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A Histological and Qualitative Analysis of Muscle Spindle Density in the Distal Head of M. Extensor Longus Digiti Majoris

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ABSTRACT

Inconspicuous muscles in avian taxa have been previously discussed as vestigial remnants, however, evidence has shown that such muscles may hold a function in flight. Further investigations into inconspicuous muscles, such as *M. coracotriceps*, have demonstrated a muscle spindle density much greater than surrounding muscles. A higher density of muscle spindles suggests a proprioceptive function during flight. We investigated another diminutive muscle that may bear a similar function, the distal head of *M. extensor longus digiti majoris* (ED). Studies included a qualitative histological analysis comparing the density of muscle spindles in the ED to the adjacent proximal head of the *M. extensor longus digiti majoris* (EP) in 15 total samples excised from *Columba livia* (rock pigeon) and *Gallus gallus* (jungle fowl). EP is a functional muscle that serves as an osteo-pulley for the major digit, which we analyzed the ED against. Based on our qualitative histological analysis of ED, this muscle does not demonstrate a greater muscle spindle density compared to the EP. Although further research of this muscle in other Avian species may be necessary to discern its function, our research demonstrates no evidence of a proprioceptive function.

Keywords: Avian Species, Histology, Muscle Spindles, Flight

INTRODUCTION

Muscle spindles are intrafusal fibers that function to provide proprioceptive cues to the central nervous system for coordination via stretch receptors and provide reflex activity for motor neurons (*1*). Higher densities of muscle spindles are reported in avian muscles presumably to serve as kinesiological monitors during flight (*2*). Muscles with higher spindle density generally function in fine movements, postural control, and provide important mechano-sensory feedback to the central nervous system, indicating a proprioceptive function (*3*). Muscle spindles have also been seen in higher densities within avian skeletal muscles with slow-twitch fibers as opposed to their fast-twitch counterparts (*4*). This demonstrates that muscles with a higher density of muscle spindles are likely involved in sustained flight as opposed to the fast twitch muscles involved in takeoff and landing (*4*). These kinesiological monitors are often smaller, shorter muscles that act across a joint in parallel to a larger, longer muscle (*5*).

An example of a muscle with a proprioceptive function is the inconspicuous *M. coracotriceps* (*6*). This is a minute muscle that extends between the tendons of *M. expansor secundarium* and tendon of *M. humerotriceps*, and because of its size, it was previously regarded as vestigial. Eventually, research and analysis of this muscle discovered that *M. coracotriceps* was a slow tonic muscle with a muscle spindle density significantly greater than the adjacent, parallel, and functional *M. humerotriceps* (*7*). This discovery demonstrated that small and undervalued muscles can play an important role during flight.

Another example of a similar anatomical system in Aves is the distal head of *M. extensor longus digiti majoris* (ED). The ED has a tendinous insertion onto the tendon of the proximal head of the *M. extensor longus digiti majoris* (EP), which is located along the proximal end of the major metacarpal *(8)*. This orients the smaller muscle belly, ED, in parallel with the larger muscle belly, EP (Fig. 1). The EP functions as an osteo-pulley to guide muscle tendons and change the direction of pull of the major digit (*8*). However, there are limited details available on the function of the ED and ideas on its function have been varied among sources with some researchers regarding it as nonfunctional or rudimentary (*6*). Alternatively, the use of ED as a kinesiological monitor with a dense concentration of muscle spindles is an additional possibility. There is limited evidence to support either argument, as there are few details available describing the function of the muscle.

