat et al. 1998).

ANOVAs of the anterior, cannula, and posterior electrode in stage 1 of ipsilateral fast-starts showed significant differences among species in the durations and amplitudes of EMG bursts but there were no significant differences in muscle onset times. *Polypterus palmas* had significantly longer muscle activity in stage 1 than *A. calva*, and *A. calva* activity duration was greater than that of *L. osseus* and *O. mykiss* for all three ipsilateral electrode positions (Table 2, Fig. 3). Amplitude of EMG signals measure the intensity of activation of locomotor muscle. The general trend in EMG amplitude across most electrode positions was that *L. osseus* and *O. mykiss* had significantly lower amplitudes than *A. calva* and *P. palmas*. However, comparing the amplitude differentials between ipsilateral and contralateral muscle activity, we found that there is no significant difference among the species (ANOVAs for *P. palmas* and *A. calva* = 0.54 mV, SD = 0.22; for *L. osseus* and *O. mykiss* = 0.55 mV, SD = 0.32; $P = 0.96$).

ANOVAs of the anterior, cannula, and posterior electrode in stage 2 of contralateral fast-starts (Table 3, Fig. 4) showed no significant differences among the species except for the fundamentally different *P. palmas* in which stage 2 EMG activity is absent.

Evolutionary Analysis of Fast-Start Characters

Analysis of discrete characters on the four-taxon phylogeny revealed a diversity of evolutionary patterns in fast-start

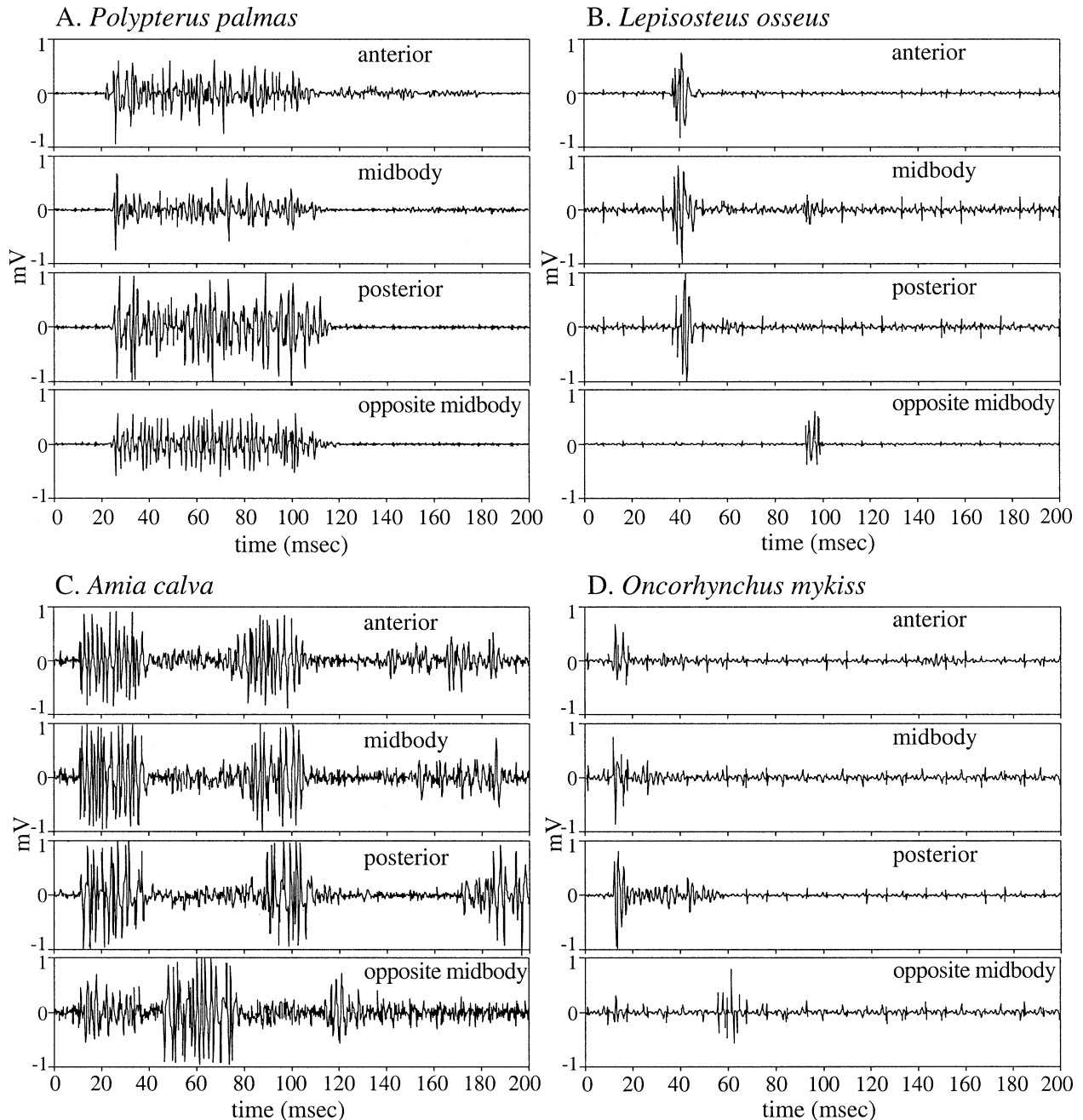


FIG. 2. Representative electromyograms (EMGs) of startle responses. Fast-start trials presented for (A) *Polypterus palmas*, (B) *Lepisosteus osseus*, (C) *Amia calva*, and (D) *Oncorhynchus mykiss* show the muscle activity patterns during an ipsilateral C-start (bending toward the three electrodes and cannula in stage 1). *Polypterus palmas* and *A. calva* had bilateral activity in stage 1, whereas *L. osseus* and *O. mykiss* activity was unilateral. *Polypterus palmas* usually had one stage of muscle activity, whereas the other three species had a second stage of activity following the initial EMG burst. EMG profiles for *P. palmas* and *A. calva* from Westneat et al. (1998).

characters (Fig. 5). Characters that were constant (plesiomorphic) in the group (Fig. 5A) included the presence of a two-stage escape behavior, stage 1 EMG onset variables, and the magnitude of intramuscular pressure. *Polypterus palmas* was either autapomorphic (unique) or plesiomorphic (ancestral) for the characters of stage 1 curvature, a single EMG stage, and several stage 2 EMG variables (Fig. 5B). *Amia calva* and *P. palmas* shared the characters of bilateral EMG

activity in stage 1 and EMG amplitude in stage 1 (Fig. 5C). Stage 2 maximum velocity increased in the *Amia/Oncorhynchus* clade (Fig. 5D).

