THESIS

Fiber type distribution and tail muscle function in birds

Submitted By:

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KYLE B. SPAINHOWER ENTITLED "FIBER TYPE DISTRIBUTION AND TAIL MUSCLE FUNCTION IN BIRDS" BE ACCEPTED AS FULFILLING PARTIAL REQUIREMENTS FOR THE THESIS IN ZOOLOGY.

Thesis Committee

Advisor

Department Chair

Abstract

The tail apparatus of birds is adapted to perform multiple functions. Muscles contract to produce movements that lift, lower, turn, and fan the tail, which creates an auxiliary flight surface for takeoff, turning, braking, and landing. Tail movements are also incorporated into sexual and territorial displays. This muscle group must also possess a postural function for the tail; many birds spend time foraging on the ground, creating the need to hold the tail horizontally, presumably to avoid tail feather damage. Locomotor, display, and postural functions in tail muscles are mostly known, yet fiber composition is not well studied. These muscles facilitate multiple functions, thus their morphology and fiber composition are intriguing and warrant investigation. I used immunohistochemistry to quantify fast and slow fiber populations in four tail muscles from four Black-billed Magpies (*Pica hudsonia*), one Western Scrub-Jay (*Aphelocoma californica*), five Northern Flickers (*Colaptes auretus*), two Red-winged Blackbirds (*Agelaius phoeniceus*), three Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*), and two American Robins (*Turdus migratorius*) to ascertain a relationship between fiber composition and postural muscle function. M. levator caudae showed slow fibers in every individual. Flickers consistently showed slow fibers in M. depressor caudae. Slow fibers in M. levator caudae are probably associated with the ubiquitous need to maintain an elevated tail posture while foraging on the ground and are likely to be found in all birds with a tail. Slow fibers in M. depressor caudae may be associated with sustained tail depression in woodpeckers, a behavior that provides additional stability for excavating cavities and clinging to tree trunks.

Introduction

The evolution of flight has played a critical role in the diversity and success of birds. Morphological adaptations for flight reduce weight and increase rigidity, improving aerodynamic performance. For example, feathers are lightweight, resilient integumentary structures made of keratin that occur in a variety of shapes (Stettenheim 2000). Feathers on the wings are capable of creating lift because they are asymmetrical. The central shaft of the feather is closer to the leading edge than to the trailing edge, creating an airfoil that generates lift and powers flight (Lucas and Stettenheim 1972). Additionally, skeletal fusion simultaneously decreases weight and increases rigidity. The sacrum and pelvis are fused into a single unit, the synsacrum, which creates a rigid, compact platform that reduces deformation during flapping flight (Hildebrand and Goslow 2001). Reduction of distal forelimb skeletal elements reduces weight to accommodate large-scale wing oscillations (Norberg 1990). In modern birds, the tail skeleton is reduced to five or six free caudal vertebrae terminating with the rudder-shaped pygostyle (Feduccia 1996).

Over evolutionary time, the tail transitioned from a long structure resembling a palm frond to a shortened, fan-like structure, reflecting a shift in function (Pittman et al. 2013). The transition from a frond-like tail to a fan-like tail occurred via reduction in the number of caudal vertebrae and allowed for greater manipulation of the tail. Gatesy and Dial (1996) discussed that primitive bird tail skeletons, like that of *Archaeopteryx*, are made of 15-20 vertebrae, each with associated right and left tail feathers that protrude laterally. These feathers are fixed to the bone, limiting the movements of the tail. In contrast, modern bird tails can alter aerodynamic properties by movements in the vertebrae, feathers, or both. Thomas and Balmford (1995) demonstrated that a forked tail

maintains a higher lift-to-drag ratio than an unforked tail and is the most optimized shape for maintaining stable flight over various speeds and for generating lift during turning and slow flight.

The tail plays an important role in flight; it augments the wings in turning, braking, and landing. However, birds have evolved a variety of tail shapes and sizes, and aerodynamic performance only partially explains the diversity. For example, sexual selection produces extreme tail plumages in male birds (Winquist and Lemon 1994); the exaggerated peacock tail is selected because of its ability to attract mates despite its apparent detriment to flight control (Askew 2014). Additionally, tail movements can also be part of behavioral displays; blackbird species, for example, use tail postures and movements in their territory displays. A male Yellow-headed Blackbird (*X. xanthocephalus*) will perch at his boundary and spread his tail feathers and waggle the tail while calling to mark his territory (Twedt and Crawford 1995). The tail muscle group must also function posturally; many birds forage on the ground and hold their tails horizontally, presumably to avoid damaging the tail feathers. The multifunctional use of the tail appendage likely affects the composition of the underlying musculature.

