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Evolutionary divergence of facial muscle physiology between domestic dogs and wolves

Anne M. Burrows[1] | Leo W. Smith[2] | Sarah E. Downing[1] |
K. Madisen Omstead[1] | Timothy D. Smith[3]

[1]Department of Physical Therapy, Duquesne University, Pittsburgh, Pennsylvania, USA

[2]Department of Chemistry & Biochemistry, Duquesne University, Pittsburgh, Pennsylvania, USA

[3]Department of Health and Rehabilitation Sciences, Slippery Rock University, Slippery Rock, Pennsylvania, USA

Correspondence
Anne M. Burrows, Genentech, San Francisco, CA, USA.
Email: burrows@duq.edu

Present address
K. Madisen Omstead, Genentech, San Francisco, California, USA.

Abstract

Domestic dogs (*Canis familiaris*) are descended from gray wolf (*Canis lupus*) populations that inhabited Western Europe and Siberia. The specific timing of dog domestication is debated, but archeological and genetic evidence suggest that it was a multi-phase process that began at least 15,000 years ago. There are many morphological differences between dogs and wolves, including marked divergence in facial muscle morphology, but we know little about the comparative physiology of these muscles. A better understanding of comparative facial muscle physiology between domestic dogs and gray wolves would improve our conceptual framework for the processual mechanisms in dog domestication. To address these issues, we assessed the myosin profiles (type I and type II) from the zygomaticus and orbicularis oris muscles of 6 domestic dogs and 4 gray wolves. Due to small sample sizes, statistical analyses were not done. Results reveal that sampled domestic dogs have almost 100% fast-twitch (type II) muscle fibers while gray wolves have less than 50%, meaning that dog faces can contract fast while wolf faces are able to sustain facial muscle contraction. Sample sizes are limited but the present study indicates that dog domestication is associated with not only a change in facial muscle morphology but a concomitant change in how these muscles function physiologically. Selective pressures in the development of communication between dogs and humans using facial expression may have influenced this evolutionary divergence, but the paedomorphic retention of barking in adult dogs may have also played a role.

KEYWORDS
barking, myosin, orbicularis oris, paedomorphism, zygomaticus

1 | INTRODUCTION

Dog domestication goes back at least 15,000 years ago with some studies indicating more than 30,000 years, and it characterizes the evolution of human cultures (Galeta et al., 2021; Galibert et al., 2011; Janssens et al., 2018; Ovodov et al., 2011; Thalmann et al., 2013). Dogs (*Canis familiaris*) were domesticated from gray wolves (*Canis lupus*) and the domestication processes seem to have started at roughly the same time as other technological innovations of the Upper Paleolithic (UP) such as art, personal adornment, a wide range of small, specialized tools, and the emergence of language (Bar-Yosef, 2002; Hoffecker, 2011). While humans and Neanderthals overlapped in several regions during the UP, there is no evidence that Neanderthals participated in dog domestication; thus, the dog/human bond appears to be unique to *Homo sapiens* (Galeta et al., 2021; Shipman, 2021).

Domestication was a multi-phasic process and resulted in dogs that are socially tolerant toward humans and actively seek out social and physical contact with humans (Herbeck et al., 2022; Tancredi & Cardinali, 2023). While there is debate, it appears that the process of dog domestication had two general phases. The first phase would have been the development of a new ecological niche for gray wolves (Herbeck et al., 2022; Tancredi & Cardinali, 2023) wherein they lived in close proximity to UP humans and benefitted from this association. There are multiple theories as to how this set of interactions began but most focus on improved access to food for wolves who remained near humans, resulting in "synanthropes" (Herbeck et al., 2022), non-domesticated wolves that lived near humans and benefited from this proximity via access to new food resources in the form of "garbage" that humans generated. It is unlikely that UP humans were selecting for specific behavioral traits in these "synanthropic wolves" but wolves that had depressed fear responses would have been the ones to stay near humans (Herbeck et al., 2022; Mech, 1981; Mech & Janssens, 2022; Tancredi & Cardinali, 2023). These changes were occurring prior to the development of agriculture and may have occurred relatively simultaneously in Western Europe and Eastern Asia (Bergström et al., 2020; Frantz et al., 2016).