Reconstruction of ancestral states at the nodes of the phylogeny (Table 4) showed that some characters (e.g., intramuscular pressure) underwent little evolutionary change. Other characters showed evolutionary trends (e.g., curvature, duration) but had high standard errors associated

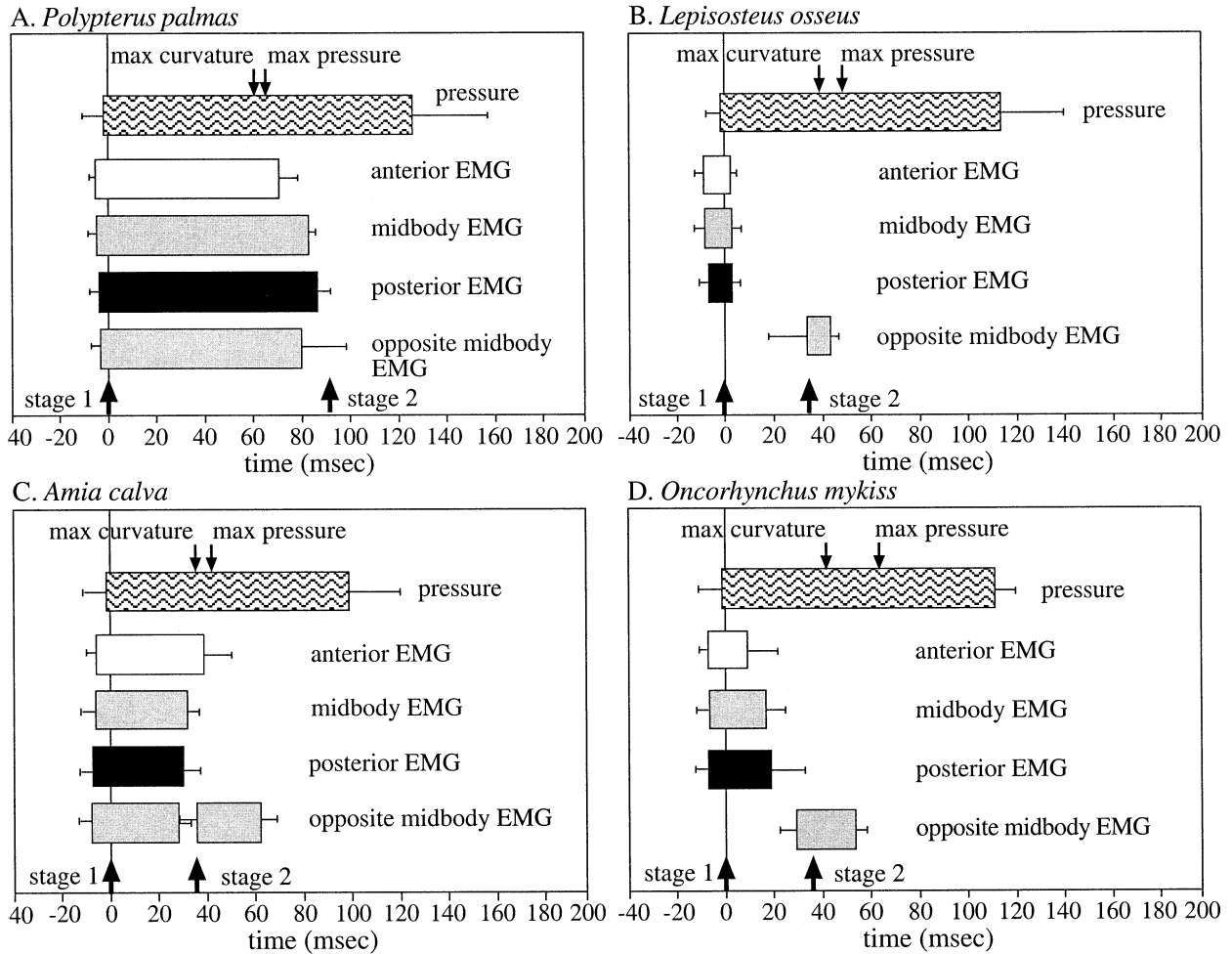


FIG. 3. Summary diagrams of ipsilateral C-starts (bending toward the cannula and three electrodes in stage 1), for (A) *Polypterus palmas*, (B) *Lepisosteus osseus*, (C) *Amia calva*, and (D) *Oncorhynchus mykiss*. Lengths of bars represent mean duration of muscle activity or elevated pressure. Error bars on the right indicate the standard deviation of the durations. Relative onset time is indicated by the left margin of each bar and the error bar associated with it represents the standard deviation in onset time of EMG activity relative to the first movement of the head. Data for *P. palmas* and *A. calva* from Westneat et al. (1998).

with ancestral estimates. The unusual fast-start of *P. palmas* had a large effect on nodal values for characters of curvature, stage 1 duration and angle, and several stage 2 EMG variables (Table 4). The pattern in these cases is for the root ancestor of the phylogeny below *P. palmas* to have values similar to the *P. palmas* tip state, with the ancestors at nodes 1 and 2 being intermediate between states of the other three species.