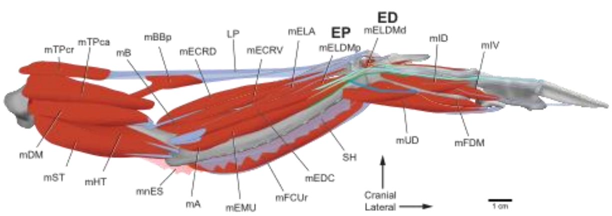

Figure 1. Shown is a figure of muscle architecture of the forelimb in Columbia Livia. The elongate proximal head of the M. extensor longus digiti majoris (EP), labeled above mELDMp , can be visualized here with its tendon course into the major digit. The ED, labeled above mELDMd, is a tiny muscle belly located adjacent to the EP with a tendinous insertion into the tendon of EP. Copyright 2016. Adapted with permission *(15).*

Additionally, the ED is present in only certain avian taxa. Distal limb reduction is an evolutionary pattern used by certain birds to eliminate unnecessary weight that is costly to takeoff, inertia, and landing during flight (*9*, *10*). The evolutionary pattern depicts that birds that had distal limb reduction have lost the ED, while those without distal limb reduction have retained it. Species such as swifts and members of the "ground bird" assemblage, Cavitaves, have eliminated this muscle belly (*11*). These birds also have a flight style of greater wingbeat frequency. However, this muscle is preserved in rock pigeons, which do not exhibit distal limb reduction nor have a pattern of high wingbeat frequency (*11*). This evolutionary pattern raises additional unanswered questions about the function of ED. To investigate the function of the ED, we examined the quality of muscle spindle density to determine if it is a feasible kinesiological monitor during flight.

METHODS

The ED and EP were excised from five-frozen *Columba livia* (rock pigeon) and four *Gallus gallus* (jungle fowl). Four samples of ED and 4 EP were excised from *Columba livia* while 3 ED and 4 EP were excised from *Gallus gallus.* In total, we excised 7 ED and 8 EP (15 total samples). Each muscle belly was isolated and left overnight in 10% formalin, then washed two times in PBS solution, and finally dehydrated and stored in 70% ethanol. Next, the muscle bellies were processed in the Leica ASP300 Enclosed Tissue Processor (Leica Biosystems, Buffalo Grove, IL) for 24 hours. The following day, the muscle tissue was embedded in paraffin blocks and sectioned at 5-7µm thickness using a microtome and left overnight to dry on microscope slides. The sections were next stained using Hematoxylin and Eosin (H&E). The muscle tissues were examined under a light microscope. Fifteen sections were qualitatively examined for muscle spindles, 8 ED and 7 EP. The muscles were qualitatively inspected to examine the muscles under a light microscope and record if there were any obvious differences in muscle spindle density between the EP and the ED in both species. Based on the results of muscle spindle density in the *M. coracotriceps*, 6.33 muscle spindles per gram of muscle, we kept our threshold at six muscle spindles per gram of muscle to qualify it as a kinesiological monitor. We estimated muscle mass based on linear measurements. Samples were approximately 1 cm in length and diameter. Based on published estimates of muscle density at 1.06g/cm^3 we estimated mass of ED to be 1.06g (*12*).

RESULTS

Examination of the 8 samples of ED showed no qualitative difference in muscle spindle density in comparison to the 7 samples of EP. We did not find muscle spindles in the density expected of a kinesiological monitor. Out of the 15 samples examined, we did not notice a higher density of muscle spindles in the ED in comparison to the EP. Each muscle belly demonstrated approximately 0-2 total muscle spindles (Fig. 2), with consistent results across the ED and EP. The lack of a large quantitative count of muscle spindles allowed for quick inspection for each section. This finding was consistent across both species and there were no qualitative differences between them.

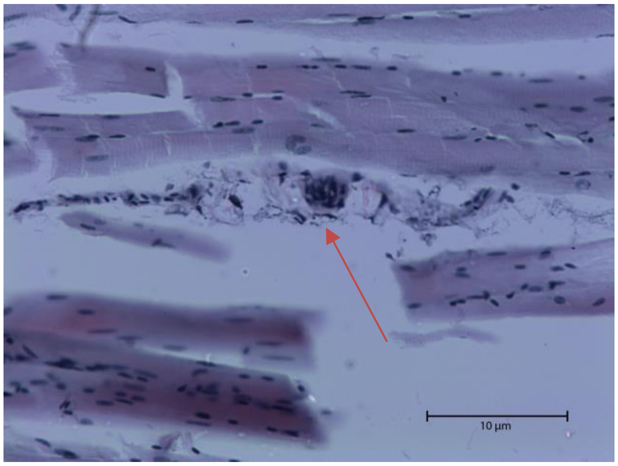

Figure 2. Pictured is a light microscopic photograph of a muscle spindle (red arrow) in the proximal head of the M. extensor longus digiti majoris (EP) in *Columba livia* (rock pigeon). Stained with hematoxylin and eosin. 20X objective. Image courtesy of Dr. Tobin Hieronymus.