The second phase likely involved changes in interactions between humans and early dogs, or "proto-dogs." It is thought that this phase involved selection by humans for specific behavioral traits in early dogs which likely included both aggression and bite suppression as well as social and emotional bonding, resulting in domesticated dogs (Herbeck et al., 2022; Tancredi & Cardinali, 2023).

While the specific timing and mechanisms involved with these processes are unclear, they resulted in the contemporary dog that is now abundant across the globe and shares a deep socioemotional bond with us. One of the diagnostic features that resulted from domestication is dogs' ability to read human facial expressions and communicate with humans in ways that other domesticated animals cannot (Burrows et al., 2021; Correia-Caeiro et al., 2023; Hare & Tomasello, 2005; Marshall-Pescini & Kaminski, 2014; Miklósi et al., 2003).

This unique partnership with dogs includes communication in addition to facial expressions, such as gesture and mutual gaze. Unlike chimpanzees, our closest living relatives, dogs are uniquely adapted to read and process human facial expressions and gestures, and humans are better at reading dog facial expression than we are at reading chimpanzee expression (Correia-Caeiro et al., 2020; Hare et al., 2002; Kaminski & Nitzschner, 2013; Sullivan et al., 2022). Recent studies have demonstrated that humans place high value on specific movements of the eye region in domestic dogs that are not produced by gray wolves (Waller et al., 2013). It is becoming increasingly clear that humans selected specific traits during domestication, be it consciously or not, that focused on facilitating facial communication between humans and dogs (Correia-Caeiro et al., 2020; Gácsi et al., 2004; Hare & Tomasello, 2005; Kaminski et al., 2019; Kaminski & Nitzschner, 2013; Miklósi & Topál, 2013; Waller et al., 2013).

Facial expressions are made by facial expression (mimetic) musculature, skeletal muscle developed from the 2nd pharyngeal arch and innervated by the facial nerve/CN VII (Burrows, 2008; Standring, 2020). These muscles typically attach to the bony or cartilaginous aspects of the head and to the dermis of the face, ears, and neck. Upon contraction, these muscles produce the movements that we describe as facial expressions, but they also produce movement of the vibrissae, or whiskers, and squeeze shut the openings of the eyes, nares, and oral cavity (Burrows, 2008; Standring, 2020). Mimetic muscles are also used in feeding, vision, hearing, "face touch" (or using vibrissae of the face), and in vocalizations (Burrows, 2008). Unlike most other skeletal muscles, the evolution of facial expression musculature includes a strong social behavior component as a selective pressure (Burrows et al., 2016). Our understanding of facial expression muscle evolutionary morphology is continuously improving, and a better understanding of these muscles allows us to make connections to the evolutionary ecology of social behavior among mammals.

Dog facial musculature morphology is divergent from that of gray wolves (Burrows et al., 2021; Kaminski et al., 2019). For example, Kaminski et al. (Waller et al., 2013) found that domestic dogs typically have musculature around the eye region that gray wolves seem to not have. Studies on facial musculature can inform our understanding of what movements both dogs and wolves

can make with their faces. However, we know little about the physiology of these muscles, which can inform our understanding of how these muscles are used and the selective pressures that were important during the dog domestication process.

1.1 | Muscle physiology

Mammalian skeletal muscle physiology can be categorized in numerous ways, but it can be useful to think about speed of contraction and fatigue-resistance. Slow-twitch (type I) muscle fibers contract relatively slowly but are fatigue-resistant and have a small motor unit, meaning that they can contract with specificity. These fibers are used more often for low-power, sustained contractions that happen frequently over an extended time. Fast-twitch (type II family) muscle fibers contract relatively quickly but also fatigue quickly and have larger motor units with lower specificity. Type II fibers can generate high power but cannot sustain the contraction (Hoh, 1992; Lieber, 2009). These physiological characteristics of skeletal muscle can inform our understanding of how these muscles are used, how muscles may have evolved over time, and the selection pressures associated with the evolutionary process.

