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Manduca sexta as an Animal Model of Muscle Aging

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MANDUCA SEXTA AS AN ANIMAL MODEL OF MUSCLE AGING

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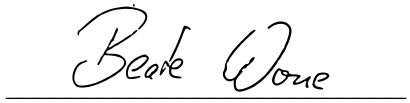

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ABSTRACT

Manduca sexta as an Animal Model of Muscle Aging

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Aging has a multisystemic effect on an organism's functional physiology and quality of life. One such effect is the progressive loss of muscle mass, strength, and function, known as sarcopenia. A thorough investigation to the multiple factors that contribute to sarcopenia is required. Models are used to dissect the mechanistic understanding of the muscle aging process. *Manduca sexta* is emerging as a novel and complementary muscle aging model because their flight muscles are metabolically and functionally similar to vertebrate skeletal muscle. *Manduca sexta* is already a prominent invertebrate model organism in neurobiology, immunology, developmental research, and flight mechanics because of its short life span, economic benefits, feasible handling, and extensive molecular toolkit for future research and therapeutics. In this review, I analyze the research showing that *Manduca sexta* is an emerging and ideal invertebrate muscle aging model that enhances the comparative approach for muscle aging research.

KEYWORDS: *Manduca sexta*, muscle aging, model organism

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Introduction

Biological aging is an inevitable physiological process that afflicts the organism as a whole. The age-related progressive degeneration of an organism can lead to cardiovascular disease, heart failure, neurodegenerative diseases like Alzheimer's, type 2 diabetes, osteoarthritis, sarcopenia, and many more conditions (Augustin and Partridge 2009; Campisi 2013; De Nobrega and Lyons 2020; Naylor et al. 2013; Selman and Withers 2011). These degenerative aspects of aging significantly decrease the quality of life for the individual. With a rapidly aging human population, due to a massive increase in average life expectancy, there is a need to more readily identify and treat age-related diseases before current healthcare capacities become obsolete (Arking 2003; De Nobrega and Lyons 2020; Selman and Withers 2011).

Although linked with aging, the exact pathogenesis of sarcopenia is unknown; however, the factors that are associated with sarcopenia are multifactorial, making the identification of the cellular pathways and mechanisms a priority for muscle aging research (Augustin and Partridge 2009; Christian and Benian 2020; Mankhong et al. 2020). A well characterized factor for sarcopenia is cellular senescence, which is the arrest of cellular proliferation; in vertebrates, senescent cells accumulate in the skeletal muscle of aging organisms which contributes to the loss of regeneration in muscle cells (He and Sharpless 2017; Mankhong et al. 2020; Wone et al. 2018a). Cellular senescence has well-researched biomarkers that make it an attractive indicator of aging muscles and thus sarcopenia. To better understand these molecular underpinnings of sarcopenia, experimental models are imperative to identify the underlying factors.

Because studying aging is dependent on time to accurately track the longitudinal changes in an organism, research into muscle aging of longer life span organisms, like humans, is particularly difficult. Ideal animal models for aging studies should be cost-effective to rear and

maintain, have an extensive molecular toolkit, rapid development, relatively short lifespan, and provide unique qualities for comparative analysis (Christian and Benian 2020; Mankhong et al. 2020). Currently, models for sarcopenia, and muscle function and performance include, but are not limited to rodents, *Drosophila, Caenorhabditis elegans,* African killifish, and even dogs, all provide invaluable information on the mechanism and therapeutics for muscle aging (Christian and Benian 2020; Demontis et al. 2013; Holmes and Kristan 2008; Mankhong et al. 2020; Puppa and Demontis 2015). Despite these models and more, an animal model for other disciplines is appearing as a promising muscle aging system. *Manduca sexta* is an emerging model for muscle aging that can aid in the comparative research of muscle aging.

Manduca sexta, known as the tobacco hornworm as a larva and a hawkmoth as an adult moth, is a lepidopteran insect that is widely used as a model for neurobiology, olfaction, immunity, development, immunity, and pesticide resistance (Cao et al. 2015; Dacks et al. 2009; Gershman et al. 2021; Goodman et al. 1985; Kanost et al. 2016; Kanost et al. 2004; Martin et al. 2011; Willmott and Ellington 1997b; Wone et al. 2018a). With a relatively short entire life cycle (~40 days) of four distinct life stages, from embryo, larvae, pupae, and then adult moth (life expectancy of ~10 days) (Figure 1). The short lifespan of *M. sexta* is an advantage in muscle aging and developmental studies (Goodman et al. 1985; Reinecke et al. 1980; Wone et al. 2018a). The *Manduca's* eggs are deposited on host solanaceous plants, like tobacco and tomato plants, and once hatched feed on their host plant. The tobacco hornworm goes through five instars, where the larvae continue to feed on the surrounding plants and grow progressively larger with each molt (Byron and Gillet-Kaufman 2017). At the fifth instar, the larva is at its largest (Figure 1) and wanders for a pupation site. After eclosing, the emerging moth is crepuscular or nocturnal and feeds on the nectar of flowering plants. They will feed until mating

and oviposition, using olfactory cues to search for nectar and oviposition sites, and produce as much as 1,000 eggs making their large population an agricultural problem when hungry larvae feed on their host and surrounding plants (Byron and Gillet-Kaufman 2017).

The tobacco hornworm moth is an agricultural pest distributed amongst the southern United States, parts of Canada, Central America, and the Caribbean. As the name suggests, the larvae of *M. sexta* live off tobacco plants due to their fundamental nicotinic resistance, making it an attractive model for ecologist as it decimates host plants and surrounding crops, depending on oviposition site (Baldwin 2001; Byron and Gillet-Kaufman 2017; Gershman et al. 2021; Kanost et al. 2016). *Manduca sexta* is also unique in its body size, the insect can grow up to 10 g just in the larval stage, making it an attractive insect model for larger tissue samples when compared to other insects (Byron and Gillet-Kaufman 2017; Cao and Jiang 2017; Kanost et al. 2016; Reinecke et al. 1980). As a current model organism with a short adult life span, cost-effective, ease of rearing, and a large body size for an insect, *M. sexta* contains base qualities that are attractive for a muscle aging animal model. This present review will examine the research surrounding *M. sexta* that makes this organism an ideal model for muscle aging and the attributes it has compared to other organisms.