Skeletal muscle is composed of long cylindrical cells called fibers that vary in force generation, shortening velocity, and stamina. Muscles include fibers that vary in contractile speed, which is determined by the physiological properties of the myosin isoform found in the fiber (Hermanson 1998). Type I fibers are highly aerobic, giving them a fatigue-resistant quality. They use adenosine triphosphate (ATP) slowly and have a lot of mitochondria, and thus are both efficient and economic for slow, repetitive movements (Goldspink 1977). Type I fibers are found in postural muscles, and henceforth referred to as slow fibers. Type II fibers are fast contracting and capable of

generating more force but fatigue more quickly because they have fewer mitochondria and therefore are less aerobic (Goldspink 1977). These fibers are found in dynamic muscles, and henceforth referred to as fast fibers. Accordingly, fiber composition is related to muscle function, and the proportion of slow and fast fibers in the muscles of a multifunctional appendage, such as the bird tail, would reflect the predominance of postural or dynamic activities.

My goals were to analyze the tail muscles of six bird species for the presence of slow fibers, and to determine if a relationship exists between fiber composition and postural muscle function. I present data from immunohistochemistry analyses of four tail muscles from six bird species and propose structure-function relationships.

Methods

Tissue Collection

Tail muscles from four Black-billed Magpies (*Pica hudsonia*), one Western Scrub-Jay (*Aphelocoma californica*), five Northern Flickers (*Colaptes auretus*), two Red-winged Blackbirds (*Agelaius phoeniceus*), three Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*), and two American Robins (*Turdus migratorius*) were dissected for anatomical comparison and fiber-typing. Specimens were selected based on availability, stored frozen, and then thawed prior to dissection. Muscle portions approximately 1 cm long were removed mid-belly and mounted in 5% gum tragacanth on cork so that the fibers lay perpendicular to the block. Samples were frozen in isopentane cooled in liquid nitrogen and stored at -80° C. Frozen tissue was sectioned at 10 μm on a freezing microtome (-20° C) and mounted on glass slides for immunohistological studies. The remaining muscles, synsacrum, hindlimbs, and tail were removed as a single unit and stored in formalin for anatomical studies.

Histology

Following protocol described by Meyers and Stakebake (2005), immunohistochemistry was used to identify fast and slow fiber types. ALD 58 anti-slow antibodies and F 18 anti-fast antibodies were obtained through Hybridoma Bank (University of Iowa). The two antibodies produce non-overlapping reactions (Figure 1). Following sectioning, tissue was reacted against the antibodies for two hours at 25° C in a humid chamber. Slides were rinsed in phosphate buffered saline, and then incubated in goat anti-mouse antibody (ImmPRESS Reagent Kit, Vector, Burlingame, CA) and stained with a peroxidase kit (AEC Peroxidase Substrate Kit, Vector, Burlingame, CA). Slides were cover-slipped, and photographed on a microscope (Canon Rebel T1i, Zeiss Axioskop 40). Photographs were merged in Adobe Photoshop 2.0 on a Macintosh computer then printed. I quantified fiber populations by counting fast and slow fibers on the printed images. For statistical analysis, percent means ± SD for slow fibers were calculated.

Anatomy

The following anatomical descriptions are for pigeon tail muscles and are included for general reference (Figure 2); see Baumel's work on the pigeon tail apparatus (1988) for full descriptions. All muscles are paired, consisting of right and left sides.

Lateral Flexion/Tilt

Lateral flexion (i.e. side-to-side movement), and tilting are produced by Mm. caudofemoralis and lateralis caudae. M. caudofemoralis arises anteriorly from the femur and inserts along a crest on the posterior border of the pygostyle. Its action for the tail is to produce lateral flexion. M. lateralis caudae arises from the posterior synsacrum and tranverse processes of the three proximal free caudal vertebrae. The muscle is narrow

anteriorly and spreads as it travels posteriorly towards insertion. The broad posterior portion inserts on the dorsal and lateral surfaces of the rectricial bulbs. When Mm. lateralis caudae and caudofemoralis are activated ipsilaterally, they cause lateral flexion. Contralateral contraction of these muscles tilts the tail; lateralis caudae pulls the tail up and caudofemoralis lowers the tail, causing the tail to rotate about the body axis.

Elevation

Tail dorsiflexion (i.e. elevation) is produced by M. levator caudae. It arises from the fixed caudal vertebrae that are fused with the synsacrum and the spinous processes of the four proximal free caudal vertebrae and inserts on the distal free caudal vertebrae by way of short digitations. The posterior-most digitation continues as a tendon beyond the free caudal vertebrae and inserts on a notch in the anterior border of the pygostyle. M. levator caudae lifts the caudal vertebrae and rotates the bulbs dorsally.

Depression

Tail ventriflexion (i.e. depression) is produced by M. depressor caudae. It arises from ventral surface of the fixed caudal vertebrae and first free caudal vertebra. The muscle continues as an aponeurosis and inserts on the distal free caudal vertebra, pygostyle, and rectricial bulbs.

Results

Histology

Table 1 summarizes the data that I collected from the fiber-type analyses. These data are recapitulated in the text below. Fibers that reacted positive against the slow antibody are presumed to be slow contracting and fibers that reacted positive against the fast antibody are presumed to be fast contracting.