Most muscles contain differential distributions of slow-twitch and fast-twitch fibers, depending on the use of any specific muscle. For example, previous studies have demonstrated that human facial muscles contain mostly fast-twitch muscle fibers (Stål, 1994; Stål et al., 1987; Stål et al., 1990), in accordance with the rapidly produced genuine facial expressions in social interactions. However, Burrows et al. (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014) demonstrated that, while human facial muscles have more fast-twitch myosin fibers than slow-twitch, humans have more slow-twitch muscle fibers than do both chimpanzees and rhesus macaques. This unique myosin profile in human facial musculature is thought to be related to the evolution of speech and the need to "slow down the face" to accurately move the lips for speech sounds. To wit, Sanders et al. (Sanders et al., 2013) demonstrated that the human tongue myosin profile was dominated by type I fibers relative to rhesus macaques and that this adaptive profile is likely to be related to the evolution of human speech associated with slowing the tongue to produce distinct and specific movements.

Since dogs have different facial musculature relative to gray wolves and that this musculature produces movements that humans prefer (Kaminski et al., 2019; Waller et al., 2013), we expect that domestic dogs also have a unique myosin profile relative to gray wolves. We test the hypothesis that dog facial muscle physiology differs from that in the gray wolf. Because human facial muscles are dominated by fast-twitch fibers but with a large minority of slow-twitch fibers (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014), we predict that domestic dogs will have a similar profile.

2 | MATERIALS AND METHODS

We used myosin immunohistochemistry to test our hypothesis. We sampled sections from the middle part of the left upper lip (containing the orbicularis oris muscle [OOM]) and from the midface (containing the zygomaticus muscle [ZM]) with overlying skin. We sampled from a broad range of domestic dog (*Canis familiaris*) breeds ($N = 6$: 1 mixed breed, 1 Labrador retriever, 1 husky, 1 chihuahua, 1 spaniel, and 1 beagle) and gray wolves (*Canis lupus*, $N = 4$). The gray wolf samples were all adults and came from the Michigan Department of Natural Resources, culled for population management (see 24). We chose these muscles because they are involved in both facial expressions and in vocalizations in these species as well as in humans (Evans & de Lahunta, 2012; Seikel et al., 2023; Stavness et al., 2013), and they are easy to isolate from other surrounding muscles. Two specimens for the wolves were purchased from the taxidermy industry but were not killed for the purpose of this study, and the two other wolf specimens were obtained from the Michigan Department of Natural Resources. We obtained the dog sample from the National Museum of Health and Medicine (NMHM). All samples were procured from cadaveric specimens that were not euthanized for our research. Thus, this study was exempt from Institutional Animal Care and Use Committee oversight at Duquesne University. All specimens were fixed with 10% buffered formalin prior to sampling.

Samples were prepared for paraffin-based histology following methodology from Burrows et al. (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014) and each muscle was sectioned at 8–10 μm thickness. We created 150 to 300 sections that were spaced five sections apart and we mounted these on SuperFrost Plus slides (Fisher Scientific). This methodology generated sections that were representative of the entire muscle. Mouse monoclonal antibodies were used as primary antibodies to slow myosin (ab11083, Clone NOQ7.5.4D, Abcam, Inc.) and fast myosin (ab51263, Clone MY-32, Abcam, Inc.). A random selection of three to five slides per individual containing three muscle sections per slide were chosen for immunohistochemistry using each primary antibody which yielded 18–40 sections for each muscle in each of the specimens.