Manduca sexta as a Model Organism in Various Fields

The *Manduca sexta* is a prominent animal model in various fields of biology, and by analyzing the contributions to these fields, and key findings, this review can establish a similar analysis for *M. sexta's* role as a model for muscle aging. By showing how other disciplines have used *M. sexta,* this review will likely show that *M. sexta* has been, is, and will be a robust model for comparative research in a multitude of interdisciplinary works. The present review will take a look at the fields that *M. sexta* has been invaluable in developing. First, this review will

investigate a more in-depth view of the field of neurobiology, specifically concerning olfaction surrounding the moth, and then discuss immunology research and how it has expanded *M. sexta's* toolkit. Then, this review will briefly discuss the role of *M. sexta* as a model for insect development and life stages and as a unique model of flight mechanics. The goal is to gain a more complex understanding of the importance of *M. sexta* as a model organism and set up the main review of the moth as a model organism for muscle aging.

Neurobiology: Olfaction

Manduca sexta is already a model in comparative olfactory neurobiology, which has contributed to the research of sensory biology, evolution, ecology, biological computational models, and natural behaviors. *Manduca sexta* is at the forefront of olfactory research as it relies on olfaction for mates, flowers for food, and oviposition and, due to its nocturnal behavior, cannot rely on sight (Dacks et al. 2009; Martin et al. 2011; Reisenman et al. 2009). The olfaction system in these moths is among the most studied for, not only for lepidopterans, insects, or even invertebrates but for all animal systems making *M. sexta* a well-developed model for comparative research in the field (Dacks et al. 2009; Kanost et al. 2016; Martin et al. 2011; Oland and Tolbert 1996; Reisenman et al. 2009). *Manduca sexta's* olfactory system is so advanced that not only can the moth identify and discriminate odors, but it can also determine their concentration, giving the moth a comprehensive map of odors and the ratios at which they are presented (Dacks et al. 2009). This sensitivity allows *M. sexta's* neural system to be capable of learning new odors, which is a process that has interested researchers in studying the relationship of learning and the olfactory system, even to create a computational model that can also perform like the *Manduca's* olfaction system in terms of both physiological interactions and odor learning which can provide critical insight into the structure and function of other olfactory

systems (Dacks et al. 2009; Delahunt et al. 2018). This type of learning also aids the wild *M. sexta* to determine when their food source is sparse and then learns to seek out different flowers for feeding (Martin et al. 2011).

As a comparative model, *M. sexta* shares basic organizational characteristics with nearly all vertebrate primary olfaction centers and glomerular function (Oland and Tolbert 1996). This characteristic makes the functional anatomy of the *Manduca sexta* olfactory system a prime model for both vertebrates and invertebrates. For instance, the moth's antennal lobe has a unique developmental process of the olfactory glomeruli that offers invaluable information into olfactory function and brain development (Dacks et al. 2009; Martin et al. 2011; Oland and Tolbert 1996). Research highlights the development of olfactory glomeruli in *M. sexta* to be a favorable model to study detailed cellular interactions, specifically research done by Oland & Tolbert (1996), detailed the complex developmental comparisons between *M. sexta's* antennal lobe and the vertebrate olfactory lobe (Oland and Tolbert 1996). Although more complex, vertebrates share many characteristics with *M. sexta* in the development of olfactory glomeruli. The olfactory system's structure, function, and physiology contain similarities that make studying insects, such as *M. sexta,* attractive models. Vertebrates, like humans, use their noses while the insects use their antennae. Both contain hair-like olfactory receptor neurons for odor detection; insects contain an antennal lobe, whereas vertebrates use an olfactory bulb for neuronal processing of the odorant (Dacks et al. 2009; Oland and Tolbert 1996; Wilson-Sanders 2011). All this research has led to an increase in the interest in understanding the complex interactions of the olfactory system in animals and their environment, providing a solid base for further exploration of the nervous system's process and response to chemical signals in their environment.

The importance of this modeling system allows us to create a more comprehensive view of the olfaction system, which is helpful for studying the complex relations in sensory neurobiology. A strong base for comparative neurobiology allows consistent research and experimentation to stimulate the growth of such topics, like olfaction, and the *Manduca sexta* has done this, proving it is already a stable model. This example is applicable to the underlying basis of this review; with any model organism, there exists a complex understanding of the research at hand that is essential to the development of future research in the specified field. These present studies give critical insight into the comparative neurobiology for olfaction and show how *M. sexta* is already a strong comparative model across species and continues to grow.

Immunology

Manduca sexta has been a widely used as a model of an innate immune system. First, due to the moth's large body size and hemolymph volume compared to other insects, combined with the economic and rearing advantages invertebrates have over other organisms, the *Manduca* is used extensively (Jiang et al. 2010; Kanost et al. 2004; Zou et al. 2008). Kanost and Jiang have been a part of numerous studies surrounding the innate immune response of *M. sexta*; starting back in 1990, Kanost and Jiang have offered invaluable research on the subject, beginning with dissecting the complex immune system's various molecular pathways (Cao et al. 2015; Cao and Jiang 2017; Cao et al. 2020; Gunaratna and Jiang 2013; Jiang et al. 2010; Kanost et al. 2016; Kanost et al. 2004; Kanost et al. 1990; Zou et al. 2008). The goal of mapping, or molecular dissection, of *M. sexta's* immune system, and any other organism's system, is to thoroughly provide future researchers with intricate details surrounding the system and its interactions. Extensive mapping requires a multifaceted approach to understanding each level of the immune system, anywhere from identification of proteins, regulator system interactions, or signal

transduction pathways. These all need a massive and precise toolkit to examine each level appropriately. The research surrounding *M. sexta*'s immunology is an example of the evolution of a model organism's ability to provide priceless information to facilitate novel and comparative research.