DISCUSSION

Given the relative muscle spindle density in ED not exceeding that of EP, this muscle is unlikely to be a kinesiological monitor. This contrasts with the *M. coracotriceps* which showed a total muscle spindle density of over 14000 spindles per 2300 grams of muscle (15 times greater than any other muscles in the avian species) (*7*). This is a much higher density than the total 0-2 spindles examined in each of 15 samples of ED and EP, which were in the same order of magnitude in size as 1 gram of *M. coracotriceps*.

The evolutionary pattern of ED shows both preservation and loss in different bird species. The presence of this muscle belly is preserved in species that do not have distal limb reduction, such as jungle fowl and rock pigeons (*11*). This muscle is not retained in species such as perching birds (Passeriformes), swifts, members of the ground bird assemblage (Cavitaves), and members of the "metavian" assemblage (cuckoos, cranes, and allies) some of which show a pattern of distal limb reduction (Fig. 3) (*11*,*13*). Species that have undergone distal limb reduction have a flight style which demonstrates high beat wing frequency (*13*). There is reasoning that extra weight with vestigial structures in avian hands may be costly to inertia during flight (*10*). It has been previously demonstrated that higher intensity action of distal wing musculature is present during takeoff and landing for Aves during flight (*10*). During active flight, birds are efficient in minimizing muscle contractions and saving metabolic energy (*10*). A vestigial structure present in many clades would go against the efficiency of birds in flight. Therefore, this evolutionary pattern suggests that the ED is not of use in Aves with distal limb reduction, however, it may still serve a function in Aves that have retained it.

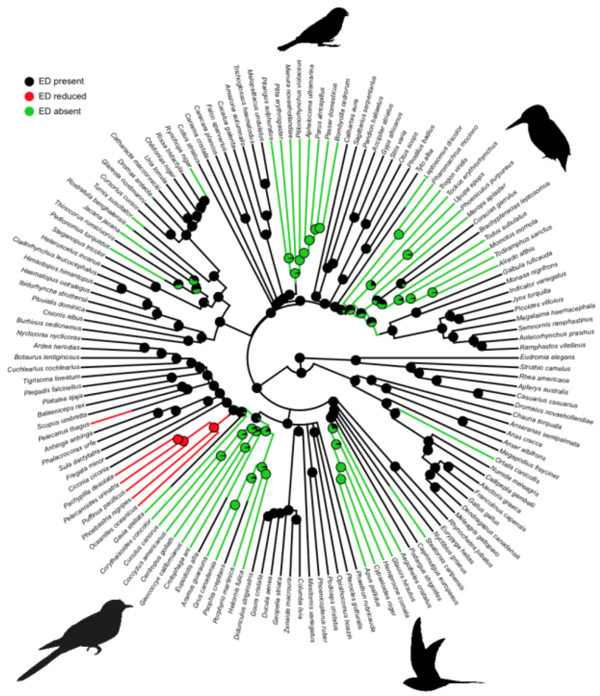

Figure 3. Phylogenetic Tree of the ED retention. Character states drawn from Livezey and Zusi (2007). Silhouettes from PhyloPic.org : *Hemiprocne mystacea* by Jerry Oldenettel (vectorized by T. Michael Keesey). *Passer domesticus* by Andrew Butko (modified). All silhouettes CC BY-NC-SA 3.0. creativecommons.org/licenses/by-nc-sa/3.0/. All other silhouettes public domain *(11)*.