Phylogenetic correlation results using independent contrasts show that several hypotheses of correlation between stage 1 muscle activity and behavior were supported by significant correlation coefficients (Table 5). Stage 1 EMG duration was significantly correlated with the total duration of stage 1 of the fast-start ($P < 0.05$), maximum curvature ($P < 0.04$), and angle of rotation ($P < 0.05$). EMG duration was also positively, but not significantly, correlated with maximum angular velocity of stage 1 and escape distance (Table 5). The amplitude of stage 1 EMG was positively but not significantly correlated with stage 1 kinematic param-

eters, and peak escape velocity was not significantly correlated with EMG variables (Table 5).

Character analysis on a phylogeny of vertebrates (Figs. 6, 7) revealed evolutionary patterns for four important fast-start characters. Character optimization of presence/absence of the Mauthner neuron that initiates the fast-start (Fig. 6A) shows that there was a single origin of the M-cell along the branch leading from Myxiniformes (hagfishes) to Petromyzontiformes (lampreys) and gnathostomes. The M-cell initiates many different types of startle behavior, from axial retraction mechanisms and pectoral fin thrusts to the fast-start escape of fishes and tadpoles and the escape jump of frogs (Fig. 6B). The activity patterns of muscles during the startle behavior are not stereotypic. Stage 1 EMG can be either unilateral (occurring on just one side of the body) or bilateral (Fig. 7A), depending largely on neural inhibition mechanisms that show homoplasy among the vertebrates. Similarly, stage 1 muscle contraction may or may not be accompanied by stage 2 muscle activity (Fig. 7B), a homoplastic character that in many

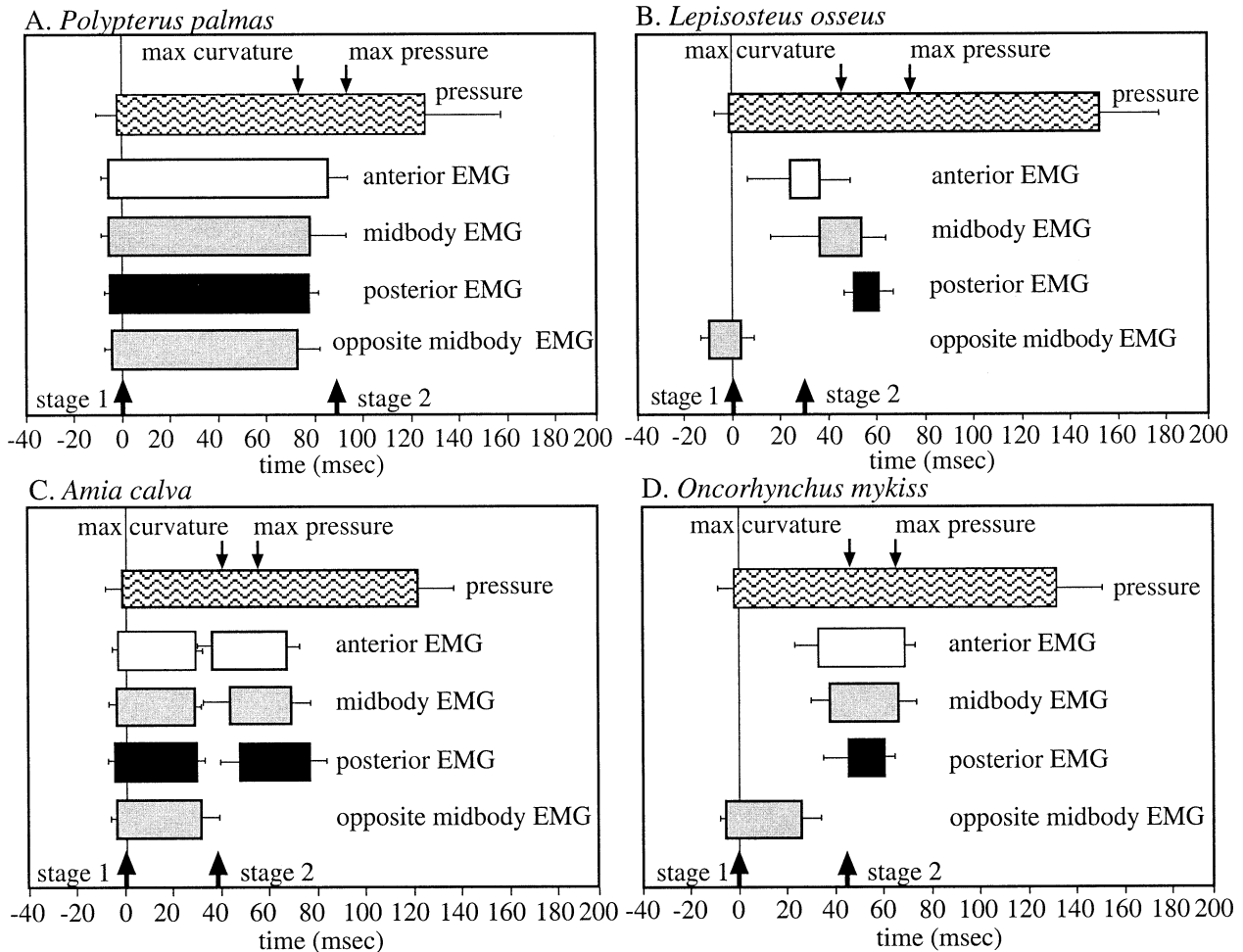


FIG. 4. Summary diagrams of contralateral C-starts (bending away from the cannula and three electrodes in stage 1), for (A) *Polypterus palmas*, (B) *Lepisosteus osseus*, (C) *Amia calva*, and (D) *Oncorhynchus mykiss*. Lengths of bars represent mean duration of muscle activity or elevated pressure. Error bars on right indicate the standard deviation of the durations. Relative onset time is indicated by the left margin of each bar and the error bar associated with it represents the standard deviation in onset time of EMG activity relative to the first movement of the head. Data for *P. palmas* and *A. calva* from Westneat et al. (1998).

fish species (and tadpoles) is used during the fast start to accelerate during the escape response.