To prepare tissues for immunohistochemistry, de-paraffinized rehydrated sections were subjected to

enzymatic retrieval with 0.5% trypsin for slow myosin, or an overnight epitope retrieval with Tris-EDTA buffer for fast myosin staining. Endogenous peroxidase activity was blocked by 0.9% hydrogen peroxide in methanol and sections were pretreated with 5% normal goat serum. The primary antibodies to slow myosin (1:1000) and fast myosin (1:750) were diluted in 5% normal goat serum and were incubated for 1 h at room temperature. After this, sections were washed with PBS and biotinylated goat anti-mouse antibody diluted 1:200 in 5% normal goat serum was applied. Sections were then again washed and incubated with Vectastain ABC reagent (Vector Laboratories). Finally, sections were exposed to 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories) for 2 min, the reaction was stopped with water, and the sections were dehydrated, cleared, and mounted with permount (Fisher Scientific). For a full description, see Burrows et al. (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014).

2.1 | Determination of fiber type distributions

We determined distributions of fiber types by selecting 3–10 sections stained for identification of each fiber type for each individual in both the OOM and ZM muscles, then photographing five random areas of the muscle section at 10X using a light microscope with a mounted camera. We then used ImageJ (NIH) to derive averages for fast and slow fiber types in each photograph by counting all reactive fibers in the photograph and then dividing by the total number of fibers present. We skipped any fiber that was partially out of the field of view and did not count those.

The resulting percentages of reactive fibers in each composite were used to calculate the mean percentage of fiber type in each muscle for each species (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014). Because sample sizes were low, statistical analyses were not performed. We instead present average percentages that have not been subjected to statistical analyses.

3 | RESULTS

Figures 1 and 2, and Table 1 show that dogs have more fast-twitch fibers in their facial muscles than do wolves. Like humans and non-human primates, dog facial muscles are dominated by fast-twitch muscle fibers, with a pooled average of 92% and a range from 90% in the OOM to 94% in the zygomaticus muscle (ZM). However, previous studies (Burrows, Durham, et al., 2014; Burrows,
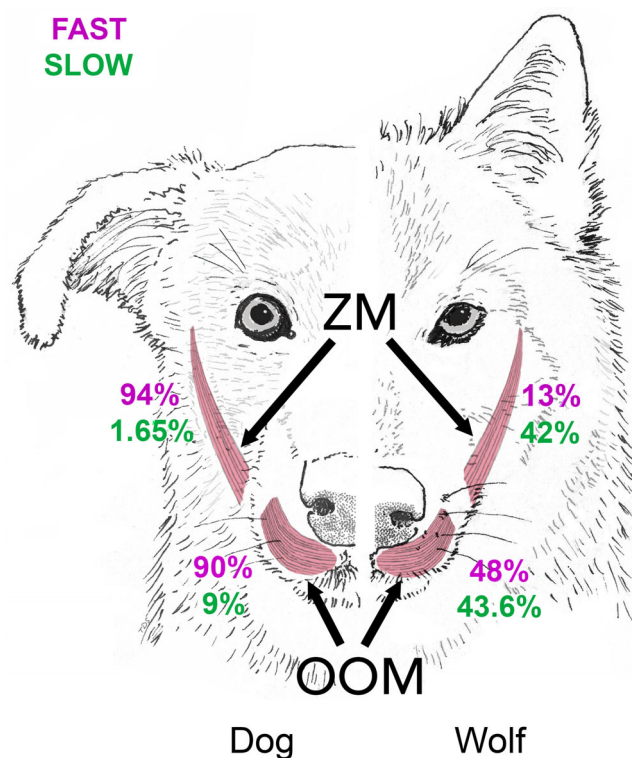
FIGURE 1 Dog face (left) and gray wolf face (right) highlighting the two facial muscles investigated in the present study. Numbers next to each muscle show the average fiber type percentages with fast percentage in purple font and slow percentage in green font. ZM, zygomaticus muscle; OOM, orbicularis oris muscle.

