Through the eyes of a wolf: Using non-invasive methods to quantify and classify the facial signalling of wolves (Canis lupus) and domestic dogs (Canis lupus familiaris)

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Through the eyes of a wolf: Using non-invasive methods to quantify and classify the facial signalling of wolves (*Canis lupus*) and domestic dogs (*Canis lupus familiaris*)

By

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2019

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Master of Science (MSc) by research.

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I confirm that no part of the material presented in this thesis has previously been submitted by me or any other person for a degree in this or any other university. In all cases, where it is relevant, material from the work of others has been acknowledged.

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Abstract.

The morphologies of wild animals typically evolved via natural selection as a result of environmental pressures leading to specific adaptations to cope with life-history challenges, such as foraging, mating and communication. However, many animals, in particular mammals, have been domesticated by humans. As a result of domestication a range of divergent morphological traits are frequently seen in domesticated mammals that separate them from their wild, ancestral counterparts. Many of these diverged traits pertain to cranium morphologies, such as different head shapes and sizes, shorter muzzles, and different ear shapes and positions. To date, there is little research into the potential implications that these diverged morphological traits of domesticated mammals may pose for their behaviour.

A model example of diverged cranium morphologies is seen in the domestic dog (*Canis lupus familiaris*), the selectively bred descendant of wolves (*Canis lupus*). It is thought that the head and facial feature morphologies of wolves aid the production of facial expressions for communicative purposes. Previous researchers have also speculated on the ability of wolves to convey affective states as well as social status via their facial expressions. Affective states are forms of motivation such as emotions, moods, attitudes, desires, preferences, intentions and dislikes. However, to date there has been no quantitative analyses of these suggested links between facial expressions, affective states and social status in wolves.

The relative shape and position of the eyes, ears, forehead, muzzle, nose and lips (the main conveyers of facial expressiveness) are the same for all wolves, throughout the world. However, selective breeding has resulted in the main conveyers of facial expressiveness of dogs greatly diverging from those of their wolf ancestors, although, it is still thought that dogs use facial expressions to convey affective states. However, to date there has been little quantitative analyses of the links between domestic dog facial expressions and affective states. This thesis aims to quantify the affective facial expressions of wolves and domestic dogs, and to determine if the various head and facial morphologies found across different dog breeds limits their abilities to successfully produce affective facial expressions like their wolf ancestors.

The facial expressions of captive, human-habituated wolves (*n* = 10) and kennelled rescue dogs (*n* = 64) were video recorded during social interactions and reactions to ‘emotive’ stimuli. To quantify the facial expressions of wolves and dogs, the video footage was decoded using the Dog Facial Action Coding System (DogFACS). The affective states of focal canids were also quantified and classified from the video footage using independent observers. The quantified facial expressions were then mapped against the reliable classifications of affective states using hierarchical cluster analyses and linear discriminant analyses. Two separate confusion matrices for wolves and dogs were generated from the linear discriminant analyses, which revealed the level of precision (agreement) between the actual and predicted affective facial expressions of wolves and dogs.

The research presented in this thesis provides the first quantification of facial expressions in wolves and relates them to reliable classifications of affective states across a range of social contexts. This research also provides the first quantitative, preliminary evidence for wolf facial expressions conveying social status, which has never been shown to exist in any other non-human animal. The affective facial expressions of wolves are shown to be similar to those seen in primates and are discussed in the framework of the social intelligence hypothesis. For the first time, this thesis also shows that the varying head and facial feature morphologies of dogs (in particular non-wolf-like morphologies) limit their ability to produce the same range of affective facial expressions as their wolf ancestors. However, this research reveals that dogs have evolved a compensatory way to convey their affective states, via the use of vocalisations.

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My second acknowledgments go to the UK Wolf Conservation Trust and Dogs Trust Darlington for allowing me to use their canids for my research. I would personally like to thank the 10 wonderful wolves of the UKWCT, Torak, Mosi, Massak, Sikko, Pukak, Motomo, Mai, Tundra, Nuka and Tala. I would also like to personally thank all of the dogs at Dogs Trust Darlington (all 64 of them!). It was an honour to observe the behaviour of all of these amazing canids.

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Dedications.

This thesis is dedicated to the memory my dad, Edmund Charles Hobkirk, a keen amateur naturalist, who initiated my love of the natural world by teaching me to observe and see what others simply dismiss. I would also like to dedicate this thesis to my German Shepherd dogs, past (Sheba) and present (Saphire and White Fang), for they are my inspiration who essentially taught me to ‘speak’ dog, an important skill for this research! Finally, I dedicate this thesis to my uncle Sean, who has stood by me when things have been incredibly tough, and who has endlessly encouraged me and told me repeatedly to never doubt myself! His words eventually sunk in, and are the reason for me embarking (pun intended) on this research, and for this thesis reaching its completion!

Elana with White Fang and Saphire.

Chapter 1: General introduction.

This chapter introduces the background to this research, detailing the importance of non-human animal communication and its role within group-living species. Vital to this research is an understanding of what ‘affective states’ are and how visual communication, in particular facial expressions, are used to convey them. Facial expressions are well studied in primates, but not in other mammals. In this chapter a rationale for the investigation of facial expressions in wolves (*Canis lupus*) is laid-out as a foundation for assessing the potential morphological constraints that may limit the ability of domestic dogs (*Canis lupus familiaris*) to convey affective states via facial expressions. Finally, the relevance of this research is discussed with regards to dog welfare and dog bite prevention.

1.1: Non-human animal communication.

Traditionally, the field of non-human animal communication has formed a major part of the discipline of animal behaviour (Lewis & Gower 1980) and has led to the formation of many evolutionary theories of the social communicative behaviour of humans, and how non-human animals are able to understand human communication (Arbib et al. 2008; Hare & Tomasello 2005; Pollick & De Waal 2007; Salmi 2015). However, in recent years, the study of non-human animal communication has become the focus of animal welfare science (Boissy et al. 2007; Duncan 1981; Ladewig 2019; Manteuffel et al. 2004), with an emphasis on the human understanding of what non-human animals try to communicate and, in particular, what ‘emotional’ states they are trying to communicate (Boissy et al. 2007; Desire et al. 2002; Ladewig 2019; Lafollette et al. 2018; Manteuffel et al. 2004; Mendl & Paul 2004).

Non-human animal communication has no singularly agreed upon definition as its meaning is highly debateable (Lewis & Gower 1980). However, it is widely considered that animal communication is the transfer of ‘information’ between two or more conspecifics or societal heterospecifics, and/or the manipulation of the receiving individual’s behaviour (Deecke et al. 2005; Laidre & Johnstone 2013; Lewis & Gower 1980; Marler 1967; Rendell et al. 2009; Seyfarth et al. 2010). Information can be transferred via a myriad of signals (or cues) through several modalities, which include (but are not restricted to), olfactory, auditory and visual (Laidre & Johnstone 2013; Lewis & Gower 1980; Marler 1967; Smith & Harper 2003; Rendell et al. 2009; Seyfarth et al. 2010).

Communication and social behaviour are intertwined (McGregor & Peake 2000; Pollard & Blumstein 2012). Successful communication is essential for highly social, group-living animals as it mediates important social behaviour, upholds social hierarchies and maintains strong social bonds (Fernald 2014; McGregor & Peake 2000; Smith et al. 2011; Smuts & Watanabe 1990; Whitham & Maestripieri 2003). Social communication has been studied in a vast array of species, including insects (Alexander 1967; Ali & Morgan 1990; Lloyd 1983; Virant-Doberlet & Cokl 2004), fish (Liley 1982; Van der Sluijs et al. 2011), reptiles (Barnett, Cocroft & Fleishman 1999; Blrghardt 1977; Ferguson 1977; Mason & Parker 2010; Stuart-Fox & Moussalli 2009) birds (Butcher & Rohwer 1989; Hart et al. 2015; Kumar 2003; Marler 1957), and mammals (Eisenberg & Kleiman 1972; Hotchkin & Parks 2013; Randall 2001; Townsend & Manser 2013) which highlights the value of communication for group-living animals. However, for the purposes of this research, the social communication of mammals will be discussed in greater detail.