DISCUSSION

We draw three major conclusions about the evolution of the startle response, a widespread behavior used in the life-or-death response of these animals to their predators. First, we find that the motor pattern of the startle response varies considerably among species, particularly in the extent of bilateral stage 1 activity and in the presence of a distinct second stage of muscle activity. From this we conclude that the organization of neural circuits controlling escape behavior has undergone extensive evolution among fishes. Second, our data show that the startle response is not stereotypic across taxa. Instead, we find that there is extensive evolutionary change in startle kinematics. Third, we combine our data and previous results with a phylogeny of vertebrates to reconstruct the evolutionary history of the startle response.

Startle Motor Patterns and Behavior: Implications for Neural Circuits

A major difference in motor pattern of the startle among the four species we studied is the laterality of muscle activity in stage 1. Axial muscles of *L. osseus* and *O. mykiss* are active unilaterally on the side of the body opposite to the stimulus (Figs. 2–4). In contrast, *P. palmas* and *A. calva* axial muscles are active simultaneously on both sides of the body (Figs. 2–4) with higher EMG amplitude in the direction of bending ($P < 0.003$; Westneat et al. 1998). We found that the amplitude differential between the contralateral and ipsilateral sides was not significantly different among species, suggesting that an amplitude differential in muscle activity between the sides of the body rather than complete inhibition of the contralateral muscle is critical for fast-start behavior.

The fact that the startle response can be generated with bilateral muscle activity has implications for neural circuit design for the startle response. The C-start type of startle response has generally been thought to involve unilateral

TABLE 2. Stage 1 EMG data for ipsilateral fast-starts (stage 1 to the left, toward the canula and three electrodes) of *Polypterus palmas*, *Lepisosteus osseus*, *Amia calva*, and *Oncorhynchus mykiss*. Data are species means (standard deviations). ANOVA results for species effect are listed at right. Sample size is four individuals, four ipsilateral fast-starts, for all species except *P. palmas*, for which sample size is three individuals and three fast-starts. Significant differences are in bold.

	<i>P. palmas</i>	<i>L. osseus</i>	<i>A. calva</i>	<i>O. mykiss</i>	F-ratio
Anterior EMG duration (msec)	75 (9)	11(2)	44 (22)	18 (13)	15.4**
Canula EMG duration (msec)	88 (4)	12 (1)	37 (8)	22 (13)	50.0*
Posterior EMG duration (msec)	90 (10)	10 (3)	38 (10)	26 (17)	30.7*
Anterior EMG onset (msec)	-6 (3.6)	-9 (5.8)	-7 (3.4)	-10 (2.0)	0.7
Canula EMG onset (msec)	-5 (1.9)	-9 (6.4)	-9 (3.4)	-10 (2.0)	0.9
Posterior EMG onset (msec)	-5 (1.3)	-8 (6.9)	-9 (3.5)	-9 (2.8)	0.7
Anterior EMG amplitude (mV)	0.85 (0.17)	0.57 (0.18)	0.88 (0.18)	0.35 (0.05)	10.8**
Canula EMG amplitude (mV)	1.15 (0.30)	0.78 (0.12)	1.01 (0.08)	0.37 (0.15)	15.3**
Posterior EMG amplitude (mV)	0.86 (0.11)	0.65 (0.26)	0.96 (0.05)	0.60 (0.46)	1.4

* $P < 0.0001$.

** $P < 0.005$.

muscle activity. Models of the startle neural circuit based on electrophysiology and anatomy in goldfish (Furukawa and Furshpan 1963; Fetcho and Faber 1988; Fetcho 1990) include several cell types that have been shown to inhibit muscle activity contralateral to the direction of bending. The generation of significant bilateral muscle activity indicates that there is species-specific variation in this system. In fact, bilateral muscle activity during the startle may be common. EMGs from goldfish (Foreman and Eaton 1993) and sunfish (Jayne and Lauder 1993; Czuwala et al. 1999) also show low levels of muscle activity on the opposite side of the body from the main muscle activity in the direction of bending suggesting that the startle response may involve additional complexities not explained by current models of the startle neural circuits.

The withdrawal type of startle response is also generated by bilateral activity. In larval lamprey, a rostral startle stimulus results in a withdrawal response in which, as in *P. palmas* and *A. calva*, there is asymmetry in the strength of muscle contraction between the sides of the body (Currie and Carlsen 1985, 1987). However, the asymmetries in activity appear to result from asymmetries in initial body position rather than from the direction of the stimulus (Currie and Carlsen 1985). Similarly, eels have independently evolved a withdrawal type startle response and, like the lamprey, lack the axon cap structure that is involved in inhibiting contralateral activity (Meyers et al. 1998). By analogy to lampreys, it seems likely

that their response also involves bilateral muscle activity. Thus, neural circuits in some lineages of fishes may provide for nearly complete inhibition of muscle activity, whereas other taxa have a circuit design that allows simultaneous contraction on both sides of the body for a retraction response or for increasing stiffness during the escape (Foreman and Eaton 1993; Westneat et al. 1998).

Whereas the neural basis of stage 1 of the fast-start has been studied in depth, little attention has focused on the neural basis of stage 2 activity. Recent studies examining a combination of kinematics and EMGs (Foreman and Eaton 1993; Westneat et al. 1998) have proposed mechanisms by which the propulsive stage of the fast-start is generated. Foreman and Eaton (1993) found that propulsion can occur from the stage 1 burst of muscle activity alone and that stage 2 muscle activity is responsible for directional change in stage 2. They suggest that bilateral activity in stage 1 EMGs observed in goldfish may help stiffen the body to generate propulsive movement in stage 2. The ability of passive mechanisms to generate stage 2 propulsion is illustrated by the fact that *P. palmas* can perform stage 2 propulsion without stage 2 muscle activity.