The implications of this research have cumulated into what can only be described as an instrumental asset to the field of immunology and *M. sexta's* toolkit. The initial development of this research, beginning in 1990, was limited to the lack of a reference genome and adequate transcript profiling required for accurate analysis of the immune system's complexity (Jiang et al. 2010; Kanost et al. 2004; Kanost et al. 1990; Zou et al. 2008). As advanced sequencing technology evolved, becoming cheaper and more efficient, the understanding of *M. sexta's* immune system components grew. For example, in 2013, researchers Gunaratna and Jiang (2013) sequenced a large number of immunity-related genes putting *M. sexta's* immunogenome on par with its more researched counterparts (*Bombyx mori, Drosophila melanogaster,* and *Apis mellifera,* that have extensively sequenced genomes and transcriptomes) which further extended the analysis of these genes (Gunaratna and Jiang 2013; Jiang et al. 2010). Without a comprehensive map of the model's immunogenome, comparisons with other species, especially with humans, would be difficult.

The study of the immune system of the *Manduca sexta* places this insect as an emerging comparative model organism. For example, while studying signal transduction pathways in *M. sexta*, researchers improved the overall immunogenomic profile, complete with annotations (Cao et al. 2015). This improvement paved the way to create a draft genome for the moth, which would facilitate any research surrounding this modeling system and provide an in-depth genomic toolkit for detailed comparison (Kanost et al. 2016). This toolkit led to a study that analyzed

RNA-seq datasets for the first time in an insect at all different life stages providing an improved modeling system under *M. sexta* that facilitates gene function research for all other organisms (Cao and Jiang 2017). Additionally, researchers very recently updated Kanosts' team's reference genome for *M. sexta* (Gershman et al. 2021; Kanost et al. 2016).

The purpose of this discussion was to provide a brief overview our understanding of *Manduca sexta* as an immunology animal model. Because of the lack of genomic data in the initial immunology research for *M. sexta*, there exists a continuity of improved techniques and references that only assist in the versatile toolkit for *M. sexta*, which provides researchers, from all disciplines, with an improved and extensively studied model that can keep up with the rigors of modern scientific testing. *M. sexta* is continuously growing as a model organism; however, more research is required to provide a more robust comparative model.

Development

As a large insect, *M. sexta* has been used to characterize and compare developmental stages. Specifically, neural, and hormonal changes in *M. sexta's* development, molting, and metamorphosis, neural and hormonal changes provide insight into a comparative neuroendocrine system across similar species, like *B. mori,* the silkworm moth (Kanost et al. 2016). *Manduca sexta* employs an extensively mapped autophagic pathway that is consistently used to study metamorphosis in other lepidopterans (Gershman et al. 2021; Jones et al. 1995; Zakeri et al. 1996). Through researching *M. sexta* development, a detailed map of transcriptomic and proteomic data can annotate each life stage of *M. sexta* (Cao and Jin 2020; Cao and Jiang 2017). Additionally, experiments with *M. sexta's* development utilized an 'experimental-evolution' approach that resulted in creation of three genetically different moths, differing in key development traits (Davidowitz et al. 2016). By changing developmental traits, researchers were

able to dissect the underlying effects of how and why the traits assist in organismal development, not just for *M. sexta* but for any homologous traits in different species (Davidowitz et al. 2016). The use of changing key developmental traits contributes to *M. sexta's* use as a model organism with differing genetic lines, as the ability to make these different genetic lines is already being used. This detail is useful for comparative 'omics of other model species that exhibit similar development stages.

Flight Mechanics

As a means of locomotion, flight is extensively researched due to its unique mechanics across species; as a large model insect, *M. sexta* provides a means to research flight attributes. Unlike *Drosophila* and two-thirds of other flying insects, *M. sexta's* flight muscles are synchronous, meaning with each contraction of the flight muscle, there is a coupled neural stimulation (Gong et al. 2020; Kanost et al. 2016; Yuan et al. 2015). This difference allows *M. sexta* to be a model for synchronous flight in insects making their flight muscles highly researched. *Manuda sexta's* flight mechanics led to a comprehensive study on kinematics and aerodynamic modeling for flight (Willmott and Ellington 1997a; 1997b). Synchronous muscles are considered less efficient for flight than asynchronous due to a constant need for neuronal stimulus for a muscle contraction, asynchronous muscles provide high power output and contraction frequencies for smaller insects to conserve energy but still meet the power demands of flight (Pennycuick 2008; Tu and Daniel 2004). Because of this difference, *Manduca*, is considered heterothermic as it needs to generate enough energy to take flight using synchronous muscles, the moth 'powers' up by extremely heating its thorax to an average of 40-42 ℃, once this temperature is achieved the moth can begin free flight at a wing-beat frequency of 25 Hz making it have a comparative wing-beat frequency and internal temperature to a similar-sized

hummingbird for flight (Cao and Jin 2020; Heinrich 1971; Heinrich and Bartholomew 1971; Tu and Daniel 2004). Due to the similarities to vertebrate cardiac and skeletal muscles, the large synchronous flight muscles of *M. sexta* are of particular interest to those researching comparisons of the structure and function of similar muscle systems (Gong et al. 2020; Tu and Daniel 2004). Furthermore, the *Manduca's* flight muscles has a similar twitch length-tension curve as mammalian cardiac muscle (Figure 2), further investigation into this also revealed a similar Z-band thickness as vertebrate slow and cardiac muscle fibers and similar A-band, and I-band structure as vertebrate skeletal muscle making the *Manduca's* flight muscle attractive for mutli-species muscle performance and function (Gong et al. 2020; Tu and Daniel 2004; Yuan et al. 2015). This research further indicates that *M. sexta* has a current role as a model in comparative research; by supplying extensive and brief examples of *M. sexta's* use in experimental modeling systems, this review has shown that *M. sexta* is already a prominent model organism in these respective fields.

Current Muscle Aging Models

Before exploring how *Manduca sexta* is emerging as a model organism for muscle aging, this review will briefly look at current models of muscle aging. In order to assess the *Manduca* properly, this review will take a comparative approach. By looking at current models of muscle aging, one can examine where *Manduca* fits in this picture. This present review will first examine two prominent invertebrate models and then some mammalian models.