The social communication of mammals has been well studied and includes a vast array of research on auditory and visual signalling. For example, Vervet monkeys (*Cercopithecus aethiops*) produce predator specific ‘alarm calls’, to which group conspecifics respond to collectively, to avoid predation (Ducheminsky et al. 2014; Seyfarth & Cheney 1990; Seyfarth et al. 1980). Orca (*Orcinus orca*) produce auditory ‘food calls’ to announce the presence of prey to conspecifics (Deecke et al. 2011; Samarra 2015), and use their conspicuous white eye patches as visual cues to coordinate group (pod) hunting behaviour and obtain located prey (Pitman & Durban 2012). During the ritualised social greetings of Spotted hyena (*Crocuta crocuta*), the erect ‘penis’ is used as a visual cue of social status, which helps maintain social hierarchies among clan members (East et al. 1993). In addition, the ‘laugh’ vocalisation of *C. crocuta* is also used to convey not only social status, but also sex, age and individual identity, which allows receiving conspecifics to assess the societal position of the signaller (Mathevon et al. 2010). Indeed, the ability to discern between group members and non-group members is essential for gregarious animals, as it maintains social bonds and allows groups to protect resources from intruders (Henkel et al. 2015; Cheney & Seyfarth 1992). Identification of individual group members has been studied in many species, for example, African elephants (*Loxondata africana*) use infrasonic ‘contact calls’ to identify group from non-group members (McComb et al. 2000). Rhesus macaques (*Macaca mulatta*) use scent to identify group from non-group members (Henkel et al. 2015), and ‘coo’ calls to identify kin from non-kin (Pfefferle et al. 2015). Chimpanzees (*Pan troglodytes*) can also identify kin from non-kin via facial recognition (Parr et al. 1999; Tomonaga et al. 2004), which is interesting as mammalian faces are capable of conveying a wealth of information via communicative signals (Burrows et al. 2006; Calder & Young 2005; Somppi et al. 2014; Thunstrom et al. 2014), and as a result, facial expressions are considered highly important for social communication amongst mammals (Somppi et al. 2014). Although the definition of non-human animal communication is unresolved (Lewis & Gower 1980), it is evident from the research to date (described above), that group-living animals do indeed communicate with one another through a wide variety of mechanisms, and that influences the behaviour of group individuals. Moreover, the use of facial expressions is a familiar form of communication for humans and therefore, can prove useful for social interactions between humans and non-human animals.

1.2: Affective states and facial expressions.

While conveying information about one's external state (such as alarm and resource calls described above) is important for social animals, conveying one's internal 'affective state' is also essential for social animals as this allows for the selection of appropriate behavioural decisions to be made by receivers, in response to external or internal cues from others (Albuquerque et al. 2016; Briefer et al. 2015; Parr et al. 2005b). Affective states are forms of motivation such as emotions, moods, attitudes, desires, preferences, intentions and dislikes (Sloman et al. 2003). There has been growing interest in the study of animal affective states in recent years (Figure 1.1), which span a range of mammalian species, including livestock (Briefer et al. 2015; Desire et al. 2002), rodents (Burman et al. 2008; File 2001), dolphins (Kellerman 1966), elephants (Bates et al. 2008; Russell & Barrett 1999), non-human primates (Kemp & Kaplan 2013; Parr 2001; Parr et al. 2005b, 2007), and canids (including companion and wild captive canids, Burman et al. 2011; Haidt 2001; Moe et al. 2006; Plutchik 1971; Walker et al. 2010). These recent studies show that non-human animals are capable of perceiving the affective states of conspecifics (and in some cases heterospecifics, Barber et al. 2017; Muller et al. 2015; Smith et al. 2016; Yong & Ruffman 2015) in addition to displaying them and can even display empathy toward group members (Bates et al. 2008), which means animal affective states are not purely subjectively fabricated notions, but do have a functional purpose in social communication (Ballesta & Duhamel 2015; Bates et al. 2008; Kemp and Kaplan 2013).

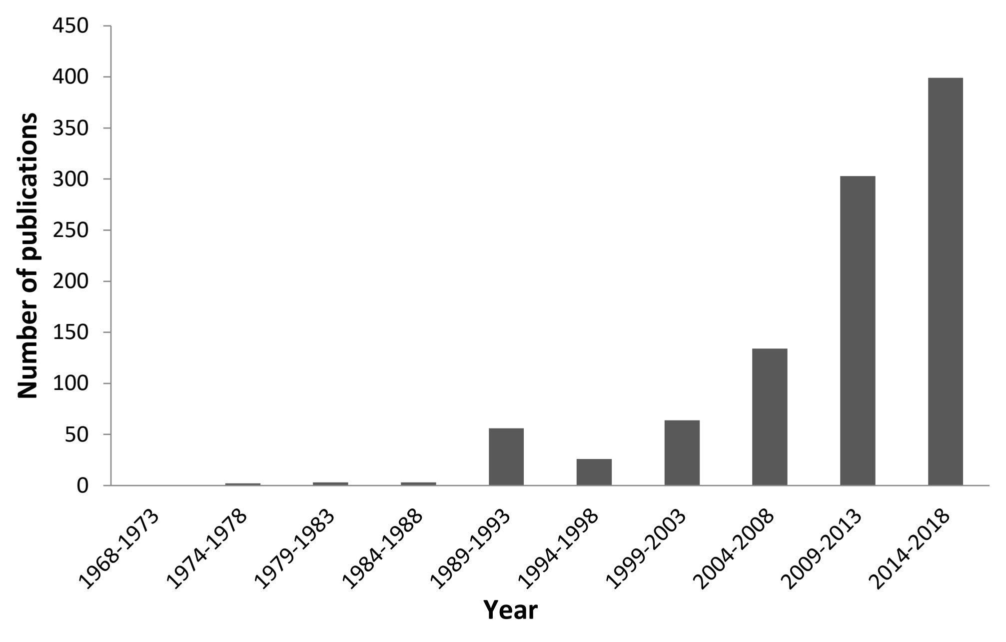

Figure 1.1. The number of publications produced between 1968 and 2018, in intervals, relating to non-human animal affective state research. Produced using 'Web of Science' search engine and the following key words; non-human, animal, affective states, emotions. Searches were refined to 'behavioural sciences' and 'zoology', and all peer reviewed journals were selected.

Indictors of affective states can be measured physiologically, which often includes measures of stress indicators. Stress is considered an affective state because, both stress and emotions result from hormone releases in response to specific external or internal cues (Canamero 1997; Joels et al. 2006; Le Roux, Bouic & Bester 2007; Yeh et al. 2015). Consequently, changes in the function of the hypothalamic-pituitary-adrenal (HPA) system, such as changes in glucocorticoid levels (Paul et al. 2005) can be indictors of stress, and changes in the sympathetic-adrenal-medullary (SAM) system can provide indictors of stress, measured as proxies such as Heart Rate Variability (HRV), blood pressure, skin conductance and temperature (Chapman et al. 1985; Desire et al. 2002; Paul et al. 2005; von-Borell et al. 2007). However, many physiological sampling methods are invasive (such as blood extraction) and can induce additional stressors in animals due to capturing procedures (Millspaugh et al. 2002). As a result, the additional stressors can have confounding effects on the affective state of an animal, and therefore, on baseline levels of the affective state indictor being measured (Paul et al. 2005). However, affective states can also be measured using non-invasive behavioural observation methods, which include the quantification of vocalisations and 'body language' (Briefer et al. 2015; Chapman et al. 1985; Desire et al. 2002; Paul et al. 2005; Siniscalchi et al. 2013), but in particular facial expressions (Cohn et al. 2007). In fact, examining facial expressions have been the foundation of many leading affective state theories (Cohn et al. 2007; Ekman 1992), and as a result of their value in affective state research, several systems have been developed to quantify them (Cohn et al. 2007). The most comprehensive system is the Facial Action Coding System (FACS, Cohn et al. 2007; Ekman & Friesen 1978; Ekman et al. 2002), which describes human facial expressions according to the movements of the underlying mimic muscles (Ekman & Friesen 1978; Ekman et al. 2002). FACS has been adapted for use in many other mammalian species, such as chimpanzees (*C*himpFACS, Vick et al. 2007), cats (*Felis catus*, CatFACS, Caeiro et al. 2013a), orangutans (*Pongo spp.*, OrangFACS, Caeiro et al. 2013b), gibbons (*Hylobatidae spp.,* GibbonFACS, Waller et al. 2012), macaques (*Macaca spp*., MaqFACS, Parr et al. 2010), horses (*Equus caballus*, EquiFACS, Wathan et al. 2015), and dogs (DogFACS, Waller et al. 2013). Linking affective states to facial expressions has the potential to provide a useful non-invasive tool for monitoring animal welfare (Paul et al. 2005). Being able to discern an animal's 'emotional' state is key to maintaining the psychological well-being and health of captive animals (Desire et al. 2002; Yeates & Main 2008), and could provide a useful non-invasive tool for assessing affective states (for example stress) in wild animals due to reduced risks of disturbance effects (Millspaugh et al. 2002; Paul et al. 2005). In addition, discerning the affective states of non-human animals would in general provide a better understanding of animal communication.