The Evolution of Behavior and Motor Control of the Fast-Start

We explored fast-start features in the context of both basal actinopterygian (Fig. 5; Patterson 1982; Lauder and Liem

TABLE 3. Stage 2 EMG data for contralateral escapes (stage 1 to the right, stage 2 accelerates the fish back toward the left) of *Polypterus palmas*, *Lepisosteus osseus*, *Amia calva*, and *Oncorhynchus mykiss*. Data are species means (standard deviations). ANOVA results for species effect are listed at right. Sample size is four individuals, four fast-starts for all species, except *P. palmas*, for which only a single trial from one individual had stage 2 activity. Significant difference is in bold.

	<i>P. palmas</i> ¹	<i>L. osseus</i>	<i>A. calva</i>	<i>O. mykiss</i>	F-ratio
Anterior EMG duration (msec)	55	16 (13)	29 (8)	40 (5)	0.7
Canula EMG duration (msec)	31	18 (12)	23 (10)	27 (7)	1.7
Posterior EMG duration (msec)		11 (8)	27 (9)	15 (6)	7.3
Anterior EMG onset (msec)	107	22 (19)	31 (7)	33 (11)	0.6
Canula EMG onset (msec)	79	35 (22)	41 (12)	35 (10)	0.2
Posterior EMG onset (msec)		51 (3)	44 (11)	43 (15)	0.3
Anterior EMG amplitude (mV)	0.12	0.33 (0.02)	0.33 (0.13)	0.08 (0.03)	16.9**
Canula EMG amplitude (mV)	0.14	0.43 (0.25)	0.31 (0.12)	0.36 (0.28)	0.03
Posterior EMG amplitude (mV)		0.22 (0.03)	0.34 (0.08)	0.29 (0.20)	1.7

¹ Stage 2 muscle activity was rare in *P. palmas*.

** $P < 0.005$.

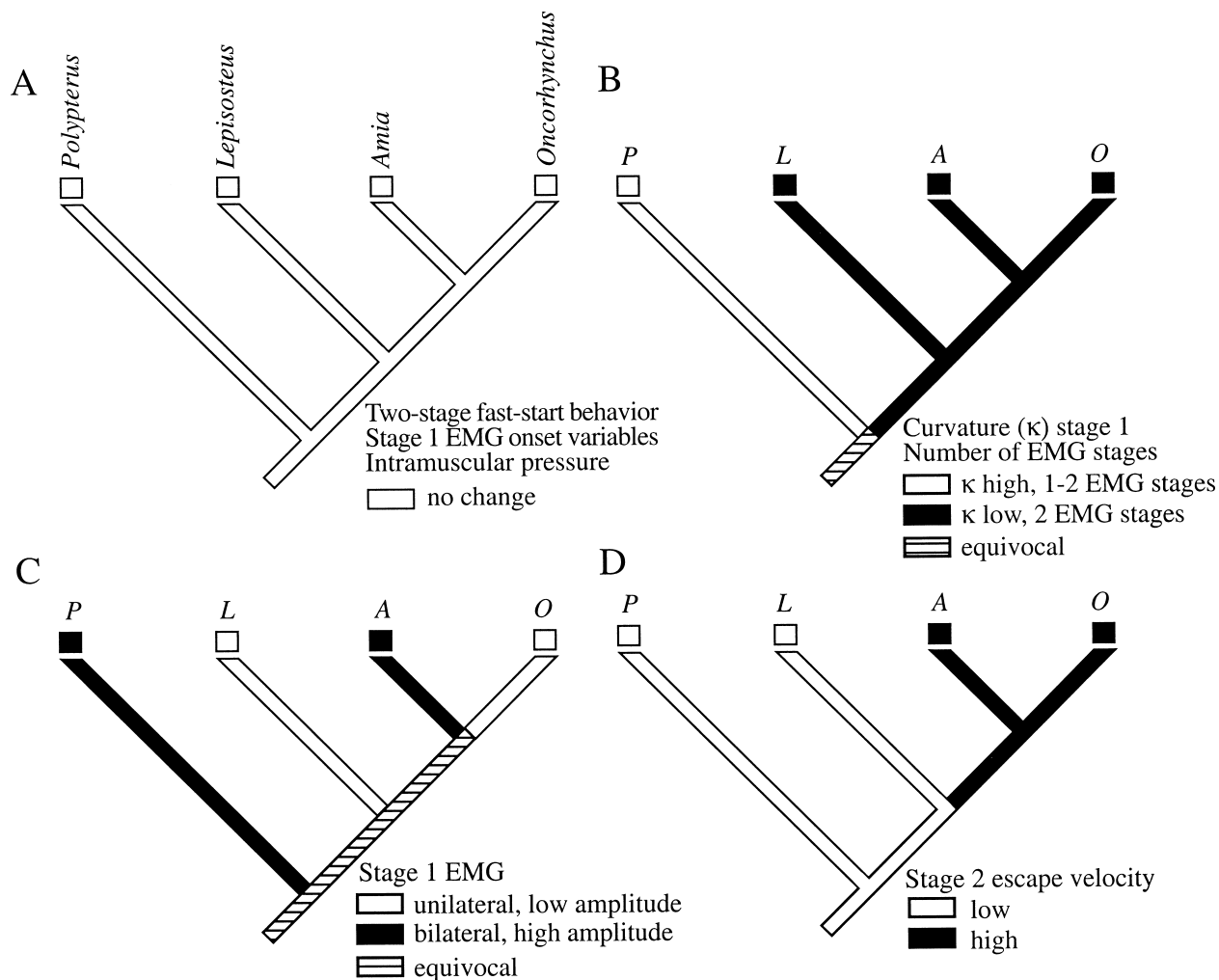


FIG. 5. Evolution of startle response characters mapped onto the phylogeny of basal actinopterygian fishes. Each tree illustrates a character evolution pattern for one or more characters: (A) conservative characters that show no difference among the taxa; (B) characters that differ between *Polypterus palmis* and the other taxa; (C) *Polypterus palmis* and *Amia calva* share characters that are different from *Lepisosteus osseus* and *Oncorhynchus mykiss*; and (D) performance character shared by *A. calva* and *O. mykiss*.