Lateral Flexion/Tilt

M. caudofemoralis was uniformly fast in four species and had small numbers of slow fibers in two species. All samples from Black-billed Magpies ($n = 4$), Western Scrub-Jay ($n = 1$), Yellow-headed Blackbirds ($n = 3$), and Red-winged Blackbirds ($n = 2$) were composed of 100% fast fibers. One American Robin ($n = 2$) sample had no slow fibers and the other had 0.16% slow. Three of the five Northern Flickers had slow fibers in M. caudofemoralis (Figure 3). The sample with the greatest proportion of slow fibers contained 1.42%. Slow fibers appeared to be distributed with no particular spatial arrangement (Table 1).

M. lateralis caudae was completely fast in three species, mixed in one species, and composition varied in two species. I found 100% fast fiber composition Northern Flickers (Figure 4), Western Scrub-Jay, and Red-winged Blackbirds. All four Black-billed Magpie samples possessed mixed fiber populations, averaging 4.57±4.01% slow fibers in one sample. Slow fibers appeared to be evenly distributed through the muscle. I found few slow fibers in one robin (0.89%) and two Yellow-headed Blackbirds (0.84%, 6.76%) (Table 1).

Depression

M. depressor caudae was mixed in two species, completely fast in three species, and of varied composition in one species. I found slow fibers in all samples from Northern Flickers (Figure 5), averaging 7.40±4.95%. Slow fibers clustered medially and thinned laterally. Both robins had very small numbers of slow fibers, corresponding to 0.31% and 0.43% composition. Slow fibers appeared near the edges of the muscle. I found no slow fibers in M. depressor caudae of the Western Scrub-Jay or in either blackbird species (Table 1).

Elevation

All species examined had both fast and slow fibers in M. levator caudae. I found the fewest number of slow fibers in Northern Flickers (Figure 6), ranging from 0.9% to 4.12% composition. I found the greatest number of slow fibers in Black-billled Magpies, ranging from 7.34% to 11.71% composition. The Western Scrub-Jay had 2.93%, American Robins averaged 6.1%, Yellow-headed Blackbirds averaged 6.28%, and Red-winged blackbirds averaged 4.79% slow fibers (Table 1).

Discussion

Functional Interpretation

As mentioned above, fiber composition is related to muscle function. In general, dynamically active muscles have more fast fibers than slow fibers and postural muscles are reversed, increasing slow fiber populations and decreasing fast fiber populations. Olson et al. (2016) investigated scratch-digging behavior in nine-banded armadillos and showed that the powerful limb retractor muscles are composed primarily of fast fibers, contributing to high force output of these muscles, whereas the elbow extensors have a large population of slow fibers, increasing fatigue resistance. Like the forelimb of the nine-banded armadillo, bird tails are multifunctional appendages, and the distribution of fast and slow fibers should indicate functional demand. Thus, we can make functional predictions based on patterns of fiber distribution in the tail muscles.

Functionally Significant Fiber Populations

One of the challenges of interpreting muscle fiber proportions is determining how many fibers constitute a functionally significant population in mixed-fiber muscles. I have observed a variety of slow fiber proportions in different muscles, ranging from less than 0.5% to nearly 14%. Can a 0.5% slow fiber population be functionally meaningful? Sokoloff et al. (1998) showed that three motor units from pigeon pectoralis averaged 274

fibers, producing an average force of 0.033 N, which corresponds to 3.4 grams (mass = force/gravity). Theoretically, 200 fibers in one tail muscle can hold an eight-gram tail (because muscles are paired).

Lateral Flexion/Tilt

M. caudofemoralis is functionally fast in all species studied. This corresponds well with the high activity cost of locomotion. The fiber composition of M. caudofemoralis is well suited for its primary function as a leg retractor. Fast fibers are appropriate for powering high-speed activities like running.

M. lateralis caudae was uniformly fast in the Northern Flickers, Western Scrub-Jay, American Robins, Yellow-headed blackbirds, and Red-winged Blackbirds. This also corresponds well with the dynamic activity for this muscle. In contrast, Black-billed Magpies showed slow fibers in all individuals. Having a slow fiber population in M. lateralis caudae is perhaps an adaptation for magpie courtship display. The slow fibers in M. lateralis caudae of the magpie can be explained by functional specialization. Magpies are known to angle their tail towards a potential mate during courtship (Trost 1999), and presumably none of the other species are known to exhibit this behavior. Slow fibers in M. lateralis caudae may increase the efficiency of tail pointing.

Depression

What sort of postural function might M. depressor caudae in flickers serve? Northern Flickers are a species of woodpecker, which is a group of birds known for drumming and adaptations that accommodate the behavior. For example, the central tail feathers have a thickened shaft that allows woodpeckers to prop the tail against a tree trunk for support while drumming. M. depressor caudae causes the tail to deflect ventrally; the fatigue-resistant nature of the slow fibers in this muscle likely enhances

efficiency in sustained tail depression. Slow fiber type distribution in M. depressor caudae is likely an energy saving adaptation associated with flicker drumming behavior. Just as we see adaptations like a sturdy bill, strong tail feathers, and complex toe arrangements - characteristics that enhance climbing and drumming - I predict that all woodpecker species possess slow fibers in M. depressor caudae.