1.3: Wolf facial expressions and affective states.

One species that has frequently been used as a model for describing the social behaviour of group-living animals is the wolf (*Canis lupus*, Mech 1999). Wolves are known to have the most highly developed social system of all canids (Sheldon 1992), living in groups (known as packs), which range in size of between two and 42 individuals (Mech & Boitani 2003). Successful communication within wolf packs is vital in reducing aggression among pack members, thus maintaining pack cohesion and stability (Mech 2007). Wolves have a vast repertoire of communicative behaviour, utilising olfactory, auditory and visual modalities of communication (Fox 1975), to convey information about their affective states and social status to other pack members (Mech 2007). Wolves also attempt to elicit behavioural responses from other pack members and are capable of conveying and perceiving intensities of affective states during social interactions (Schenkel 1947). It is for these reasons that wolves will be used as a model, baseline species for this research to investigate intra-pack social communication.

The majority of research on olfactory communication in wolves is based on inter-pack communication via territorial scent-marking (Barja et al. 2004; Briscoe et al. 2002; Petak 2010: Peters and Mech 1975; Rothman and Mech 1979). Similarly, the majority of research on auditory communication in wolves has been conducted on the howl, which mainly focuses on inter-pack territorial behaviour (Dungol et al. 2008; Harrington and Mech 1982; Harrington and Mech 1979; Nowak et al. 2007; Theberge and Falls 1967; Tooze et al. 1990). However, wolves are capable of producing a wide diversity of vocalisations which accompany other communicative signals, such as facial expressions (Table 1.1, Busch 2007; Feddersen-Petersen 2000; Fox 1970; Harrington and Mech 1978). In fact, the main mode of intra-pack communication in wolves is visual, which involves the utilisation of the entire body (Mech 2007), including tail shape and positioning (Schenkel 1947; 1967), and postural displays (Schenkel 1947; 1967). But, the most visually expressive part of a wolf's body is its head (Schenkel 1947; 1967), with a variety of facial expressions (Fox 1970; Schenkel 1947).

Table 1.1. Comparison of Simian primate and canid facial expressions, and the social situations in which they are utilised, adapted from Fox (1970).

Simian primate facial expressions.	**Canid* facial expressions.**	**Social situations.**
Tense mouth face.	1. Agonistic pucker. Vertical retraction of lips in W, C and D.	Tendency to attack.
Open-mouth face.	2. Threat gape (distinct in F).	Tendency to attack. Signals bit intention.
Staring bared-teeth scream face.	3. W, C and D. Threat gape with vertical and horizontal retraction of the lips.	Signals bite-intention with some flight tendency.
Frowning bared-teeth scream face.	4. W, C and D. Above with wider gape and greater vertical lip retraction.	Defensive threat when escape is blocked.
Silent bared-teeth face.	5. Submissive grin (horizontal retractions of lips).	Low tendency to flee: Ritualised appeasement.
Bared-teeth ‘gecker’ face.	No comparable expression.	In infants when disturbed. Low tendency to approach and flee in adults.
Teeth-chattering face.	6. W, C and D. Agonistic tooth snapping.	Tendency to flee.
Lip-smacking face and Tongue-smacking face.	7. W, C and D. Licking intention.	Strong approach tendency and weaker flight tendency.
Chewing-smacking face.	8. W, C and D. Nibbling intention.	No comparable social situation
Snarl-smacking face and Protruded-lip face.	No comparable expression	Approach (often to mate).
Pout face.	9. W, C and D. Submissive rooting approach and forepaw rising.	Approach (mother-infant) intention movement to take nipple in mouth, and between adults.
Relaxed open-mouth face.	10. Play-face.	During play or advances to play.

Key: Canid* vocalisations accompanying facial expressions;

1 = low growl; 2 = louder growl or explosive ‘Tch Tch’ sound in F; 3 and 4 = silent or successive growl and whine; 5 = silent, or whining; winnowing call in F; 6 = silent or successive whining and growling; 7 = silent or whining; 8 = silent or whining; 9 = silent or whining; 10 = silent; barking only in D.

*****Except where indicated, facial expressions are seen in all canids; W = seen in wolves (*Canis lupus)*, D = seen in dogs (*Canis lupus familiaris)*, C = seen in coyotes (*Canis latrans)*; F = seen in foxes (*Vulpes vulpes).*

Studies suggest that wolf facial expressions convey affective states and social status (Fox 1970; Schenkel 1947). Schenkel's work covered more than 20 variations of wolf facial expressions (some of which are seen in Figure 1.2). Fox (1970) compared the facial expressions of wolves (highly social canids), coyotes (*Canis latrans*, semi-social canids), and several fox species (least social) to primates. He found that wolves were capable of a broad range of facial expressions, which are used in varying social interactions, and are similar to those seen in Simian primates (Table 1.1). It was also proposed by Fox that the ontogeny of canid facial expressions recapitulates phylogeny, in that facial expressions typical of foxes (less social canids) develop earlier in coyotes and wolves (semi-social and highly social canids, respectively), thus increased complexity of facial expressiveness occurs with increased levels of sociality in canids. It is known that non-vocal communication in primate societies is highly important for group members (Burrows et al. 2014), and is often achieved by complex variations in olfactory, auditory and visual communication, and by the ability of conspecific receivers to perceive and manipulate information being conveyed by signals during social interactions (Blumstein & Armitage 1997; Dunbar 1998; Joffe and Dunbar 1997; Dunbar and Shultz 2007). It is argued that primates evolved such complexity to cope with their increased sociality (Blumstein & Armitage 1997, McComb & Semple 2005; Pollard & Blumstein 2012), thus it is possible that wolves may have also evolved complex forms of communication to cope with their increased sociality (especially when living as part of a large pack, of 40+ individuals, Mech & Boitani 2003).

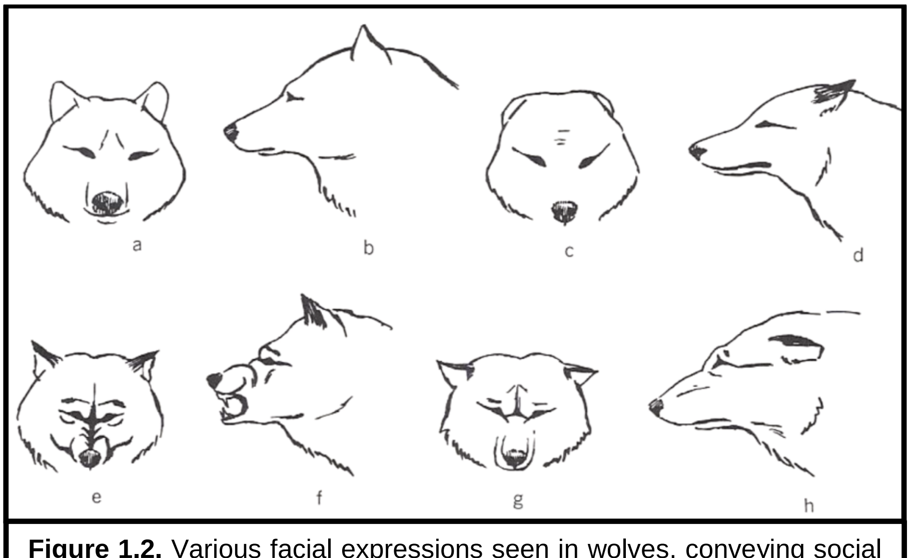

Figure 1.2. Various facial expressions seen in wolves, conveying social status and affective states; a and b show the relaxed facial expressions of a dominant, high ranking wolf; c and d show the facial expressions of an anxious wolf; e and f are the facial expressions of a threatening wolf; g and h portray suspicion or doubt. Adapted from Schenkel (1947).