TABLE 4. Reconstructions of ancestral character states for 13 kinematic and EMG characters of the fast-start, for the phylogeny of four actinopterygian fishes (Fig. 6). Reconstructed character states (standard errors) are shown. Ancestral states were reconstructed using the PGLS ancestor method of Martins and Hansen (1997). Root is the ancestor node of the clade, below *Polypterus palmis*; node 1 is the ancestor node between *P. palmis* and *Lepisosteus osseus*, and node 2 is the ancestor node of *Amia calva* and *Oncorhynchus mykiss*.

	Root	Node 1	Node 2
Maximum curvature	9.0 (4.7)	6.8 (3.7)	6.5 (3.7)
Stage 1 duration (msec)	69 (37)	46 (29)	41 (29)
Stage 1 angle (degrees)	108 (62)	71 (48)	82 (48)
Stage 1 maximum angular velocity (degrees/msec)	2.3 (1)	2.1 (0.8)	2.7 (0.8)
Stage 2 duration (msec)	267 (147)	202 (114)	174 (114)
Stage 2 angle (degrees)	47.5 (26)	31 (20)	30 (20)
Distance stage 2 (body length)	0.5 (0.6)	0.4 (0.5)	0.4 (0.5)
Stage 2 maximum center of mass velocity (body length/sec)	3.7 (2.1)	3.8 (1.7)	5.4 (1.7)
Maximum pressure (kPa)	38.4 (19.4)	43.1 (15.2)	44.7 (15.2)
EMG Stage 1 duration (msec)	61 (35)	34 (27)	31 (27)
EMG Stage 1 amplitude (mV)	1.0 (1.0)	0.8 (0.8)	0.7 (0.8)
EMG Stage 2 duration (msec)	8 (7)	16 (5)	22 (5)
EMG Stage 2 amplitude (mV)	0.2 (0.7)	0.3 (0.5)	0.3 (0.5)

TABLE 5. Independent contrasts results for correlations between stage 1 EMG (duration and amplitude) and several kinematic parameters of the fast-start. Contrasts were performed using phenotypic diversity analysis programs of Garland et al. (1993, 1999) and the four-species phylogeny for *Polypterus palmas*, *Lepisosteus osseus*, *Amia calva*, and *Oncorhynchus mykiss*.

Hypothesis of muscle activity effect on kinematics of the fast-start	Independent contrasts correlation	P-value
EMG duration: maximum curvature	0.98	0.04
EMG duration: stage 1 duration	0.96	0.05
EMG duration: rotation angle	0.94	0.05
EMG duration: maximum angular velocity	0.57	0.17
EMG duration: escape distance	0.88	0.06
EMG duration: escape velocity	0.29	0.32
EMG amplitude: maximum curvature	0.51	0.20
EMG amplitude: rotation angle	0.58	0.14
EMG amplitude: angular velocity	0.54	0.16
EMG amplitude: escape distance	0.28	0.32
EMG amplitude: escape velocity	0.37	0.26

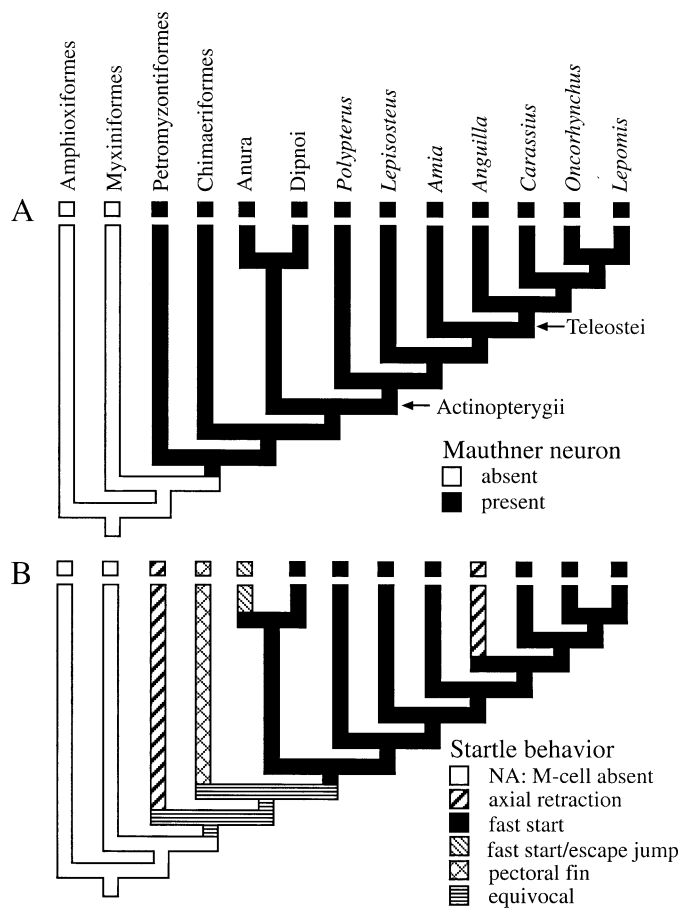


FIG. 6. Evolution of the Mauthner cell and startle behavior for Mauthner-cell initiated responses, mapped onto the phylogeny of chordates. (A) The presence of the M-cell is a synapomorphy of vertebrates, although it is apparently lost in tetrapods and sharks. (B) Startle behavior is axial retraction basally, may be a pectoral fin thrust in chimaeras or an escape jump in adult anurans, and is a fast-start escape in most ray-finned fishes.