Elevation

M. levator caudae is a mixed-fibered muscle in all individuals examined; the reason for this may be the need to resist the gravitational downward pull of the tail. In other words, force produced by M. levator caudae counteracts the weight of the tail. Based on this thinking, and that all M. levator caudae samples had slow fibers, I predict that all birds with a tail possess mixed fibers in this muscle.

The number of slow fibers in M. levator caudae may correspond with tail size, feather size, and species-dependent behaviors. For example, of all the species that I examined, flickers had the fewest slow fibers in M. levator caudae, corresponding to an average of 170 fibers, or about 2% of the muscle. In contrast, magpies showed the greatest proportion of slow fibers in M. levator caudae, corresponding to about 1480 fibers, or about 10% of the muscle. The slow fibers in M. levator caudae may represent an adaptation for bird species with terrestrial habits, and the difference in slow fiber composition between flickers and magpies may be due to species-dependent requirements with respect to maintenance of the tail feathers. Flicker tail feathers are shorter relative to body length and the distal barbs do not interlock, creating a resilient feather tip. In terms of force production, slow fibers in the paired M. levator caudae muscles of flickers could produce enough force to lift a four gram mass, and a 2% slow fiber population likely accommodates functional demand. On the other hand, slow fibers in the paired M.

levator caudae muscles of magpies could produce enough force to lift a thirty-seven gram mass. It is known that magpie tail length and quality are secondary sexual characteristics for both sexes; having a healthy, long tail increases the likelihood of finding a mates (Fitzpatrick and Price 1997), which creates a greater need prevent damage to the tail that might occur when ground-foraging.

Baier and Gatesy (2000) reported finding slow fibers in turkey levator caudae, but did not provide any numbers. Turkey courtship display involves erecting the tail, maintained by isometric contraction of M. levator caudae. It is likely that bird species with erected tail displays have a higher proportion of slow fibers in M. levator caudae than those that do not display.

Individual Variation

A developmental explanation reconciles the fact that some individuals had a small number of slow fibers when other samples from the same species had no slow fibers. Rosser et al. (1998) showed that slow fibers were present in developing pigeon pectoralis and persisted as a small proportion in adults. Muscle fibers do transition from slow to fast myosin isoforms. The small numbers of slow fibers found in the muscles of some individuals may be evidence of developmental holdover.

Concluding Remarks

In summary, this study presents immunohistological data that appear to support postural functions in the bird tail muscle group. I used immunohistological reactions to identify slow fiber populations in M. levator caudae that may be associated with the maintenance of an elevated tail posture. Slow fiber proportions may be related to tail size and species-dependent behaviors; bigger tails require more slow fibers for support and if tail quality is a secondary sex characteristic, more slow fibers are required to maintain an

elevated tail posture in order to avoid damage. Future work could investigate whether the proposed relationship between slow fiber proportions and tail elevation requirements exists.

I also discovered functionally meaningful slow fiber populations in magpie M. lateralis caudae and flicker M. depressor caudae that appear to be related to species-dependent postural and locomotor requirements. The slow fibers in the magpie lateral flexor a likely involved in courtship display, decreasing energy requirements for sustained muscle contraction. The slow fibers in the tail depressor of flickers are likely to sustain tail depression without succumbing to fatigue quickly. In the future, I hope to quantify muscle fiber types in M. depressor caudae in additional woodpecker species to confirm my prediction that this muscle is mixed-fibered in all woodpeckers.

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List of Figures

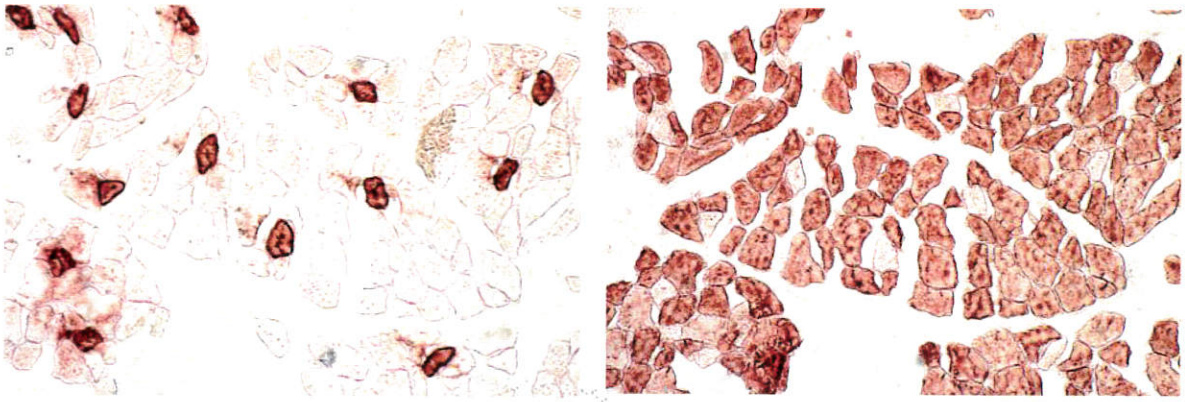

Figure 1. Serial sections of muscles reacted against ALD 58 (A) and F 18 (B). A red staining indicates a positive reaction. The red stained fibers in the muscle on the left are unreacted in the right image, showing that these are non-overlapping reactions.