Parr, et al., 2014) found that, while human facial muscles are dominated by fast-twitch fibers, the variance between muscles in humans is much higher than observed in dog muscles (60% in the zygomaticus major muscle and 91% in the OOM). Dogs, conversely, only had 5.3% pooled distribution of slow-twitch fibers, ranging from 1.65% in the ZM to roughly 9% in the OOM.

Unlike dogs, wolves presented with a high distribution of slow-twitch fibers at 42% pooled average across both the ZM and OOM (Table 1). This value is almost 9 times greater than that of dogs. However, it is much closer to the slow-twitch distribution seen in humans (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014).

Table 1 shows that there is a wide range between maximum and minimum values for fast and slow-twitch fiber types in the domestic dogs, from 8% to 100%, along with large standard deviations. This wide range is absent in the wolf samples. The main driver of both the range and standard deviations is the husky sample. In the present study, the husky sample had 37% fast twitch distribution in the zygomaticus major muscle and 8% fast twitch distribution in the OOM (see Figure 2), whereas

FIGURE 2 Graphs showing fiber type distributions between dogs and gray wolves. The values within parentheses represent the standard deviations and the values following represent the minimum and maximum values. Graph "a" shows the fiber type distributions in pooled samples from the zygomaticus and orbicularis oris muscles; graph "b" shows the fiber type distributions in the zygomaticus muscle samples; graph "c" shows the fiber type distributions in the orbicularis oris muscle samples. Note that values in this figure are calculated without the inclusion of the husky sample, but the locations of the husky sample values are indicated.
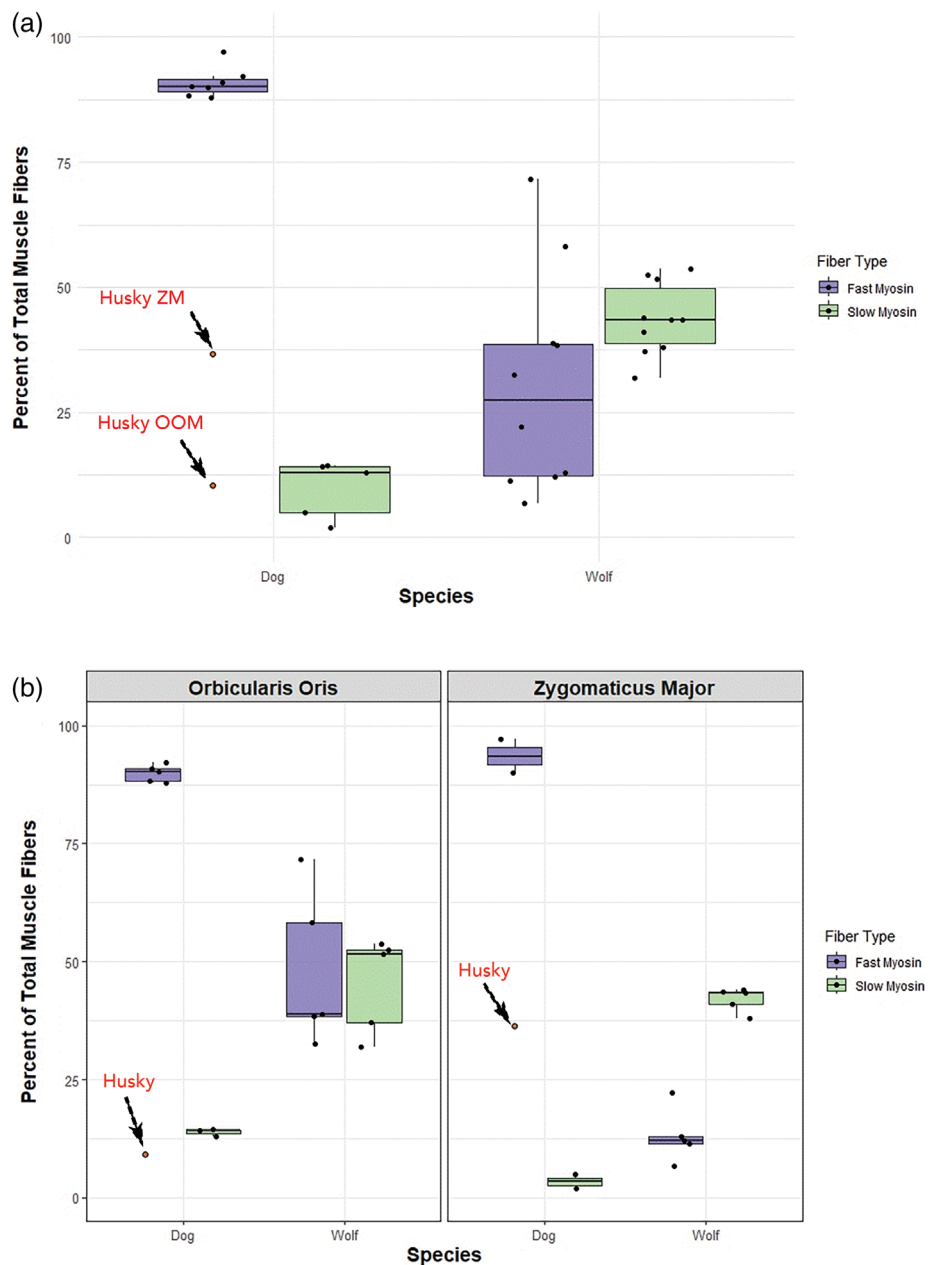
TABLE 1 Average myosin percentages (mean percentages followed in parentheses by standard deviations; minimum and maximum values follow standard deviations).