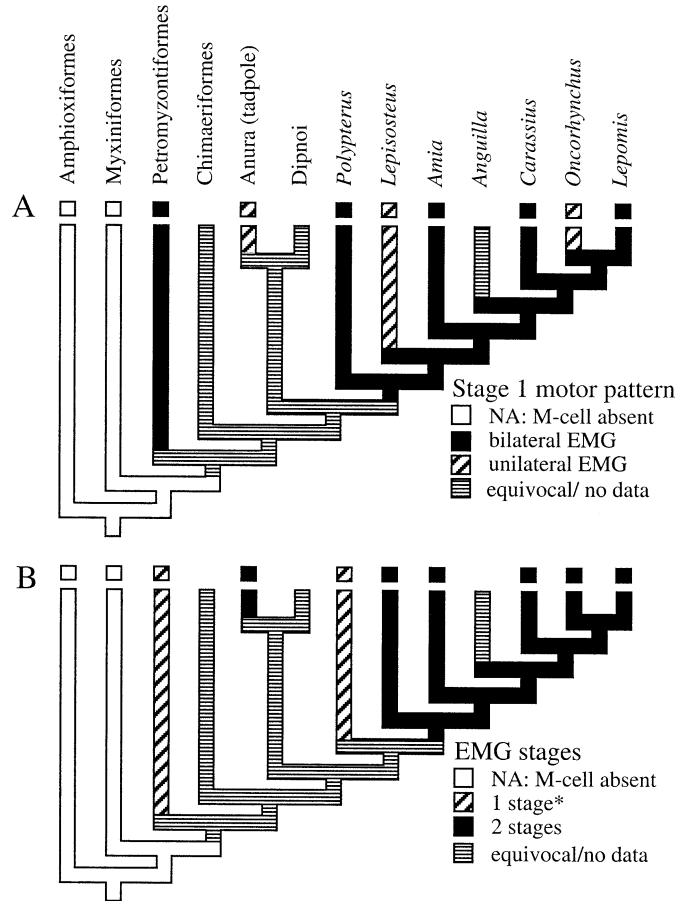


FIG. 7. Evolution of motor pattern characters for Mauthner-cell initiated responses, mapped onto the phylogeny of chordates. (A) The stage 1 motor pattern is bilateral throughout most of the tree, but unilateral muscle activity has evolved multiple times and the basal condition below lampreys is equivocal. (B) The number of EMG stages (not kinematic stages) is equivocal at the base of the clade that has M-cells. **Polypterus* may have either 1 or 2 EMG stages during the fast-start.

1983; Coates 1999) and vertebrate phylogenies (Figs. 6, 7; Forey and Janvier 1993; Bemis et al. 1997; Janvier 1997). Phylogenetic analysis of fast-start characters among the four fish species studied reveals several evolutionary patterns (Fig. 5). Conserved characters include a two-stage fast-start, a strong stage 1 muscle contraction, presence of intramuscular pressure, and several motor pattern features discussed above (Fig. 5A). Despite these common features, homoplasy (independent origin or reversal) of fast-start features occurs several times even in this simple phylogenetic tree (Fig. 5C). A key conclusion from these data is that a fast-start response can be achieved with several alternate motor strategies and variable behavior.

We found significant differences between species in stage 1 kinematic parameters (Table 1). The most striking difference was in the behavior of *P. palmas*, which had a higher turning angle, a longer stage 1 duration, and greater curvature than the other species. Reconstruction of ancestor values (Table 4) reflects this, as the root node of the phylogeny retains higher values for these traits than the two descendant nodes.

Maximum angular velocity for *P. palmas*, however, fell within the range of the other species, suggesting comparable stage 1 performance. In stage 2, *A. calva* and *O. mykiss* had greater escape velocities than *P. palmas* and *L. osseus*. Maximum stage 2 velocity has been shown to reflect escape success (O'Steen et al. 2002) during a predatory attack. Our analysis shows that escape performance, as measured by body velocity, shows an evolutionary increase in more recently derived clades (Fig. 5D). This pattern is demonstrated in the higher value for escape velocity in the ancestor (node 2) of *A. calva* and *O. mykiss*.

Kinematic comparisons must be interpreted conservatively due to a possible size bias because our specimens of *L. osseus* and *O. mykiss* were larger than the other two species. Studies that examined smaller *L. osseus* and *O. mykiss* provide insight into size trends in escape behavior. Webb et al. (1992) studied startle responses of smaller (average total length of 29.8 cm) specimens and found duration to be considerably greater in stage 1 and lower in stage 2 than we recorded. However, the total startle duration was similar, suggesting a possible difference in our determination of kinematic stages. A size series of *O. mykiss* (Webb 1976) had comparable stage 1 durations to the fish we examined although our stage 2 durations were longer. Species differences remain the same using these other data and in some instances (as with stage 1 duration of gar), increase support for our conclusions of interspecific trends.

Phylogenetic correlations between muscle activity and kinematics (Table 5) support several hypotheses regarding the biomechanics and physiology of the fast-start. The significant positive correlations between stage 1 EMG duration and stage 1 curvature, duration, and angle of rotation (Table 5) are consistent with the hypothesis that increased turning performance during the escape is enabled by greater muscle activity and perhaps total muscle force. However, it should be noted that a trade-off between force and velocity in musculoskeletal mechanisms operates, in which an increase in duration of muscle activity, while providing greater total force, decreases the rapidity with which the muscle performs its task.

Phylogenetic Patterns in the Vertebrate Fast-Start

Analysis of fast-start characters using the vertebrate phylogeny (Figs. 7, 8) reveals the current state of our knowledge of evolutionary trends in startle behavior. A major question in the evolution of startle behavior is: What is the basal state of the startle response in vertebrates? The lamprey (Petromyzontiformes) is the most basal taxon known to have M-cells (Fig. 7A; Zottoli 1978). The larval lamprey withdrawal response involves a single behavioral stage with bending on both sides of the body and a single stage of axial muscle activity that is bilateral, with greater activity in the direction of bending (Currie and Carlsen 1985). The lamprey thus has an eel-like body shape, relatively simple axial musculature, lacks true vertebrae, and performs a single stage withdrawal behavior.