Combinations of facial features, including pelage patterning (colouration and fur slope), mimic muscle movements, and the activities of the eyes, nose and ears, emphasise the appearance of the muzzle, lips, eyes, forehead and ears (the main conveyors of facial expressiveness, Bolwig 1964; Fox 1970; Schenkel 1947). For example, with regards to pelage colouration, many wolves have a conspicuous white coloured muzzle that extends backwards and merges with a less noticeable facial cheek area (Figure 1.3). It is theorised that this white muzzle and cheek area may increase the perceptibility of the black lips, for example, during a 'submissive grin' (Table 1.1) the black lips are retracted horizontally and become distinctly noticeable (Fox 1970). The region around the supraorbital vibrissae in wolves is also emphasised by paler contrasting fur, forming bilateral structures resembling small 'eyebrows' (Fox 1970). The positions of the eyebrows are altered (from a neutral, relaxed state) during numerous facial expressions. Slight elevation of the eyebrows occurs during alertness and when the eyes are wide open, for example during the 'direct stare', where a dominant individual will stare intensely at a subordinate (Fox 1970). Medial elevation and lateral depression of the eyebrows occurs during conflict or anxious affective states (Fox 1970). This suggests that these facial features are fundamental in the effectiveness of communication in wolves.

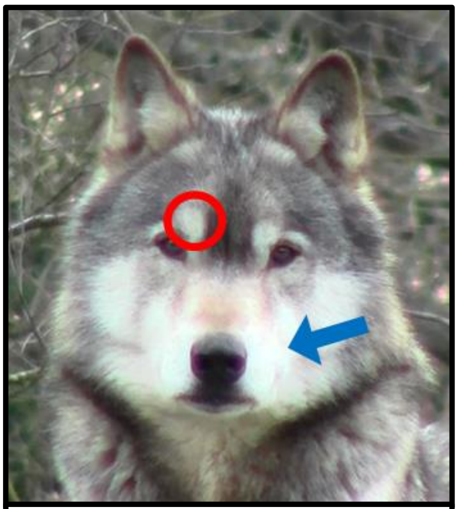

Figure 1.3. Portrait of a wolf showing the conspicuous white muzzle (blue arrow) that extends down to the cheek area, and the paler contrasting fur that forms the 'eyebrows' (red circle). Photograph by ER Hobkirk.

Some wolves appear monochrome in their colouration, such as pure white or pure black wolves and therefore, do not have the same facial patterning seen in Figure 1.3. Instead the facial patterning of these monochrome wolves is very inconspicuous, in that their eyebrows, muzzle and cheek areas are discreet and blend in with the colour of the rest of the face. This colour blending suggests the use of distinct facial patterns (such as conspicuous eyebrows, muzzle and cheek areas) to emphasise facial expressions is absent in these monochrome wolves. However, the main conveyers of facial expressiveness of every species of wolf around the world remain relatively the same; the shape and position of the eyes, ears, forehead, muzzle, nose and lips are the same for every wolf. For example, the wolf depicted in Figure 1.3 is in fact a hybrid of two separate sub-species, North American grey wolf X Eurasian wolf (*Canis lupus* X *Canis lupus lupus*) and yet, the morphologies of the main conveyers of facial expressiveness of this wolf are the same as those seen in any other wolf. Yes, wolves do vary in typical body size, but the shape and positon of their physical features remain the same. Furthermore, the fur length of different sub-species of wolves does change depending on local climates. For example, Arabian wolves (*Canis lupus arabs*) have quite short fur in comparison to Arctic wolves (*Canis lupus arctos*) as they inhabit environments with much warmer climates. However, the slope of the fur (the way in which the fur lies on the face and head of the wolf) of the Arabian and the Arctic wolf remains the same. This suggests that perhaps overall head and facial morphology is more important for the emphasis of facial expressions when visually communicating, as opposed to facial colour patterns.

Unfortunately, the studies conducted to date on wolf facial expressions and affective states (discussed above) are dated and lack quantitative data, they only cover agonistic behavioural interactions, and they do not fully consider the individuality (such as social status, sex and age) of the wolves being studied. There does not appear to be any recent research detailing the facial expressiveness of wolves and therefore these dated studies may not be representative of the full range of facial expressions wolves may be capable of, especially if they are indeed comparable to Simian primates. There is some renewed interest in the field of canid facial expressions, with regards to domestic dog-dog and dog-human communication (Albuquerque et al. 2018; Bloom & Friedman 2013; Catia, Kun & Daniel 2017; Kaminski et al. 2017; Waller et al. 2013) yet, these studies have been conducted in the absence of a quantitative natural baseline, such as that of the wolf.

1.4: Domestic dog and wolf divergence.

The domestic dog is considered to be the selectively bred descendent of wolves (Brauer et al. 2013; Clutton-Brock 1995; Goodwin et al. 1997; Scott 1967). Like wolves, dogs also express affective states (Nagasawa et al. 2013) and are capable of producing facial expressions (Bolwig 1964) due to their complex facial musculature (Figure 2.6, section 2.4.2, Bolwig 1964). However, as a result of selective breeding, the head and facial feature morphologies of dogs have diverged greatly from those of their wolf ancestors (Clutton-Brock 1995; Scott 1967), with dog breeds consisting of a vast array of body shapes, fur types and colourations. For example, the facial pelage of typical Rottweillers have conspicuous brown eyebrows set against a solid black background (Figure 1.5C), whereas typical Komondor dogs do not have any visible eyebrows (Figure 1.5E). Furthermore, the fur length and slope of Rottweillers and Komondors are quite different to each other, and to that of wolves (Figure 1.5A). Fur length and slope (such as that of a Komondor) also pose a great risk of reduced visibility of the eyes and ears, two of the main conveyers of facial expressiveness (Bolwig 1964; Fox 1970; Schenkel 1947), which will result in a lack of signal reception from conspecifics. Other morphological divergences of the domestic dog include head shape, the shape and position of the ears, and the shape and length of the muzzle, with many dog breeds having brachycephalic faces (Figure 1.5D). Do these divergences in head and facial morphology among dog breeds limit their ability to successfully communicate like their wolf ancestors? Some breeds have retained a more ‘wolf-like’ appearance (Figure 1.5B), so, are these breeds more capable of successful communication? Indeed, a single pilot study conducted has suggested that breeds with flopped ears and brachycephalic faces have less pronounced facial expressions compared to more wolf-like breeds (Meridda et al. 2014). If communicative abilities are limited, what potential impacts could this have on social interactions between different dog breeds? Could there be increased aggression among certain breeds due to an inability to successfully communicate? Furthermore, it is known that dogs will often ‘yelp’ when subjected to pain or discomfort (Yeon 2007). However, not all dogs make vocalisations to announce they are in pain or discomfort. Many dogs express early signs of pain and discomfort via ‘body language’ (visual communication, including facial expressions, Hubrecht 1995; Pers. Obs.). If dog breeds differ in their abilities to produce facial expressions due to physical limitations, could this result in humans misinterpreting their own companion dogs and increasing the risks of becoming bitten if they cannot recognise a particular affective state such as pain, of their dog? Indeed, what if the dog owner is deaf and cannot hear their dog (perhaps a hearing guide dog) vocalise due to pain? It would be beneficial to visually assess the affective states of dogs from a distance (perhaps via easily interpretable, breed specific descriptors of key facial expressions denoting corresponding affective states) as not only would this aid dog bite prevention, but would also prove useful for authorities involved in cruelty cases. For example, video footage could be taken of neglected dogs and used in court cases, thus essentially giving neglected dogs a ‘voice’.