Invertebrates

Both, *Drosophila melanogaster* and *Caenorhabditis elegans* are models used to study muscle aging in relation to humans. Both *Drosophila* and *C. elegans* share common attributes

that make them attractive models for any study. Both share features that are beneficial for aging studies, having a short lifespan, a massive genetic toolkit, economic cost, and ethical considerations (Christian and Benian 2020; De Nobrega and Lyons 2020; Demontis et al. 2013; Piccirillo et al. 2014; Puppa and Demontis 2015). One of the main features that both the nematode and fly share is the absence of muscle stem cells which is found in mammalian muscles (Christian and Benian 2020; Demontis et al. 2013). This absence is beneficial when studying the mechanisms concerning muscle senescence since the muscle cells would not be affected by regeneration. Current mammalian models, like rodents and the rhesus monkeys, contain muscle stem cells, which is beneficial for studying sarcopenia with the effects of regeneration over time (Christian and Benian 2020). The present review will examine *Drosophila* and *C. elegans* in more detail and then briefly compare mammalian models.

Drosophila

It is no question that *Drosophila melanogaster* is one of the most important organisms for scientific research and development. In fact, as of 2016, the most mentioned species on PubMed was *Drosophila*, with over 40,000 more papers written on it than the next most written species (Cao and Jiang 2017). In terms of muscle aging, *Drosophila* has similar muscle organization, development, and metabolism as mammals but present muscle aging at a markedly rapid pace compared to their mammal counterparts (Demontis et al. 2013; Piccirillo et al. 2014; Puppa and Demontis 2015). Researchers reported that due to the fruit fly's short lifespan of 2-3 months, sarcopenia could be assessed more readily than mammals across the fly's lifetime (Demontis et al. 2013; Piccirillo et al. 2014; Puppa and Demontis 2015). Despite not having the same satellite cells mammalian muscle contains, *Drosophila* uses stem cell-like adult muscle precursors (AMPs) for muscle cell formation after metamorphosis (Augustin and Partridge 2009).

Drosophila has mainly been used to research the cellular and genetic implications of skeletal muscle aging because there are readily available genetic resources for *Drosophila;* this gives the benefit of rapid testing of specific processes made relevant during research (Puppa and Demontis 2015). *Drosophila* has emerged as an attractive model for aging, like other invertebrates, because of its efficiency in the research setting and its contribution to muscle aging research.

Caenorhabditis elegans

Caenorhabditis elegans is another invertebrate model used in aging research. In terms of muscle aging, the nematode has an even shorter lifespan than *Drosophila* (at 18-21 days) and offers a larger sample size than *Drosophila* (Christian and Benian 2020). Because *C. elegans* is a nematode, they use their whole body for movement, making age-related declines more apparent during the model's lifetime. The nematode's muscles are mononucleated and post-mitotic, like vertebrates, hence muscle senescence observed in this model is closely related to mammalian aging (Augustin and Partridge 2009). Like *Drosophila*, there is plentiful research on cellular and genetic implications of aging; specifically, day by day, genetic changes were observed in *C. elegans* muscles (Augustin and Partridge 2009; Christian and Benian 2020). This precision is useful in determining aging-related genetic markers for comparative analyses with other species and humans. Interestingly, *C. elegans* has undefined muscle fiber types, unlike *Drosophila's* flight muscle which is mainly oxidative slow twitch, or type I muscle fiber (Hu and Brunet 2018; Piccirillo et al. 2014; Puppa and Demontis 2015). However, like the *Drosophila*, it is clear that *C. elegans* has a place as a model for muscle aging.

Vertebrates

African Turquoise Killifish

As a vertebrate, the African turquoise killifish (*Nothobranchius furzeri)* offers a significantly shorter life span (3-7 months) than its mammal counterparts (Hu and Brunet 2018; Poeschla and Valenzano 2020; Reuter et al. 2018). The killifish has a fully sequenced genome and transcriptome while also exhibiting biomarkers of aging, such as a decrease in mitochondria and cellular senescence signatures, making it an emerging model for aging studies (Poeschla and Valenzano 2020; Reuter et al. 2018). The killifish also shares many phenotypical and genetic characteristics of aging with other organisms such as mitochondrial disfunction, loss of mass and muscle function, and telomere shortening, which offers the already established genetic modification of this organism as an attractive comparative technique to analyze further the molecular processes surrounding aging (Kim et al. 2016; Poeschla and Valenzano 2020). Because the killifish is a vertebrate, it offers the advantage of including the dynamics of stem cell involvement in the aging process, unlike the other invertebrates in this review which are mainly post-mitotic in adulthood (Hu and Brunet 2018; Poeschla and Valenzano 2020). By filling this niche, the killifish offers another comparative model in the aging process.

Mammals

Mammalian models are attractive due to their massive similarities to humans, however, in a research setting, mammalian models like rodent, pig, dog, or monkey are too expensive and time-consuming to justify extensive use as a model. For instance, despite being a model organism with the highest genetic similarity to humans and containing a large sample size, the Rhesus monkey takes too much time, money, and effort to reliably study the effects of aging (Demontis et al. 2013; Uno 1997). Pigs are another example of a mammalian model. For

example, a study done with pigs looked at epigenetic changes related to skeletal muscle and aging, and the study showed some remarkable results that benefit age-related research (Jin et al. 2014). However, the study was only able to examine six pigs in two age groups with samples taken at only one point of time, suggesting that there exist limitations in studying these models longitudinally (Jin et al. 2014). Although an amazing study, the sample size was small, and the issue of not sampling the organism throughout their lifespan is disadvantage for this and other mammalian models.