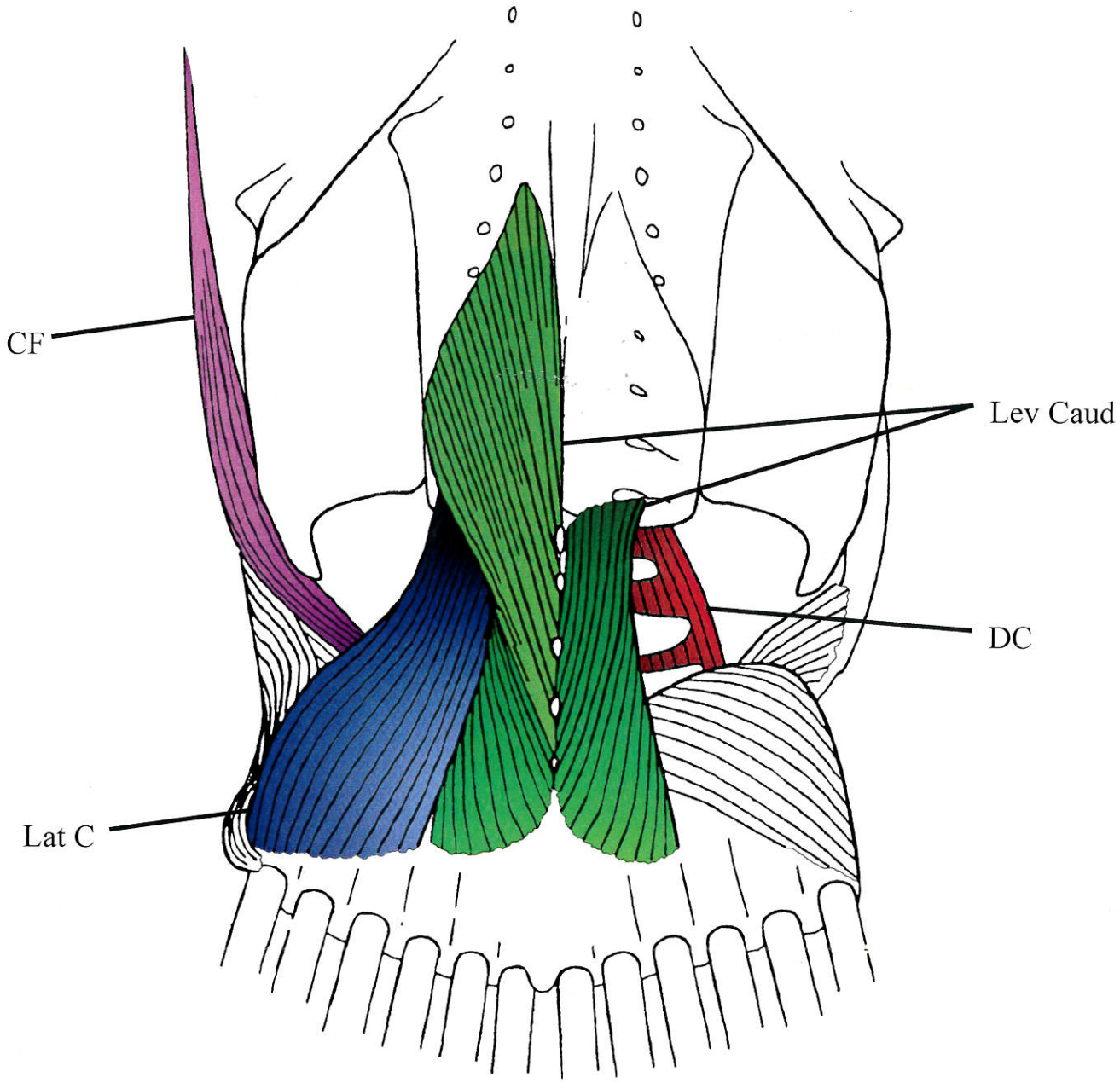

Figure 2. Pigeon tail anatomy from dorsal view adopted from Gatesy and Dial (1996). The head would be at the top of the figure, and the tail feather shafts are pointed downward. Superficial muscles are highlighted on the left and removed from the right to reveal deep musculature. All muscles have left and right pairs. Tissue was taken from the muscle mid-belly. CF: M. caudofemoralis; DC: M. depressor caudae; Lat C: M. lateralis caudae; Lev C: M. levator caudae