	Fast myosin distribution				Slow myosin distribution	
	ZM	OOM	Pooled	ZM	OOM	Pooled
Domestic dog[a] (*Canis familiaris*)	94.0% (25.5); 37,97	90.0% (37.0);8100	**91.9%**	1.7% (2.1); 0,5	8.9% (4.42); 4,14	**5.3%**
Gray wolf (*Canis lupus*)	13.0% (5.5); 7,22	48.0% (16.7); 32,72	**33.0%**	41.9% (2.4); 38,44	43.6% (10.1); 32,54	**42.8%**

Abbreviations: OOM, orbicularis oris muscleZM, zygomaticus muscle.

[a]These values represent husky sample included. The wide range between minimum and maximum values in the domestic dog are driven by the husky sample.

most breeds had a much higher fast twitch distribution. Slow-twitch myosin distributions were very tight (Table 1) and present far less variation than the fast-twitch distribution.

4 | DISCUSSION

Results from the present study demonstrate that selection during dog domestication not only shaped facial muscle

morphology (Kaminski et al., 2019) but it also shaped facial muscle physiology. Furthermore, these findings support our hypothesis that domestic dog myosin profiles differ from those of gray wolves. However, our prediction that dog myosin profiles will be closer to that of humans than gray wolves is rejected. We found that dogs have a unique myosin profile and that gray wolves have a myosin profile closer to that of humans, with a large percentage of slow twitch myosin fibers.

Dogs had myosin profiles in the OOM of roughly 90% type II/fast-twitch fibers and 94% in the ZM. Gray wolves were starkly different, with 48% type II in the OOM and 13% in the ZM. This compares with values in humans of 71%–85% fast-twitch in the OOM and 60%–90% in the zygomaticus major muscle (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014; Stål et al., 1987; Stål et al., 1990). This places dogs between humans and gray wolves in fast-twitch fibers, but closer to the human profile.

Slow-twitch fiber distribution presented a more nuanced picture. Dogs had very low average slow-twitch distributions, from 1.65% in the ZM to 9% in the OOM, while gray wolves ranged from 42% in the ZM to 43.56% in the OOM. This compares with human values from roughly 15% in the zygomaticus major muscle to roughly 20% in the OOM (). This distribution, unlike fast-twitch distribution, places dog values intermediate to both human and gray wolf myosin values.