Rodent models are used extensively to test therapeutics and model human diseases, but with a lifespan of ~2.5 years, quick and comprehensive data collection regarding aging cannot be done (Christian and Benian 2020; De Nobrega and Lyons 2020). These models are also used extensively for their comorbidities to humans, with a high genetic similarity and skeletal muscle similarity both metabolically as endotherms and physiological with mixed muscle fiber types (Christian and Benian 2020; Hu and Brunet 2018). Because of these similarities, both genetic and physiological, transgenic rodents are used to examine specific genetic effects contributing to muscle aging (Christian and Benian 2020; Mankhong et al. 2020). Lastly, dogs are being utilized as muscle aging models due to their uncanny increase in life-expectancy along with humans because of similar advances in veterinary science (Nogueira and Muzzi 2010). Sarcopenia has been researched in dogs due to their similarities in human skeletal muscle and their use as a pet making them readily available for testing despite their high research cost if raised in the lab (Freeman 2012; 2018). Like the African killifish and rodent, dogs contain with both types of skeletal muscle fibers (I and II), and atrophy of muscles leading to loss of mass, function, and performance has been observed following the onset of sarcopenia in this domestic animal (Bellows et al. 2015; Freeman 2012; 2018; Hutchinson et al. 2012). Additionally, dogs contain

many breeds that have genetic predispositions that may be attractive for studies in their respective categories, this increases the ability to examine causes and effects of muscle aging (Freeman 2018). Dogs are mainly used to study muscle performance as they age and observation of the muscle structure during atrophy, as a pet, the dog is readily available to observe the long-term effects of muscle aging and the progressive decline of muscle strength and function.

Despite having more genetic similarities and comorbidities to test muscle aging effectively, mammalian models do not have the same benefits that invertebrates do. Invertebrate models offer a higher rate of analysis at the cellular and genetic level throughout their shorter lifespans, giving researchers a more detailed look at the effects of muscle aging at a significantly larger scale. However, good science is a rigorous process that requires extensive testing before concluding; using a system of observing or discovering molecular changes in invertebrate models and then doing the same for more closely related models offers the best path to tackle the issue of aging.

Manduca sexta as an Animal Model of Muscle Aging

As previously presented, *M. sexta* is already a prominent model for various subjects. *M. sexta* offers the benefits that invertebrates and other simple organisms employ, ease of rearing, shorter lifespan, and cost-effectiveness. First, this review will examine the research done currently on *M. sexta* and then examine the literature surrounding aging and *M. sexta*.

Muscle Aging Studies in *Manduca sexta*

Wone et al. (2018a) reported a comprehensive look at the metabolites in senescing muscles of *M. sexta* (Wone et al. 2018a). This study tracked the progressive shifts in metabolism of aging muscle in *M. sexta* to further extend our knowledge of muscle senescence. By using *M.*

sexta and their short lifespan, tissue sampling could be taken every day and controlled for shifts in metabolism based on sex, feeding treatments, and time of day (day or night) (Wone et al. 2018a). This type of control is beneficial because one can then further isolate contributions to changes in metabolites and genetic regulation in relation over time, which offers a more accurate estimate of muscle senescence in *M. sexta,* which might be helpful when inferring these changes in other organisms, such as humans.

Wone et al. (2018a) linked muscle senescence to age and deterioration of muscle function in *M. sexta*. This link was also consistent with the metabolomics of human aging. For example, the biotin levels in the muscle tissues of the different groups of moths and found that biotin levels were higher in advanced age male moths, which also highlights the differences in sex between muscle senescence in *M. sexta* and is consistent with aging humans (Wone et al. 2018a). As an organism ages, biotin accumulates in their adipose tissue due to muscle cell death, which is an essential cofactor in various metabolic pathways, leading to decreased overall energy available from metabolism (Wone et al. 2018a). Biotin levels are just one example of similarities in metabolisms between *M. sexta* and humans. Wone et al. (2018a) also highlighted collagen levels as another metabolite that showed promise for being a muscle aging biomarker across *M. sexta* and humans (Wone et al. 2018a).

In another study, looking at flight duration in aging adult *M. sexta*, Wone et al (2018b) showed the importance of age-related changes and muscle ultrastructure function. Flight in insects is a demanding task that requires an adequate amount of energy to maintain (George et al. 2012; Wegener 1996; Wone et al. 2018a; Wone et al. 2018b). Due to the high demand of energy, flight muscles must sustain their function throughout the insect's lifespan. Wone et al. (2018b) showed that flight duration decreases as *M. sexta* ages and further showed that the presence of

fused mitochondria also increased due to advanced age (Wone et al. 2018b). Specifically, the mitochondria fused as the as the moth aged, an effect also observed in other flying insects and humans (Fig. 3) (Wone et al. 2018b). Mitochondrial function is necessary for muscle function and can contribute to age-related loss of muscle function in mammals (Christian and Benian 2020; Mankhong et al. 2020; Wone et al. 2018a). Other studies have found a decrease in flight muscles of another insect, *Drosophila*, due to the high energy demand of flight with advanced age (Piccirillo et al. 2014; Puppa and Demontis 2015). This similarity further indicates that multiple factors contribute to decreased muscle function with age that requires a stable model for future research.

Building from recent research, McMahon (2019) and Del Grosso (2021) further added biochemical and molecular knowledge of *M. sexta's* muscle aging process (Del Grosso 2021; McMahon 2019; Wone et al. 2018a). McMahon looked at the levels of two muscle enzymes in *M. sexta* to assess the citric acid cycle and fatty acid oxidation activity (McMahon 2019). By examining diel time, fatty acid oxidation was found to increase during the night for male moths suggesting a need for energy, highlighting a key sex feature where female moths tend to feed during their active hours, but the males do not (McMahon 2019). By sustaining their fatty acid stores, the female moth can have the energy supply to live longer than their male counterparts to lay their eggs. Additionally, the citric acid cycle showed various changes in advanced age male moths and middle to late aged female moths. Metabolic activity consistent with an increase of energy demand was found in later aged females than middle-aged females and aged males, suggesting another sex difference where the longer lifespan of the female moth increased metabolic activity of the citric acid cycle to compensate for energy deficiencies in other pathways (McMahon 2019).

Further, Del Grosso (2021) examined the genetic expression in the flight muscles of middle to advanced age male and female hawk moths by obtaining RNA-Seq data. Del Grosso (2021) found age-related changes in gene expression in the muscle tissue of *M. sexta*, specifically suggesting an increase in muscle catabolism, a decrease in metabolic enzymes associated with fatty acid oxidation and the citric acid cycle, and markers of decreased mitochondrial function in advanced age male moths (Del Grosso 2021). This research offers a more comprehensive look at the transcriptome of *M. sexta* concerning muscle aging and provides further knowledge into the molecular underpinnings of muscle senescence. More research needs to be done, especially surrounding the sex differences in *M. sexta*; however, by mapping out muscle enzyme activity and RNA-Seq data, *M. sexta* becomes a more robust comparative model for muscle aging.