Figure 3. M. caudofemoralis immunohistochemical fiber profile for Northern Flicker after applying the ALD 58 antibody. This muscle was positive for seven slow fibers. Scale bar = 0.5mm

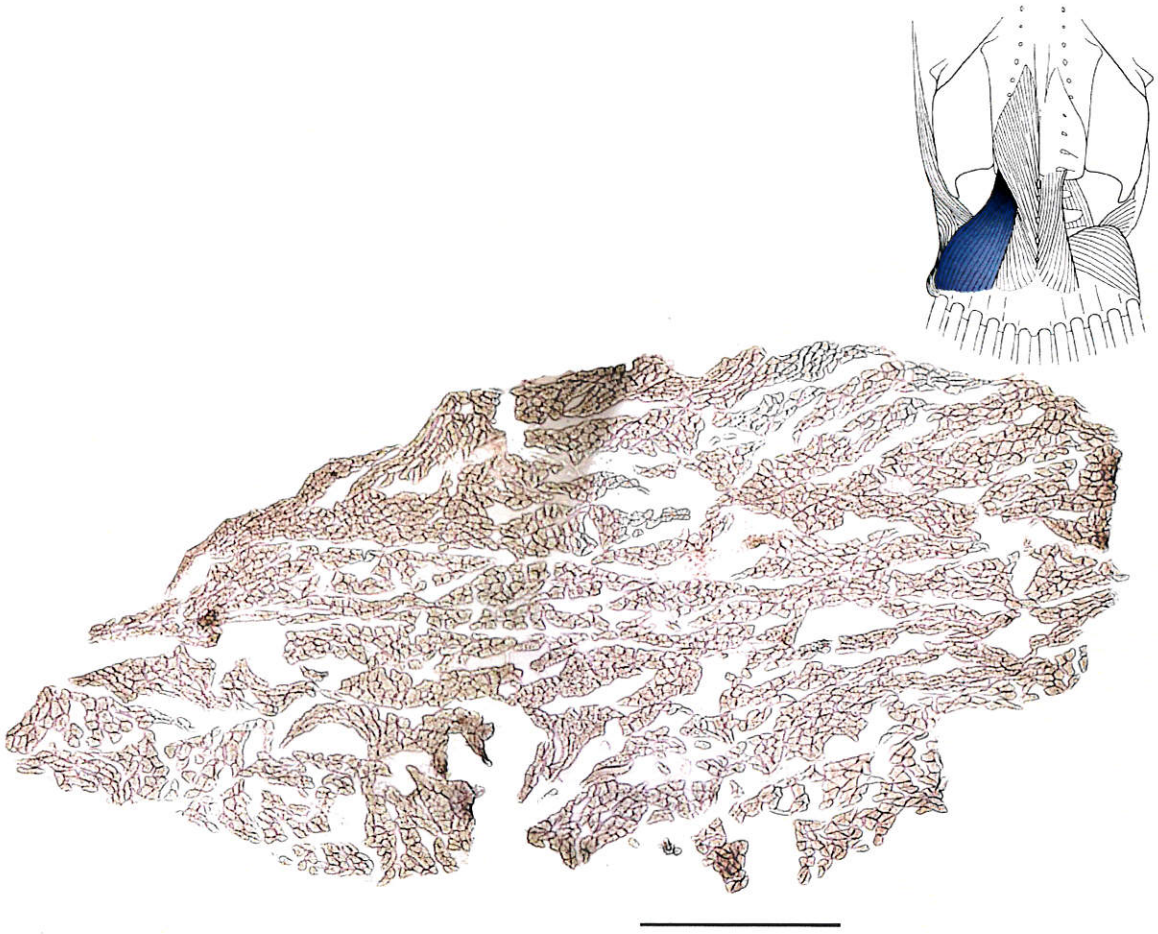

Figure 4. M. lateralis caudae immunohistochemical profile for Northern Flicker after applying the ALD 58 antibody. This reaction is negative for fast fibers.
Scale bar = 0.5mm