The speed, specificity, and endurance of facial muscle contraction are influenced by myosin fiber-type distribution. High percentages of type I (slow-twitch) fibers necessitate a longer time to initiate muscle contraction but it is specific, with small motor units, and can last a relatively long time. Type II (fast-twitch) fibers contract quickly but have low specificity of action, with larger motor units, and cannot hold the contraction (Hoh, 1992; Lieber, 2009). Facial expressions are typically spontaneous muscle contractions that contort the face, allowing for "genuine" social interactions and communication. Thus, it is no surprise that human facial musculature is dominated by fast-twitch myosin since we use facial expressions routinely in social interactions (Burrows & Cohn, 2015; Schmidt & Cohn, 2001).

Domestic dogs also use facial expressions when interacting with humans (Kaminski & Nitzschner, 2013; Marshall-Pescini & Kaminski, 2014; Waller et al., 2013) and previous studies have shown that both dogs and humans look preferentially at one another's faces during social interactions and when looking at still images, even if the dogs are feral (Albuquerque et al., 2016; Correia-Caeiro et al., 2023; Guo et al., 2010; Kaminski et al., 2017; Lazzaroni et al., 2023). Whether consciously or not, humans selected dog facial features that are more similar to humans than to gray wolves, including facial

expressions (Burrows et al., 2021; Kaminski et al., 2019; Waller et al., 2013). Here we demonstrate that the facial muscle physiology of dogs is different than that of both humans and gray wolves.

Potential explanations for this divergence in physiology between dogs and wolves include differences in the function of facial expression between dogs and wolves. Additionally, facial muscles, especially the orbicularis oris and zygomaticus (major) muscles, are used as articulators to change position of the lips during vocalizations and in human speech (Evans & de Lahunta, 2012; Seikel et al., 2023; Stavness et al., 2013). In humans these muscles (and some other facial muscles) must contract with specificity to move the lips to articulate accurate speech sounds.

Neither dogs nor wolves produce speech in the way that humans do, but they produce vocalizations that involve movements of the lips. Wolves howl. This is a stereotyped vocalization that functions in both group cohesion and long-distance communication, and it is performed in part by sustained contraction of select facial muscles. An average howl from an individual wolf lasts 3–7 s, and serial howling bouts average 35 s (Mech, 1981). Wolves also produce whimpers, growls, and barks but howling is the signature vocalization in adult wolves (Harrington & Mech, 1979; Nowak et al., 2007). Howling is a low-pitch, long-distance, sustained vocalization that may be done singly or as a community.

Howling is used in a variety of settings and includes territorial defense, social spacing, and re-assembling dispersed individuals. Wolves howl by pointing their snout up and shaping the lips in a funnel, then projecting sound in a sustained fashion. The sustained shape of the lips is accomplished by the OOM and may account for its great distribution of type I fibers in the wolves, relative to the dogs. The zygomaticus muscle draws the corner of the lips caudally and may not be involved in the howl mechanism.

Unlike wolves, the signature vocalization of the domestic dog is the bark. Dogs can and do howl but it is not their primary vocalization. As a sound, barking is an explosive onset, "noisy signal" with multiple non-harmonically related frequencies. This sound is staccato, pulsed, and varies widely in pitch during the barking bout. Bouts occur serially but are short-lived in duration, averaging 100–500 ms (Lehoczki et al., 2023; Lord et al., 2009; Pongrácz et al., 2010; Scott, 1967; Taylor et al., 2014; Yin & McCowan, 2004).

Dogs bark in a variety of contexts and it has been suggested that it is linked to communicating something specific to humans, as an alarm call, territory marking, or some internal state (Coppinger & Coppinger, 2001; Lord et al., 2009; Pongrácz et al., 2005; Taylor et al., 2014; Yin, 2002). In short, dog barking is ubiquitous relative to

the wolf howl (Faragó et al., 2013; Lesch & Fitch, 2023). Barking is related to nearby functions that do not require sustained contraction of the muscles around the lips, being a staccato, rapid onset sound. The divergence in vocal communication between dogs and wolves occurred at some point during domestication and it is likely that it occurred relatively late in domestication (Lehoczki et al., 2023).