Manduca sexta, as a Muscle Aging Model

Manduca sexta offers a better model of muscle aging compared to its invertebrate counterparts. The adult moth has the shortest lifespan compared to *Drosophila* and *C. elegans,* with a range of 5-17 days dependent on sex and feeding treatments (Wone et al. 2018a). *Manduca sexta* also offers more muscle mass per sample than *Drosophila,* giving researchers more tissue to test. *Manduca sexta* is also different from the current invertebrate models because it contains synchronous endothermic flight muscles, making it similar to humans in function and metabolism (George and Daniel 2011; Gong et al. 2020; Wone et al. 2018a). Containing synchronous endothermic flight muscles makes the *Manduca*'s muscles similar to that of human skeletal and cardiac muscles (Gong et al. 2020; Schwartz and Ruff 2002). However, like *Drosophila* and *C. elegans*, *M. sexta* lacks muscle stem cells as an adult, but as the moth develops from larvae to an adult moth, larval muscles degenerate during pupation to form the

new muscles in the adult moth, which is much like *Drosophila's* satellite cell-like AMPs (Baryshyan 2013; Rubio et al. 2020). Although both insects offer a potentially new form of muscle repair similar to vertebrates, this process is unclear and requires further study for both *Drosophila* and *Manduca sexta*.

More importantly, the structure and functionality of the *Manduca's* flight muscle shares similarities to vertebrate cardiac and slow muscle fibers. As state previously in this review, under the section of flight mechanics, the use of *Manduca* as a modeling system for flight mechanics has given researchers ample base comparisons of muscle structure, performance, and function across species. Vertebrate muscle fibers have different types, the two types of fibers this review is interested in is cardiac and skeletal or striated muscle fibers. Skeletal muscle fibers get subdivided further into slow- and fast- twitch muscle fibers, or type I and type II respectively, with type II getting even more divided to specialized subtypes; as their names imply, slow twitch fibers contract slowly generating a smaller power output over time but can usually function for longer, and fast twitch muscle contract rapidly for a larger power output but expend more energy for this work, mammalian muscle are normally made of a mixture of both fibers (Christian and Benian 2020; Curcio et al. 2020; Greenlund 2003). *Manduca sexta's* flight muscle tends to share more similarities to vertebrate slow-twitch and cardiac muscles offering a fiber-specific view of the effect of aging on these muscle fibers.

Manduca sexta's flight muscles are both synchronous and endothermic, sharing a similarity with mammals that most insects do not have (Heinrich and Bartholomew 1971; Tu and Daniel 2004; Yuan et al. 2015). Along with this similarity, the flight muscles share characteristics with slow and cardiac muscle fibers of vertebrates. Specifically with the twitch length-tension curve (Fig. 2), Z-band width, as slow and cardiac muscle fibers and alternating A-

band, I-band structure as vertebrate skeletal muscle (Gong et al. 2020; Tu and Daniel 2004; Yuan et al. 2015). The twitch length-tension curve for the dorsolongitudinal muscle in *M. sexta* has a very narrow curve that is similar to mammalian cardiac muscle, and exhibits consistent oscillations much like mammalian cardiac muscles (Gong et al. 2020; Tu and Daniel 2004; Yuan et al. 2015). Furthermore, the Z-band in the sarcomere of the flight muscle has a comparable length to cardiac and type I muscle fibers at ~142 nm for *Manduca* and ~100-140 nm wide for vertebrate slow and cardiac muscle fibers (Gong et al. 2020; Luther 2009). The similarities in structure and similar work loads of constant oscillatory contractions, *M. sexta's* flight muscle provides an interesting model for these muscle types.

Although other vertebrate models contain both type I and II muscle fibers, research has noted that this increases the difficulty of their use as a modeling system, fiber heterogeneity makes it more difficult to isolate fiber-specific deficiencies and their relationship to aging (Christian and Benian 2020). It should also be mentioned that sarcopenia, in vertebrate, has a more dramatic effect on type II cells, these fibers undergo more atrophy than their slow-twitch counterparts or lead to an inversion of fast-to-slow twitch fibers, leaving a higher density of type I fibers in its place (Curcio et al. 2020; Greenlund 2003; Lin et al. 2018). However, sarcopenia specifically concerns atrophy of muscle fibers, *Manduca sexta* offers the unique look into the relationship between sarcopenia and type I- and cardiac-like muscle fibers, which can only help the overall understanding of age-related decline in function and performance of muscles across species.

Research into how *M. sexta* contributes to the subject of muscle aging is just beginning; however, what is known further reinforces *M. sexta* as a model already. Age-related declination of muscle mass and function is readily apparent in most species. Sarcopenia is a well-

documented physiological process in invertebrates and humans alike, where muscle mass, function, and strength decline progressively (Augustin and Partridge 2009; Demontis et al. 2013; Mankhong et al. 2020; Puppa and Demontis 2015; Wone et al. 2018a; Wone et al. 2018b). Sarcopenia can progress via internal factors like mitochondrial dysfunction, or external factors, like fasting or diminished physical activity (Augustin and Partridge 2009; Campisi 2013). Cellular senescence has also been shown to be a factor for sarcopenia; it is caused naturally when the cell cycle is arrested or by various stressors (Mankhong et al. 2020; Rajabian et al. 2021). Many biomarkers have been linked as contributors to cellular senescence in muscle cells that leads to sarcopenia (He and Sharpless 2017; Mankhong et al. 2020; Rajabian et al. 2021). *M. sexta* has already shown biomarkers of muscle senescence, but more research that can examine this relationship further is required (Wone et al. 2018a). A more detailed transcriptome would benefit researchers looking to explore the effects of muscle senescence and aging in *M. sexta,* specifically a time-course sampling from young to advanced age. Translational research would paint a more comprehensive picture into the molecular underpinnings that contribute to muscle aging in *M. sexta*, and this research would bolster the testing of therapeutics for clinical settings. For instance, current experimental models take advantage of known genetic biomarkers for muscle senescence to utilize bioengineered or transgenic organisms for therapeutics or strategies in the future (Mankhong et al. 2020; Rajabian et al. 2021).