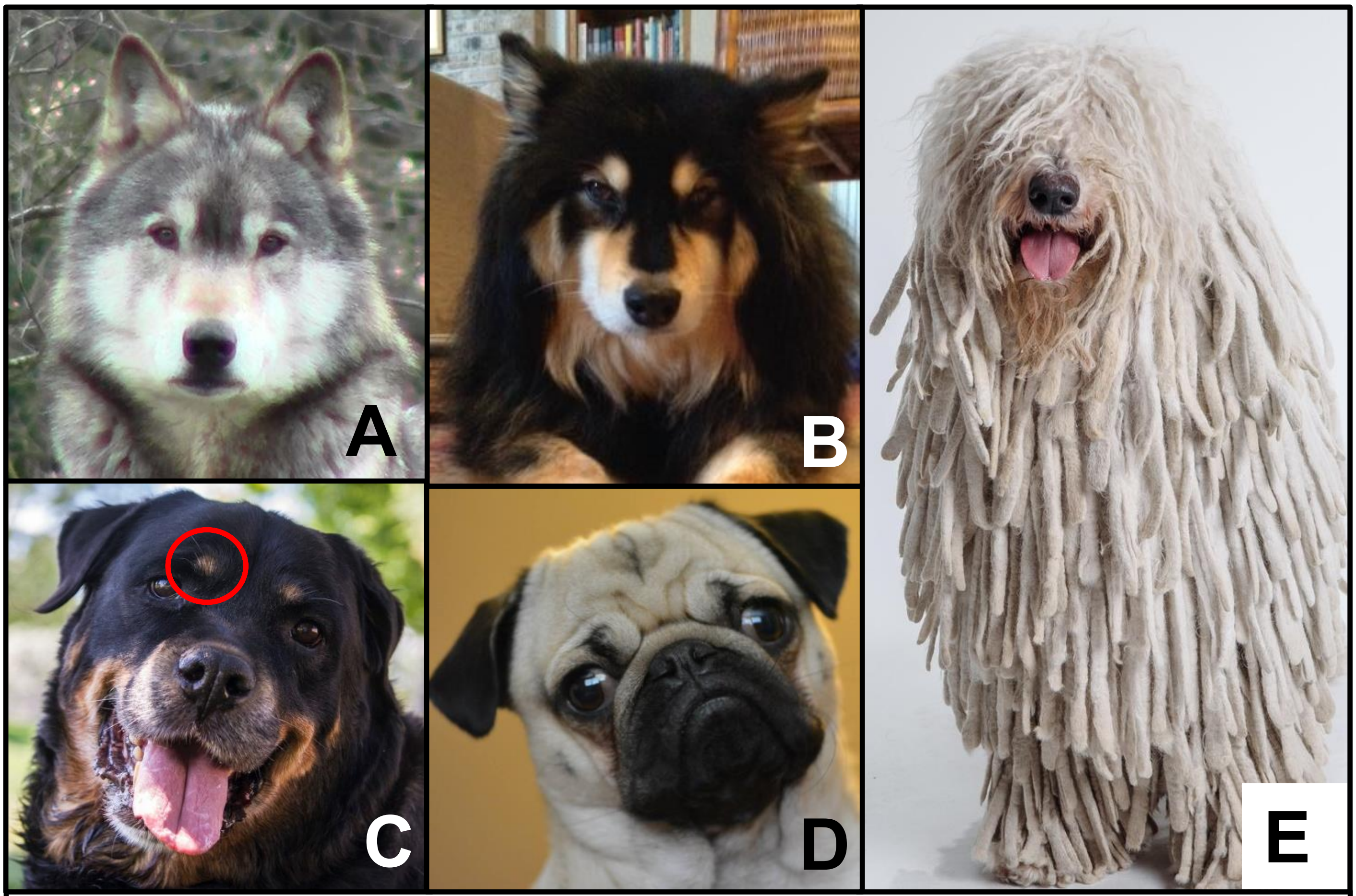

Figure 1.4. A) Wolf (*Canis lupus*) portrait depicting typical head morphologies and facial patterning. Note erect ears, head shape, fur length and slope, and facial masking as a consequence of lighter coloured 'eyebrows', muzzle and cheek area. Photograph by ER Hobkirk. **B)** 'Wolf-like' Finnish Lapphund dog, with head morphologies and facial patterning almost identical to that of the wolf. Photograph by SD Twiss. **C)** Typical Rottweiler face with conspicuous brown eyebrows (red circle), set against a solid black background. Note flopped ears and broad head shape in comparison with the wolf. Image courtesy of the American Kennel Club. **D)** Brachycephalic face of a Pug dog. Note flopped ears, bulging eyes and excessive wrinkling in comparison with the wolf. Image courtesy of the American Kennel Club. **E)** Komondor dog with less distinct facial features due to fur type (dreadlocks), length and slope. Image courtesy of the American Kennel Club.

Despite the potential inability of dogs to successfully communicate with each other (via facial expressions), they are unusually skilled at reading human social and communicative behaviour (inducing human facial expressions) more so than our closest primate relatives (Hare & Tomasello 2005). However, to date there has been little quantitative research aimed at interpreting and understanding the social and communicative behaviour of dogs, and there has been no formal quantitative analysis conducted of the full complexity and function of domestic dog facial expressiveness. Yet, the fundamental behavioural processes of domestic dog interactions are grounded in the behaviour of their ancestors (Range & Viranyi 2014a). Therefore, a full understanding of social communication in domestic dogs is only possible through a comparison with the social communication in wolves, hence the necessity to quantify wolf facial expressiveness as a baseline for that of domestic dogs. This baseline will provide the foundation for interpreting and understanding social communicative signals among the myriad of different dog breeds (Goodwin et al. 1997) and provide a means for humans to understand the social communication of their companion dogs more efficiently. To achieve this, the following objectives will be met:

(1) To quantify the affective states of wolves and dogs and relate these to specific facial expressions.

(2) To investigate the roles of different facial movements in producing successful facial signals.

(3) To identify differences or similarities between wolf and dog facial expressions.

(4) To identify differences and similarities in wolf facial expressions owing to social status.

(5) To identify constraints or limits on the diversity of facial expressiveness and, therefore, the ability to communicate affective states, in different dogs of varying head and facial morphologies.

Chapter 2: Methodology.

To meet the objectives of this research, up-close observations of both wolves and domestic dogs were required. However, wild wolves are naturally elusive and usually fearful of humans (Haber & Holleman 2013; Mech 2007; Mech & Boitani 2003; Pers. Obs.), therefore, for the purposes of this research, the use of human-habituated, captive bred and/or reared wolves was necessary. However, captive wolves tend to be housed within outdoor enclosures and have infrequent contact-time with humans. Therefore, typical pet dogs, which tend to live constantly within a human environment, and have frequent human interaction time, were not used for comparative purposes. Instead, kennelled rescue dogs housed in similar conditions to those of the captive wolves (outdoor enclosures, with infrequent human contact-time) were used.

2.1: Study sites.

All observations for this research were conducted at two separate sites; The UK Wolf Conservation Trust (UKWCT, Beenham UK, 51.419491N, -1.153433W), and Dogs Trust Darlington (Sadberge UK, 54.556676N, -1.473808W). The UKWCT is a non-profit organisation, selected as they are a member of the British and Irish Association of Zoos and Aquariums (BIAZA), who promote the scientific research of wolves, and provide opportunities for researchers to study human-habituated wolves up-close. All observations at the UKWCT were conducted between February 15th 2016 and March 4th 2016, on weekdays between 0900 and 1700 hours (GMT), amounting to 15 days in total.

Dogs Trust (formally known as the National Canine Defence League, NCDL) is the UK's largest dog welfare charity, who specialise in rehoming abandoned dogs across the UK. As a consequence, Dogs Trust consists of multiple branches spread across the UK, including Darlington. Dogs Trust was selected as they promote the scientific research of dog welfare and provide opportunities for researchers to study dogs up-close. In addition, Dogs Trust Darlington provided a wide range of dog breeds and therefore, a variety of dog facial morphologies to observe, which was fundamental to the objectives of this research. Observations were conducted at Dogs Trust Darlington between August 9th 2016 and November 11th 2016, on weekdays between 1100 and 1700 hours (BST), amounting to 21 days in total.