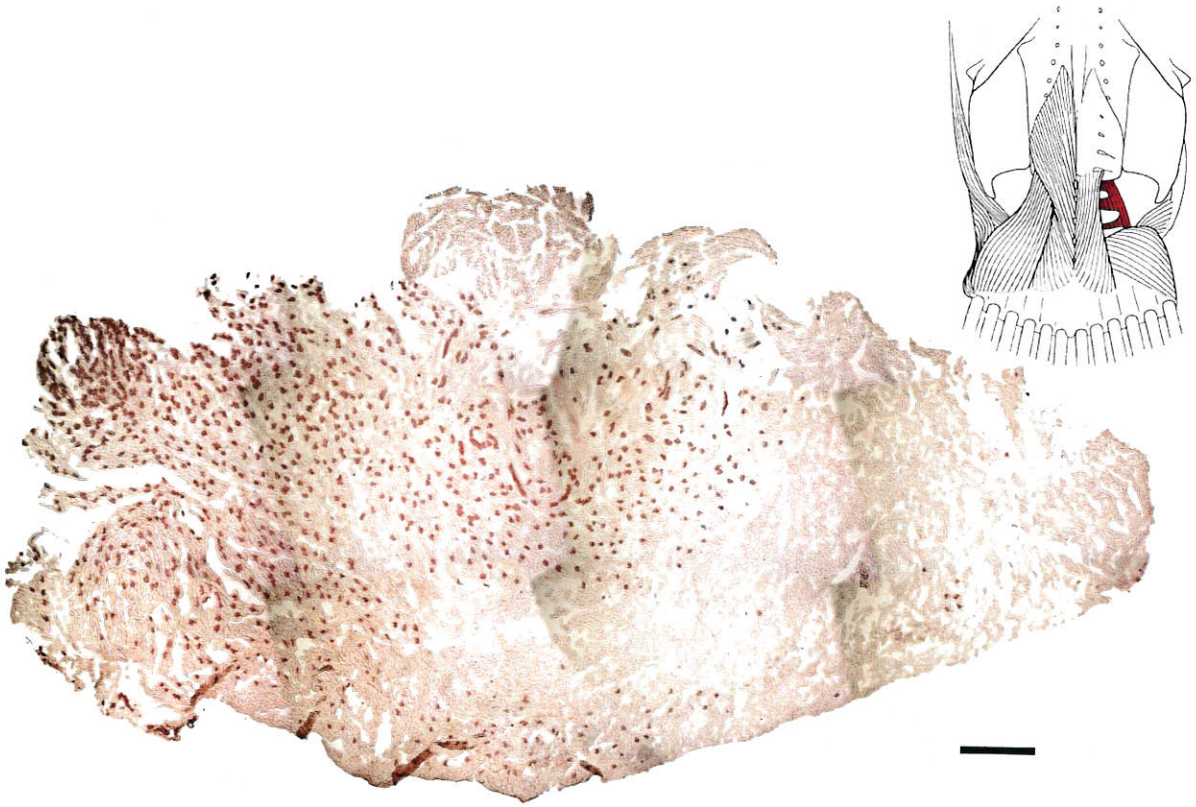

Figure 5. M. levator caudae immunohistochemical profile for Northern Flicker after applying the ALD 58 antibody. This area was positive slow fibers, which clustered medially. Scale bar = 0.5mm

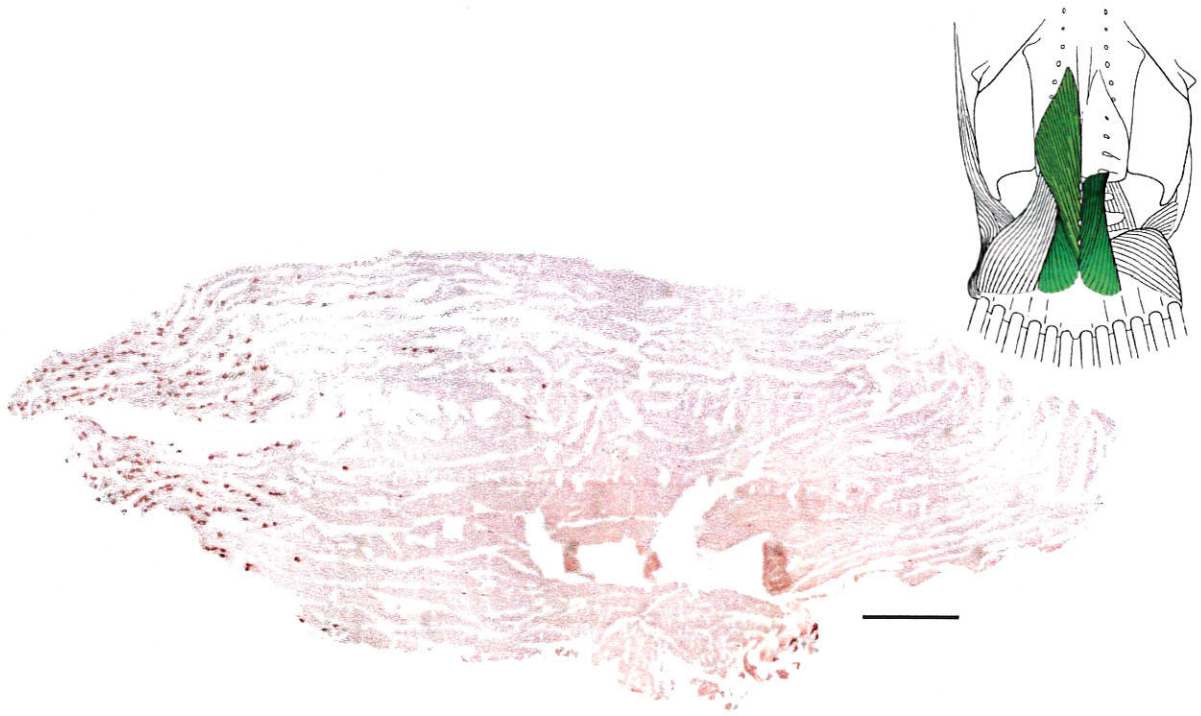

Figure 5. M. levator caudae immunohistochemical profile for Northern Flicker after applying the ALD 58 antibody. This area was positive slow fibers, which clustered medially. Scale bar = 0.5mm