Conclusion

Aging has a multifaceted effect on physiology and function of organisms, especially vertebrate. These effects can be debilitating for quality of life and functional capabilities. As aging occurs, the chronic age-related diseases get progressively worse; this creates a significant strain on the individual and the system treating them. Sarcopenia is a musculoskeletal disease

where muscle mass, function, and strength progressively worsen. As a significant age-related disease, sarcopenia has taken a keen interest from an interdisciplinary perspective because age-related muscle function has a significant effect on metabolic disease (Aversa et al. 2019). By understanding the functional causes of sarcopenia, therapeutics can be developed to treat or lessen the effects of the disease. Using model organisms to develop a comprehensive mapping of their respective age-related changes provides invaluable comparative data for muscle aging research. Current models offer a variety of benefits; however, *Manduca sexta* offers not only similar benefits with unique characteristics, but also features of continuous tracking of the muscle aging process (Table 1).

Manduca sexta is emerging as animal model to further the knowledge of the muscle aging process. *Manduca sexta* has the attributes that other invertebrate models benefit from including much shorter lifespan, inexpensive to rear and maintain, ease of handling, and large sample size to increase statistical power. Also, it contains a potential, invaluable, and comprehensive molecular and genetic toolkits that is fundamental for the comparative analysis required by this research. By a comparative approach, the molecular mechanistic underpinnings of muscle aging across species can be better identified and mapped. Using *M. sexta* as a model for muscle aging might provide significant implications in our understanding of the processes of muscle aging and the development of therapeutics that could alleviate the progression of age-related pathologies, like sarcopenia and metabolic disease.

Figures and Table

Table 1: Table comparing the attributes of key models of muscle aging in relation to research capabilities, and human muscle and muscle aging effects. *By large sampling size, this review is referring to the model's ability to sample more at a given time. For example, the sample size in the pig study described previously only used six samples total, whereas *Manduca sexta* had double digits per sampling group, 92 altogether (Jin et al. 2014; Wone et al. 2018a). ** What type of 'omics are readily available and how extensive they are, indicated with a + if present and multiple + to dictate how extensive it is compared to other models.

Attributes	Invertebrate models			Vertebrate models		
	Manduca sexta	***Drosophila***	***C. Elegans***	**African Killifish**	**Rodent**	**Dog**
Cost for rearing, housing, maintaining	$	$	$	$$	$$$	$$-$$$
Lifespan	Full cycle- 40 d, Adult - ~10 d	2-3 mo	18-21 d	3-7 mo	~2.5 yrs	~15 yrs
Tissue samples per organism	+++	+	+	+++	+++	++++

Large sampling size*	+++	++++	++++	+++	++	+
Comorbidities	-	-	-	++	+++	++++
Omics data**						
Genome	+++	++++	++++	++	++++	++
Transcriptome	++	+++	-	-	+++	-
Metabolome	++++	-	-	-	+++	-
Molecular toolkit	+++	++++	++++	++	+++	+
Time-course molecular dissection	+++++	++	++	++	-	-
Genetic lines	++	+++	+++	+++	++	No, but large genetic diversity
Functional genomics approach	+++	+++	+++	++	+++	+
Muscles						
Physiology	Synchronous	Asynchronous	Synchronous	Synchronous	Synchronous	Synchronous
Metabolism	Endothermic	Ectothermic	Ectothermic	Ectothermic	Endothermic	Endothermic

Age-related decline in function and performance	++++	+++	+	++	++++	++++
Satellite cells	Satellite-like AMPs	Satellite-like AMPs	-	+	+	+
Skeletal muscle fiber type	Type I/Cardiac-like	Type I-like flight muscles	No distinction	Both types	Both types	Both types

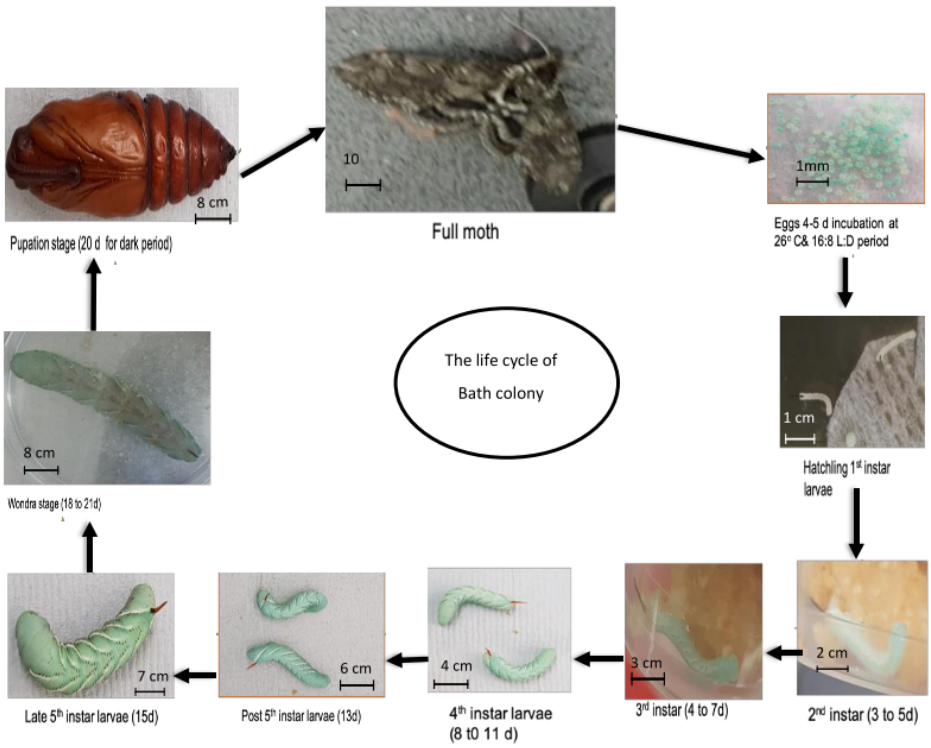

Figure 1: Figure depicting the life cycle of *Manduca sexta* in the lab setting. Full life cycle lasts an average of 40 days with adult moth life expectancy at 10 days. (Thega 2020)