Another possible function of ED is based on its anatomical characteristics and relationship with EP. The tract of the muscle tendon of the EP is from the forearm to the major metacarpal in the hand and serves as the insertion point for the distal head (Fig. 1). The distal head inserts into the tendon at the proximal head of the major metacarpal. The EP has a dual function of digit extension and wrist flexion (*8*). ED inserts directly in the middle of the tract of the tendon for EP, therefore, for EP to produce the action of wrist flexion, ED may be serving a function to decouple digit extension from EP to wrist extension.

A similar example is the *quadratus plantae,* which corrects the tendon tract of the *flexor digitorum longus* in the feet of humans (Fig. 4). This also is a smaller muscle which has a tendinous insertion onto a larger muscle's tendon in a similar structure (avian hand vs human foot). *Quadratus plantae* realigns the tendon of *flexor digitorum longus* in human feet and allows it to properly flex the second through fifth digits in the foot. The architectural resemblance between ED and EP in Aves and *Quadratus plantae* and *flexor digitorum longus* in humans supports the idea that ED may be performing a similar role as *Quadratus plantae*.

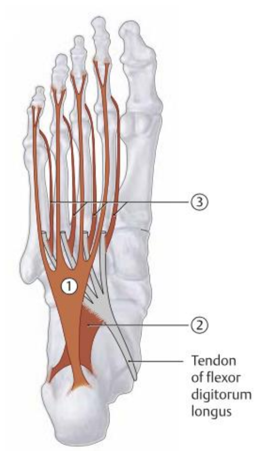

Figure 4. *Quadratus Plantae* (labeled as 2) inserting on the tendon of the *flexor digitorum longus*, allowing for proper flexion of digits 2-5. This file is licensed under the Creative Commons Attribution-Share Alike 4.0 International License. Image courtesy of Powellle 2020. https://commons.wikimedia.org/wiki/File:2_-_quadratus_plantae.png *(16)*

There are several limitations to our study. Firstly, we did not examine the muscle tendons under a light microscope, as we narrowed our focus on the muscle belly during the study. Although muscle spindles are the primary organ involved in proprioception, muscle tendons may contain Golgi Tendon Organs (GTO) which can also serve as proprioceptive monitors (*14*). Additionally, our sample size was only 15 total samples, and statistical analysis was not performed (due to the qualitative approach); additional samples and access to additional species would have made this study more effective. A quantitative analysis rather than a qualitative analysis would have allowed for a more thorough analysis and the inclusion of a statistical evaluation to definitively prove our findings; however, a qualitative analysis was performed due to time constraints and limited access to samples. Despite this, qualitative research on topics not previously explored, such as the ED and EP, can still provide meaningful insight for future studies. Therefore, future steps of this study include to expand it to multiple species with different flight patterns, such as perching birds and swifts, and additionally gathering a larger number of samples. In addition, testing the function of the muscle based on the anatomic characteristics mentioned above with techniques such as *in-vitro* contracture testing could help identify the function of the ED.

CONCLUSIONS

Our findings do not demonstrate increased muscle spindle density in the ED, in comparison to the EP, of *Gallus gallus* and *Columba livia*. Since muscles with proprioceptive functions typically demonstrate a high amount of muscle spindles, the ED is not likely to function as a kinesiological monitor. While we believe that further quantitative research on the ED among a wider variety of species may show different results, our research does not demonstrate any evidence of a proprioceptive function. Phylogenetic distribution suggests this muscle is not of use in Aves with distal limb reduction, however, it may still hold a function in the Aves that have retained it. A plausible function of ED is that it decouples digit extension of the EP to wrist extension, further supported by a similar anatomical architecture in the *Quadratus plantae* in the foot of humans. Future directions include gathering more evidence on the characteristics of the ED by expanding the study across more species of Aves with different flight patterns, and include testing the anatomical function of the ED.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: AP, TH

Methodology: AP, TH

Investigation: AP, TH

Supervision: TH

Writing – original draft: AP, TH

Writing – review & editing: AP, TH