Table 1. Slow fiber distribution of four tail muscles in six bird species

Muscle	Species	Fast Fibers	Slow Fibers	% Slow
Caudofemoralis	Flicker 1	2681	0	0
	Flicker 2	3373	7	0.21
	Flicker 3	5790	19	0.33
	Flicker 4	1256	0	0
	Flicker 5	2293	33	1.42
	Mean±SD	**3079±1698**	**12±14**	**0.39±0.59**
	Magpie 1	2873	0	0
	Magpie 2	4045	0	0
	Magpie 3	2546	0	0
	Magpie 4	3323	0	0
	Mean±SD	**3197±649**	**0**	**0**
	Scrub-Jay	1913	0	0
	Robin 1	*	0	0
	Robin 2	3102	5	0.16
	Mean	**N/A**	**N/A**	**0.08**
	Yellow-head 1	*	0	0
	Yellow-head 2	*	0	0
	Yellow-head 3	*	0	0
	Mean	**N/A**	**0**	**0**
	Red-wing 1	*	0	0
	Red-wing 2	*	0	0
	Mean	**N/A**	**0**	**0**
Lateralis Caudae	Flicker 1	3141	0	0
	Flicker 2	1228	0	0
	Flicker 3	2001	0	0
	Flicker 4	2120	0	0
	Flicker 5	1873	0	0
	Mean±SD	**2073±690**	**0**	**0**
	Magpie 1	3100	100	3.13
	Magpie 2	3648	49	1.33
	Magpie 3	2216	258	10.43
	Magpie 4	2361	83	3.4
	Mean±SD	**2831±668**	**123±93**	**4.57±4.01**
	Scrub-Jay	900	0	0
	Robin 1	1109	10	0.89
	Robin 2	1130	0	0
	Mean	**1120**	**5**	**0.45**
	Yellow-head 1	759	55	6.76
	Yellow-head 2	1536	13	0.84
	Yellow-head 3	*	0	0
	Mean±SD	**1148**	**23±29**	**2.53±3.68**

	Red-wing 1	*	0	0
	Red-wing 2	*	0	0
	Mean	**N/A**	**0**	**0**
Depressor Caudae	Flicker 1	12753	1174	8.43
	Flicker 2	7602	190	2.44
	Flicker 3	8970	981	9.86
	Flicker 4	7149	1148	13.84
	Flicker 5	7300	182	2.43
	Mean±SD	**8755±2348**	**735±507**	**7.4±4.95**
	Magpie 1	7378	414	5.31
	Magpie 2	5665	0	0
	Magpie 3	12500	516	3.96
	Magpie 4	17921	0	0
	Mean±SD	**10866±5527**	**233±272**	**2.32±2.73**
	Scrub-Jay	4879	0	0
	Robin 1	5450	17	0.31
	Robin 2	5398	23	0.43
	Mean	**5424**	**20**	**0.37**
	Yellow-head 1	*	0	0
	Yellow-head 2	*	0	0
	Yellow-head 3	*	0	0
	Mean	**N/A**	**0**	**0**
	Red-wing 1	*	0	0
	Red-wing 2	*	0	0
	Mean	**N/A**	**0**	**0**
Levator Caudae	Flicker 1	5731	246	4.12
	Flicker 2	8554	103	1.19
	Flicker 3	9719	217	2.18
	Flicker 4	8499	77	0.9
	Flicker 5	12185	208	1.68
	Mean±SD	**8938±2333**	**170±75**	**2.01±1.27**
	Magpie 1	11334	1503	11.71
	Magpie 2	15538	1740	10.07
	Magpie 3	11089	1383	11.09
	Magpie 4	16259	1288	7.34
	Mean±SD	**13555±3773**	**1479±735**	**10.05±1.93**
	Scrub-Jay	8621	260	2.93
	Robin 1	6887	598	7.99
	Robin 2	4212	185	4.2
	Mean	**5550**	**392**	**6.1**
	Yellow-head 1	5580	506	8.31
	Yellow-head 2	1952	117	5.65

Yellow-head 3	8347	428	4.88
Mean±SD	**5293±3207**	**350±206**	**6.28±1.8**
Red-wing 1	3813	167	4.2
Red-wing 2	4152	236	5.38
Mean	**3983**	**202**	**4.79**

* Indicates that fibers were not counted due to absence of mixed fiber populations.
N/A Indicates that the means and standard deviations were not calculated for muscles where fibers were not quantified.