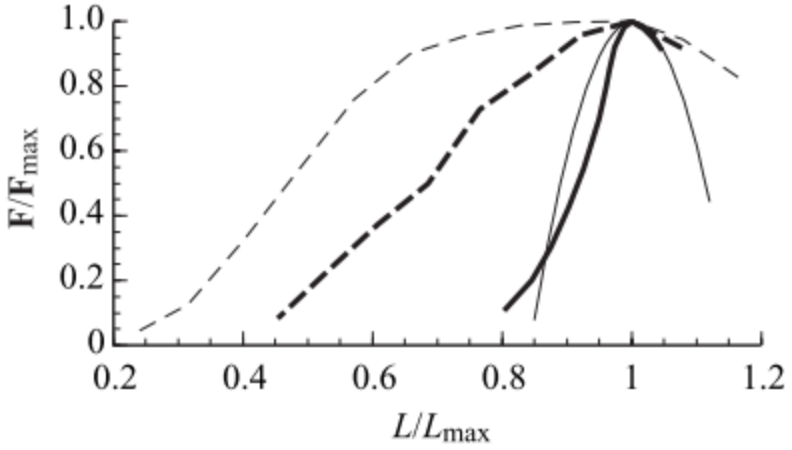

Figure 2: Active twitch length-tension curve for the dorsolongitudinal muscle of *Manduca sexta* (light solid line), mammalian cardiac muscle (heavy solid line), and two mammalian soleus muscles (broken lines) (Tu and Daniel 2004).

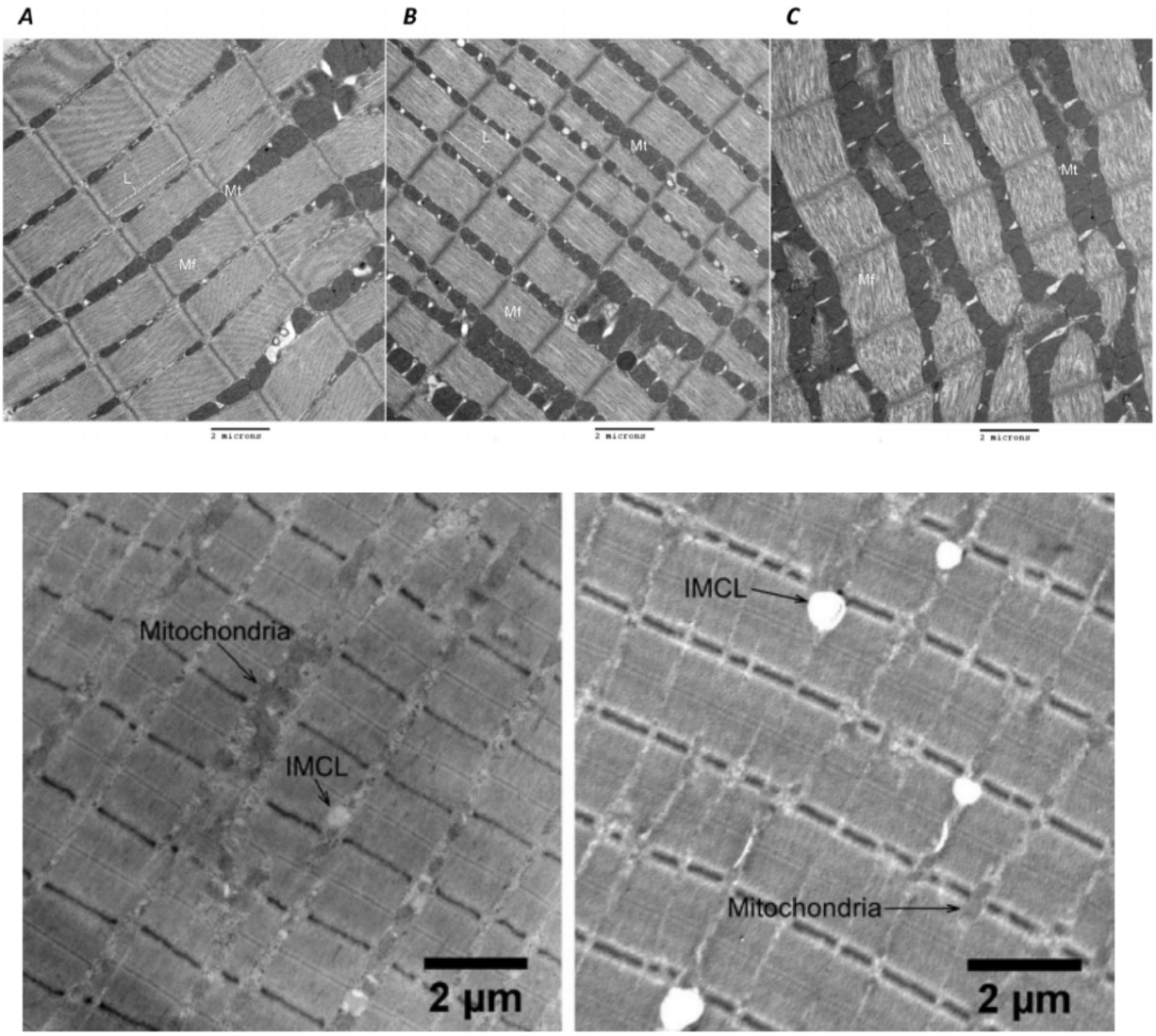

Figure 3: Muscle ultrastructures of both *M. sexta* and human muscles at various ages. (Top; A-C) Flight muscle ultrastructure depicting mitochondria at 1 day old moth with few mitochondria, 3 days old with more distinct mitochondria, and 6 day old aged moths showing fewer and larger fused mitochondria (Wone et al. 2018b). (Bottom; left-right) Skeletal muscle of young and old humans depicting fewer and fused mitochondria on the right as compared to the left (Crane et al. 2010).

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