All observations for this research were conducted during working and public open hours at both UKWCT and Dogs Trust Darlington. Therefore, observations were conducted around staff carrying out their daily duties, and around visiting public. On each day, prior to data collection, on-site staff and public were informed of the observations being conducted and verbal permission was obtained from staff and public members if they were to be included in video footage (section 2.4.1). Finally, due to seasonal changes, weather conditions varied at each study site, consisting of typical UK seasonal weather, though morning temperatures (before 1200 hours, GMT) at UKWCT were below 0°C. However, weather conditions did not appear to affect wolf and dog facial expressions, but merely affected their daily activities, with wolves tending to be more active (thus, interacting more) in cooler conditions, while dogs tended to be more active in warmer conditions (Pers. Obs.).

2.2: Study subjects.

The following two sub-sections detail the morphological features, breeding status, housing and maintenance of the wolves and domestic dogs used for this research. All study subjects were reported to be fit and healthy prior to, and throughout data collection.

2.2.1: Wolves.

The UKWCT provided 10 wolves (which included different sub-species, Table 2.1) that were habituated to the presence of humans. The wolves were habituated as they were either captive bred and/or captive reared by human handlers (Table 2.1). Wolves included adult females (*n* = 5) and males (*n* = 5), ranging in ages of between four and nine years old (Table 2.1) at the beginning of data collection. Each wolf was also sterilised prior to data collection, either via spaying (females) or castration (males) though ‘Mai’ (Table 2.1) underwent an ovariectomy (semi-spay) and ‘Torak’ (Table 2.1) underwent a vasectomy. Therefore, all wolves were unable to produce offspring (pups). However, ‘mated pairs’, consisting of a non-related female and male wolf housed together, would still engage in mating behaviour (courtship, attempted copulations and full copulations with copulatory ties) during the mating season (January to March, Haber & Holleman 2013; Mech 1974; Mech & Boitani 2003).

Table 2.1. Wolves observed for this research at the UK Wolf Conservation Trust wolf, with corresponding pack information, rearing information and sub-species classifications.

Wolf ID	**Sex**	**Age (years)**	**Pack**	**Captive bred?**	**Hand reared?**	**Species classification**
Mai*	Female	9	Mated pair	Yes	Yes	*Canis lupus*
Matomo*	Male	7		Yes	No	*Canis lupus*
Mosi	Female	9	Mated pair	Yes	Yes	*Canis lupus*
Torak	Male	9		Yes	Yes	*Canis lupus* X *Canis lupus lupus* (hybrid)
Sikko	Female	4	Siblings (‘Arctic pack’)	No	Yes	*Canis lupus arctos*
Massak	Male	4		No	Yes	*Canis lupus arctos*
Pukak	Male	4		No	Yes	*Canis lupus arctos*
Tundra	Female	4	Siblings (‘Beenham pack’)	Yes	Yes	*Canis lupus*
Tala	Female	4		Yes	Yes	*Canis lupus*
Nuka	Male	4		Yes	Yes	*Canis lupus*

*Parents to Beenham pack wolves; pups were produced four years prior to data collection, after which, all wolves were sterilised to prevent further pups being produced.

The facial colour patterns of each wolf differed between individuals (Figure 2.1), but, regardless of what sub-species they were, the overall facial morphologies of each wolf remained consistent (Figure 2.1), with the snout, lips, eyes, forehead and ears (the main conveyers of facial expressiveness) maintaining the same general shape and position, with the same general pattern of fur length and fur slope. Each wolf was also devoid of facial scars, and any neutral facial abnormalities, in that each wolf’s neutral facial expression did not present with any unusual facial features, such as constantly raised upper lips, drooping upper lips (flews), flopped or semi-flopped ears, or drooping eyes (ectropion).

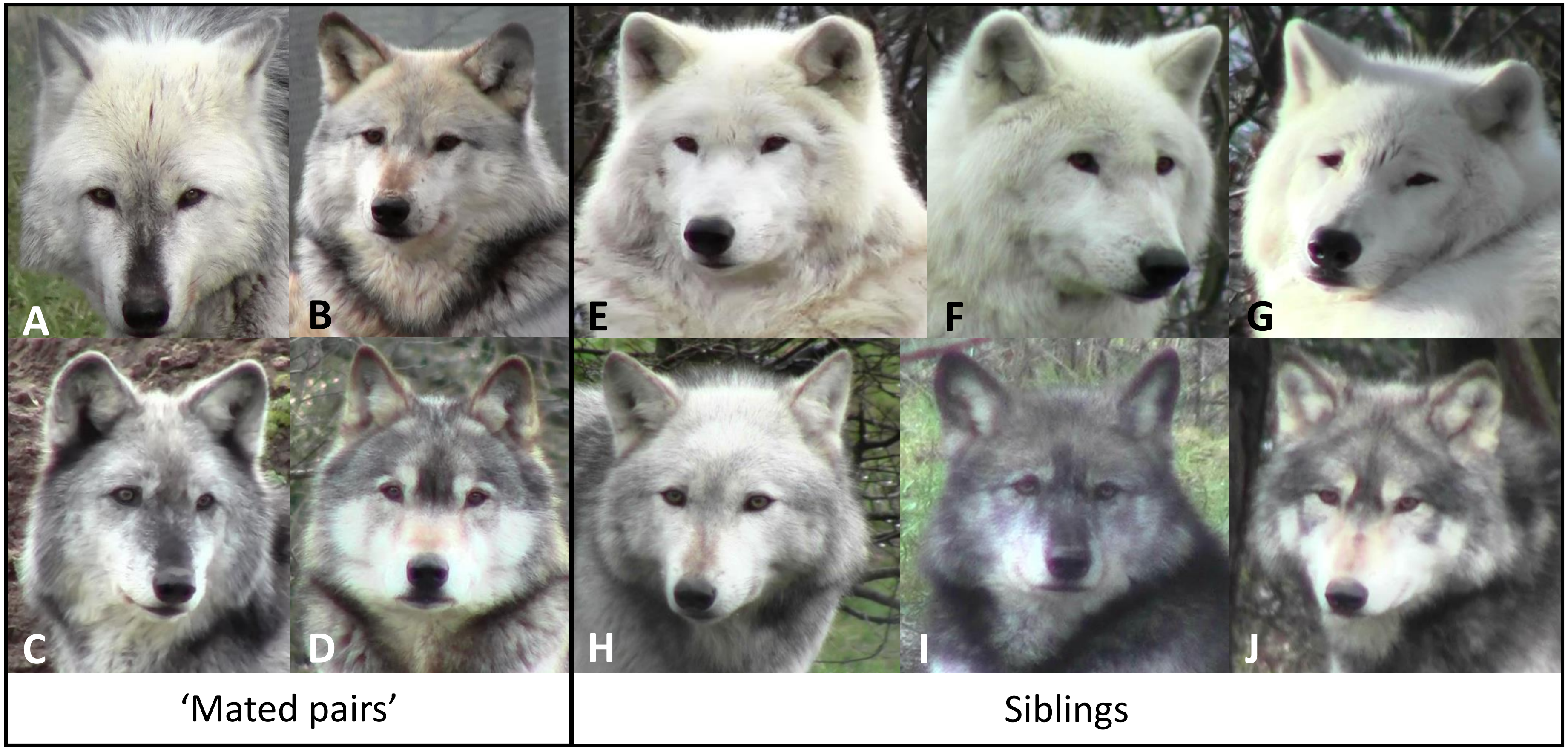

Figure 2.1. UK Wolf Conservation Trust wolf profile photos, depicting varying facial colouration patterns, yet consistent overall facial morphologies. A) ‘Mai, B) ‘Motomo’, C) ‘Mosi’, D) ‘Torak’, E) ‘Sikko’, F) ‘Massak’, G) ‘Pukak’, H) ‘Tundra’, I) ‘Tala’ J) ‘Nuka’. Wolves were identified via their facial colour patterns, except for the Sikko, Massak and Pukak, who were identified via their comparative body sizes. (Photographs by ER Hobkirk).