Is the lamprey startle mechanism the ancestral condition for the startle response? Possibly, but the phylogenetic position of living lampreys is uncertain (Forey and Janvier 1993), and the lamprey body form may or may not represent the basal form for vertebrates. Jawed vertebrates arose from

within a diverse assemblage of jawless fishes, most of which were armored and had relatively large symmetrical or asymmetrical tail fins. Living lampreys may be a modern lineage derived from within a clade of armored fossil anaspids (Forey and Janvier 1993), although recent fossils of soft-bodied agnathans from the lower Cambrian (Shu et al. 1999) appear to establish a lineage of soft-bodied lamprey-like forms at the base of the vertebrate tree. If this recent hypothesis is correct, then a soft-bodied vertebrate with a Mauthner cell and a withdrawal response, similar to the condition of the living lampreys (Fig. 7), is likely the basal condition for the escape response. If the armored jawless taxa leading to gnathostomes had Mauthner neurons, the presence of a thick, armored body and a respectable caudal fin argues against a withdrawal response as the type of startle present in these fishes, because the withdrawal requires a flexible trunk. In any case, the information on the lamprey startle is the starting point for any evolutionary analysis of fast-starts, although even at the base of the vertebrate tree there may have been a diversity of escape behavior.

Little is known of the fast-start behavior of chondrichthyans (sharks, rays, and chimaeras). Mauthner cells have been identified in just a few species (summarized by Zottoli 1978) including embryonic *Squalus* and chimaeras (Figs. 6, 7). In chimaeras M-cells are thought to trigger a bilateral response in the fish's large pectoral fins (Bone 1977). Pectoral fin adduction is part of the startle response in most fishes, although pectoral fin movement seems to be used for tucking fins close to the body to decrease hydrodynamic resistance rather than to generate force.

The Teleostomi (sarcopterygians plus actinopterygians) is the first group to arise phylogenetically in which a Mauthner cell-mediated fast-start type of escape response has been recorded. Unlike in larval lamprey, bending occurs to one side of the body in the initial stage of movement (for exceptions, see Will 1986; Meyers et al. 1998). Sarcopterygian and actinopterygian taxa also, unlike lampreys, have the ability to control the direction of bending. Rather than increasing the curvature of preexisting body bends, they bend away from the stimulus regardless of the initial position of the body. The fast-start also generally involves two stages of bending, whereas the withdrawal of lamprey involves only one stage (Fig. 6B).

Fast-start behavior has been recorded for several sarcopterygian taxa (Figs. 6, 7) including lungfish (Meyers et al. 1998), coelacanth (Fricke et al. 1987), and amphibians (Will 1986; Hoff and Wassersug 2000). Tadpole muscle activity patterns (Hoff and Wassersug 2000) indicate that the tadpole fast-start involves unilateral activity. In frogs, the Mauthner neurons are thought to elicit bilateral muscle activity and a startle jump (Will 1986). More detailed EMGs on lungfishes and amphibians are critical for broadening the scope of our understanding of motor control of the startle behavior and its evolution.

Among living actinopterygian fishes, the polypteriform lineage, including *Polypterus* (Figs. 6, 7), is the most basal (Coates 1999). During stage 1, *P. palmas* exhibits strong bilateral muscle activation. Stage 2 muscle activity was rare in *P. palmas*, and variable stage 2 muscle activity has also been recorded for *Polypterus senegalus* (Tytell and Lauder

2001). *Lepisosteus osseus* showed clear unilateral muscle activity in stage 1 (Fig. 7A) and always showed stage 2 activity, and thus represents the most basal actinopterygian fish with this motor pattern. Stage 2 EMGs are also present in *A. calva* (Fig. 7), demonstrating the ability of these taxa outside of the Teleostei to actively control stage 2 bending.

Synchronous kinematics and EMG data have been gathered for the fast-starts of just three teleost species: *O. mykiss* (rainbow trout), *Carassius auratus* (goldfish; Foreman and Eaton 1993), and *Lepomis macrochirus* (bluegill sunfish; Jayne and Lauder 1993; Czuwala et al. 1999). The trout, goldfish, and sunfish all have clear stage 1 and stage 2 kinematic bursts (Fig. 7A). Whereas the trout has slight or no contralateral muscle activity during stage 1 (Fig. 7B), both the goldfish (Foreman and Eaton 1993) and the sunfish (Czuwala et al. 1999) appear to have significant bilateral activity (but see Jayne and Lauder [1993], in which bilateral activity is discounted). The paucity of muscle activity data on the fast-start and variability among those data highlight the need for a broader survey of motor control and behavior in this model system.

In summary, the distribution of startle behaviors and motor control features in vertebrates is a mosaic pattern of ancestral and derived features with homoplasy present in all characters except the origin of the Mauthner neuron. It remains equivocal whether the fast-start escape behavior initially evolved to activate muscle unilaterally or bilaterally (Fig. 7B). Determining the distribution of the M-cell and an assessment of stage 1 motor control in chimaeras and lungfishes may help to polarize fast-start features for the rest of the vertebrate tree.

We conclude that major evolutionary events in the evolution of the fast-start response of fishes include the evolution of the Mauthner neuron, commissural inhibition of activity, and the ability to generate a two-stage startle response. Among vertebrates, the startle response varies at multiple levels including neural activity, patterns of muscle contraction, and kinematics. Because of the variation in this behavior and the neural circuits responsible for it, the startle response of fishes provides an exciting opportunity to examine the evolution of axial locomotor systems and their neural basis in vertebrates.

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