Wolf barking is rare, making up only about 2% of adult vocalizations (Schassburger, 1993) and, when done, it is typically the high-ranking adult male that barks. Wolves bark when alarmed or threatened, or when other wolf packs are in the region (Harrington & Asa, 2003; Mech, 1981; Mech & Janssens, 2022; Schassburger, 1987; Schassburger, 1993) but the barks are acoustically similar to what we find in dog barks: rapid onset, staccato sounds (Mech, 1981; Mech & Janssens, 2022).

While adult wolves rarely bark, wolf pups bark routinely, starting at day 1 of age, along with whines, whimpers, and growls (Harrington & Asa, 2003; Mech, 1981). By 4 weeks of age, pups bark in response to sounds outside the den and as they leave the den but barking diminishes in frequency as pups pass this age in favor of the development of howling (Coscia, 1995; Mech, 1988). Dog pups begin barking around 8 weeks of age, with whimpering, whining, grunting, and growling appearing prior to this, with variation among both individuals and breeds (AKC.org). Barking is a behavior that is not extinguished as dogs age. It seems likely that the dog bark is a paedomorphic retention.

Results from the husky sample are confounding in that their myosin distribution profile resembles the wolf sample more closely than it does the other domestic dog samples, having a very low distribution of fast twitch fibers. This may be due to our low sample size. However, the husky is one of the "ancient breeds," dog breeds that are genetically more closely related to gray wolves than any other breed is related to gray wolves (Bergström et al., 2020; Lehoczki et al., 2023). Ancient breeds also include the Alaskan malamute, basenji, saluki, Afghan hound, chow chow, akita, and shar pei. Many genetic, physiologic, behavioral, and anatomical traits unite these breeds including low frequency of barking (and in the case of the basenji, no barking). Huskies routinely howl, however. This behavioral trait may partially explain the very low distribution of myosin fibers in the husky sample, pushing the husky myosin profile closer to those of gray wolves. The present study only had one of these ancient breeds, but the results are intriguing and future studies should strive to examine myosin profiles of facial muscles in other ancient breeds.

With our low sample size and lack of statistical analyses, our results can only be viewed as qualitative. A past study found differences in the gross facial musculature between dog breeds (Kaminski et al., 2019), and it is possible that different breeds may have differing facial muscle physiology. Future studies should focus on increasing not only the sample sizes of domestic dogs and gray wolves, but dog breeds to explore these questions.

5 | CONCLUSION

The present study finds physiological evidence that dog facial musculature is faster than wolf facial musculature. While it is unclear what the selective pressures were that drove this divergence in myosin profiles, mutual facial communication between humans and dogs may be one explanation. However, dog samples from the present study had a significantly higher proportion of type II myosin fibers than did humans (Burrows, Durham, et al., 2014; Burrows, Parr, et al., 2014), so there may be selection pressures in addition to visual communication.

While the primary function of facial muscles seems to be in facial communication, they also play a role in articulating vocalizations, and the extended length of the howl may explain the higher proportion of slow-twitch fibers in wolf orbicularis oris and zygomaticus muscles. The staccato nature of the dog bark likely requires little extended contraction of the orbicularis oris and zygomaticus muscles to articulate the sound, related to their higher proportion of fast-twitch fibers. We suggest that the paedomorphic retention of barking in dogs may play a role in the higher proportion of fast-twitch fibers in the dog sample relative to the wolf. While we are unaware of any studies that focus on fiber typing in the facial musculature of wolf or dog pups, this could be a valuable future direction to ascertain whether muscle physiology diverges over ontogeny, especially since vocalizations between wolves and dogs diverge.

ORCID

Anne M. Burrows https://orcid.org/0000-0002-9952-8125

Sarah E. Downing https://orcid.org/0009-0002-0821-7565

Timothy D. Smith https://orcid.org/0000-0002-6883-8964

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