Wolves were housed together in small established, stable packs, of either a mated pair (Table 2.1) or a family unit of three siblings (Table 2.1) therefore, maintaining (to some extent) natural pack dynamics (Mech 1974; Mech & Boitani 2003). Each wolf pack was housed in one of four separate, yet adjacent, outdoor enclosures, which had adjoining ‘holding pens’ and ‘holding kennels’ (Figure 2.2). During data collection wolves were free to roam between their enclosures, holding pens and kennels. Each enclosure mimicked natural environments, consisting of ponds, trees and open grass areas (Figure 2.2) for the wolves to freely move about in. Providing ‘natural’ environments has shown to reduce stress and maintain the natural behaviours of captive mammals (Newberry 1995; Wells 2009). In addition, each enclosure included ‘man’-made water troughs, wooden dens and raised wooden platforms, and enclosure boundaries consisted of galvanised chain-linked fencing. Wolves were provided with various enrichment activities (again, to help reduce stress and maintain natural behaviours, Newberry 1995; Wells 2009), including foraging games, where novel food items (commercial hotdogs and tuna) were hidden around the enclosures for the wolves to find. Other enrichment activities included inanimate objects (usually straw filled hessian sacks) coated with various oil-based scents for the wolves to investigate and interact with. These enrichment activities generally allowed for various wolf-wolf social interactions to be observed and studied. Furthermore, although wolves were housed in enclosures, they were, on occasion, walked individually on leashes around UKWCT grounds, again, for enrichment purposes. When individual wolves were walked around UKWCT grounds, the wolves that remained within their enclosures would interact with the walking wolf, which allowed for more social interactions to be observed and studied.

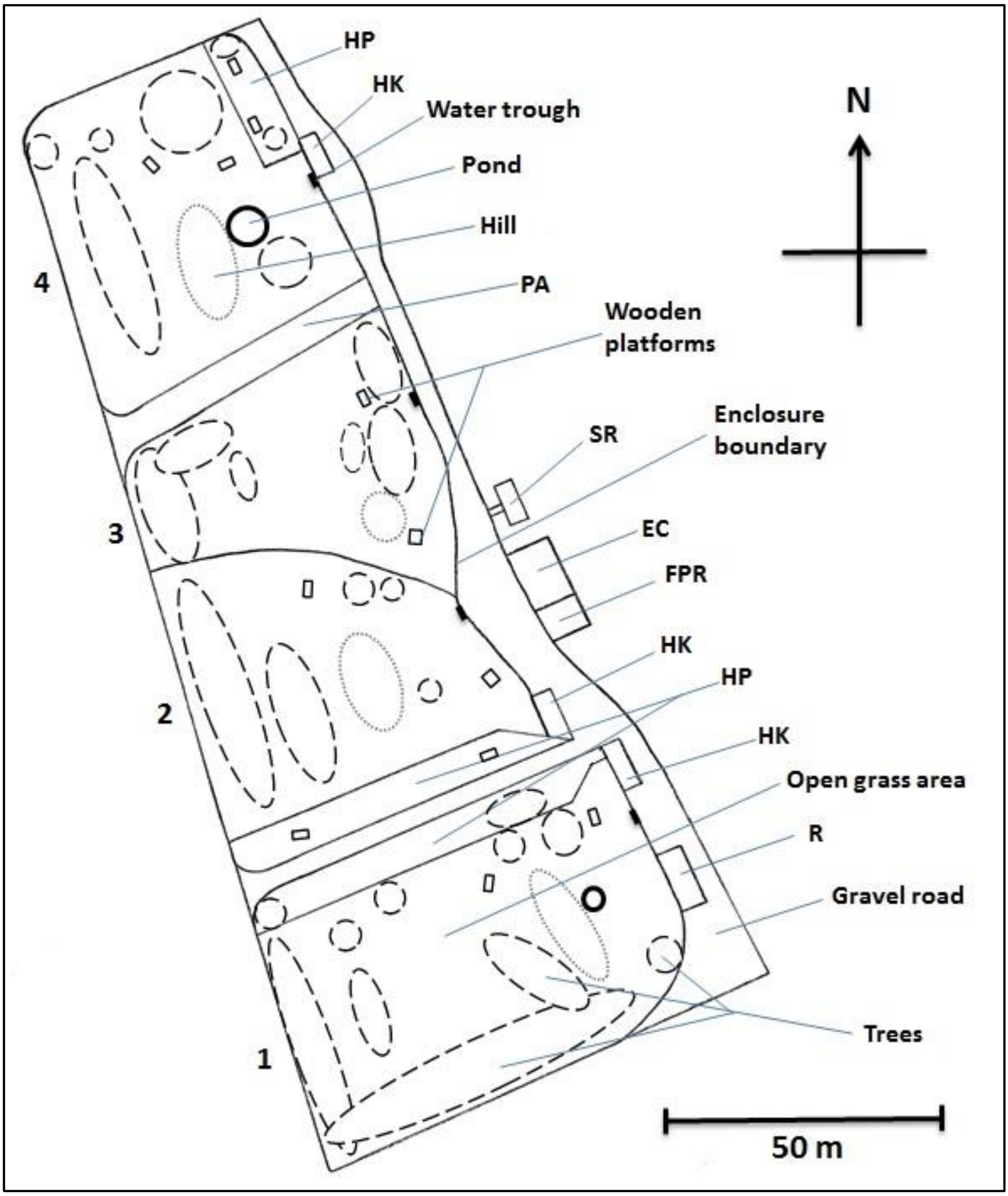

Figure 2.2. Aerial view of UKWCT grounds (drawn to scale), illustrating the layout of all four wolf enclosures and their 'natural environment' features, and surrounding buildings (note, boundary fencing was 3m in height). Enclosure 1 housed mated pair Mosi and Torak. Enclosure 2 housed the Arctic pack wolves. Enclosure 3 housed mated pair Mai and Motomo. Enclosure 4 housed the Beenham pack wolves. The design of each enclosure was similar, except enclosures 2 and 3 did not have ponds, and enclosure 4 did not have adjoining holding kennels (HK) or an adjoining holding pen (HP). However, the photography area (PA) could be used as a holding pen for enclosure 4 if required. 'Man'-made wooden platforms are shown, but 'man'-made dens are not as these are hidden under tree coverage in the enclosures of mated pairs. Surrounding buildings include the staff room (SR), education centre (EC), feeding preparation room (FPR) and reception (R) at the entrance to the grounds.

Enclosures were generally not cleaned, though on occasion holding kennels were hosed clean by UKWCT staff to remove wolf waste, and each morning (before 1100 hours, GMT) 'man'-made water troughs were replenished. Kennel cleaning and water changing routines were not observed to alter the behaviour of the wolves (Pers. Obs.). Each wolf was fed a raw diet of surplus chicken, beef and paunch from local abattoirs, though their diets were also supplemented with wild Muntjac deer (*Muntiacus reevesi*) and European rabbit (*Oryctolagus cuniculus*) supplied by local people (as either road-kill or steel-shot game). Individual wolves were fed by staff members at a randomly selected time during daylight hours. Wolves were fed six days per week, with one randomly selected 'starve day' per week, whereby wolves were not provided with any food; this feeding routine was implemented to prevent wolves from associating specific time periods with food, again, maintaining natural wolf behaviour (Bassett & Buchanan-Smith 2007; Ings et al. 1997; Shepherdson et al.1993; Young 1997). In addition, the diets of 'Torak' and 'Tundra' (Table 2.1) were supplemented with *Flexi-joints* by Healthspan© for arthritis treatment. The *Flexi-joints* tablets were crushed into powder and added to the raw food.

2.2.2: Domestic dogs.

Dogs Trust Darlington provided 64 domestic dogs; 43 standard-breeds (recognised by the UK and American Kennel Clubs), and 21 cross-breeds (including six Lurchers**, Table 2.2). Dogs consisted of both females (*n* = 21) and males (*n* = 43), ranging between the ages of nine months and 11 years old (Appendix A), though the ages of 20 dogs were unknown. However, the adult morphological development of domestic dogs depends upon their final body size (giant, large, medium or small, Table 2.2), in that adult dog ages are breed-specific (Hawthorne et al. 2004). Based upon this breed-specific development, all dogs used in this research were considered to be adults at the time of data collection, including those of unknown age, based upon their 'breed-type' (be that standard- or cross-breed, see appendix A for specific breed information).

Table 2.2. Domestic dogs observed for this research at Dogs Trust Darlington. Typical adult body sizes (bold text, in descending order) are shown per ‘breed-type’, be that standard-breed (according to UK and American Kennel Club standards) or cross-breed.

Breed-type and body size	*n*, Number of dogs		
	Females	**Males**	**Total**
Giant			
American Bulldog	1	0	1
Large			
Akita	0	1	1
German Shepherd Dog	0	2	2
Greyhound	0	2	2
Labrador Retriever	4	2	6
Boxer Dog	0	3	3
Medium			
German Shorthaired Pointer	0	1	1
Siberian Husky	1	0	1
Saluki	0	1	1
Basset Hound	0	1	1
English Springer Spaniel	0	1	1
Border Collie	1	3	4
English Cocker Spaniel	0	2	2
Whippet	0	2	2
Small			
Staffordshire Bull Terrier	3	2	5
Dachshund	0	1	1
Beagle	1	0	1
Jack Russel	2	3	5
Lhasa Apso	0	1	1
Shih Tzu	0	1	1
Japanese Chin	0	1	1
Mixed			
Lurcher**	1	5	6
Cross-breeds	6	8	15

** Explicit cross-breeds, typically of a sighthound crossed with another breed.

The facial colour patterns and fur length of the dogs used in this research varied between individuals (Appendix A), while fur slope, and facial feature morphologies varied between breed-types (Appendix A). Therefore, the size, position and shape of the main conveyers of facial expressiveness varied between breed-types. In addition, some dogs presented with consistent neutral facial abnormalities, such as constantly raised upper lips, ectropion, and malformed or scared ears (see Appendix A for details), which provided the opportunity to study the effects of increased morphological divergence from wolves.

The majority of dogs were sterilised prior to entering Dogs Trust Darlington kennels, some were not. Intact dogs were typically sterilised (females spayed, and males castrated) within one week of entering the kennels, thus not all dogs were sterilised prior to data collection. However, the dogs that were sterilised during data collection were few (*n* = 2). It has been shown that sex differences can influence the intensities (amount of facial movement) of affective ('emotional') facial expressions (Dimberg & Lundquist 1990; Thunberg & Dimberg 2000) though to date, there is no research to suggest specifically that gonadal hormones influence the presence or absence of affective facial expressions. Therefore, whether dogs were sterilised or not was deemed insignificant for the purposes of this research. In addition, dogs tended to engage in copulatory behaviour (regardless of being sterilised or not), though were not able to produce offspring, as intact male dogs were separated from females in oestrous to prevent unwanted pregnancies.

Dogs were usually housed together in small contrived packs (of two or three dogs) and may not have been familiar with each other prior to being housed together. However, dogs were only housed in packs if Dogs Trust staff assessed them to be 'dog friendly' (defined here as displaying no agonistic behaviour towards a conspecific). Thus, in general, dogs housed in packs co-existed without engaging in conflict. If a dog was assessed by staff to be 'dog aggressive' (defined here as a tendency to display agonistic behaviour towards a conspecific, often resulting in conflict), then that dog was housed alone. It should be noted that domestic dogs are capable of socially organising themselves into packs, similar to that of wolves, regardless of previous familiarity to surrounding conspecifics (Cafazzo et al. 2014; Font 1987). Therefore, for the purposes of this research, these contrived packs were considered stable and acceptable for comparison to UKWCT wolf packs.

Dog housing consisted of three separate parasol kennel blocks (Figure 2.3), with each block containing 11 kennels. Dogs were either housed in small packs or singularly within one of the 11 kennels within each kennel block. Each kennel composed of an indoor sleeping area and an outdoor 'run (Figure 2.4)', separated by a brick wall, with a single pulley-operated animal door. Indoor sleeping areas were not visible (except through the joining animal door) or accessible during data collection thus, they will not be discussed further in this chapter. However, it should be noted that sleeping areas were artificially lit and thermostatically controlled between 18 and 22°C, and dogs were free to move between their sleeping areas and runs throughout data collection. Each run composed of concrete flooring and overhead roofing, though were lit by natural light, and were not temperature controlled. Adjacent runs were separated by brick walls, while run fronts were composed of galvanised wire mesh panels, which included a door. In addition, each run contained commercial dog bedding, toys and water bowls. Due to the design and positioning of the kennel blocks, dogs in adjacent and opposing kennels were unable to visually interact with one another. However, individual dogs were frequently walked around Dogs Trust Darlington grounds by staff, past the kennels of other dogs, which provided opportunities to observe and study additional dog-dog social interactions.

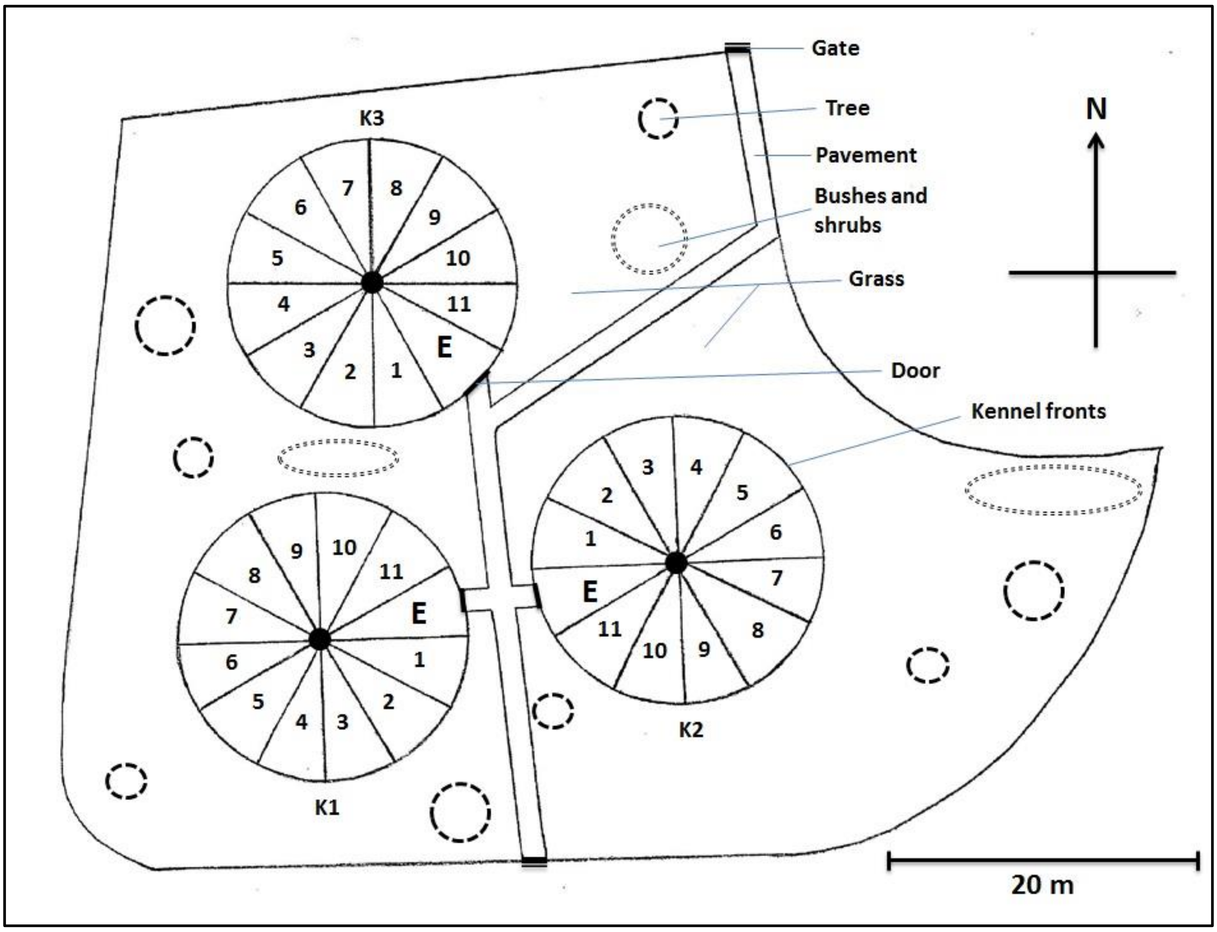

Figure 2.3. Aerial view of Dogs Trust Darlington kennel grounds (drawn to scale), illustrating the three separate parasol kennel blocks (K1, K2 and K3) used in this research. 'E' represents the entrance to each kennel block, and each kennel block contains 11 separate kennels.

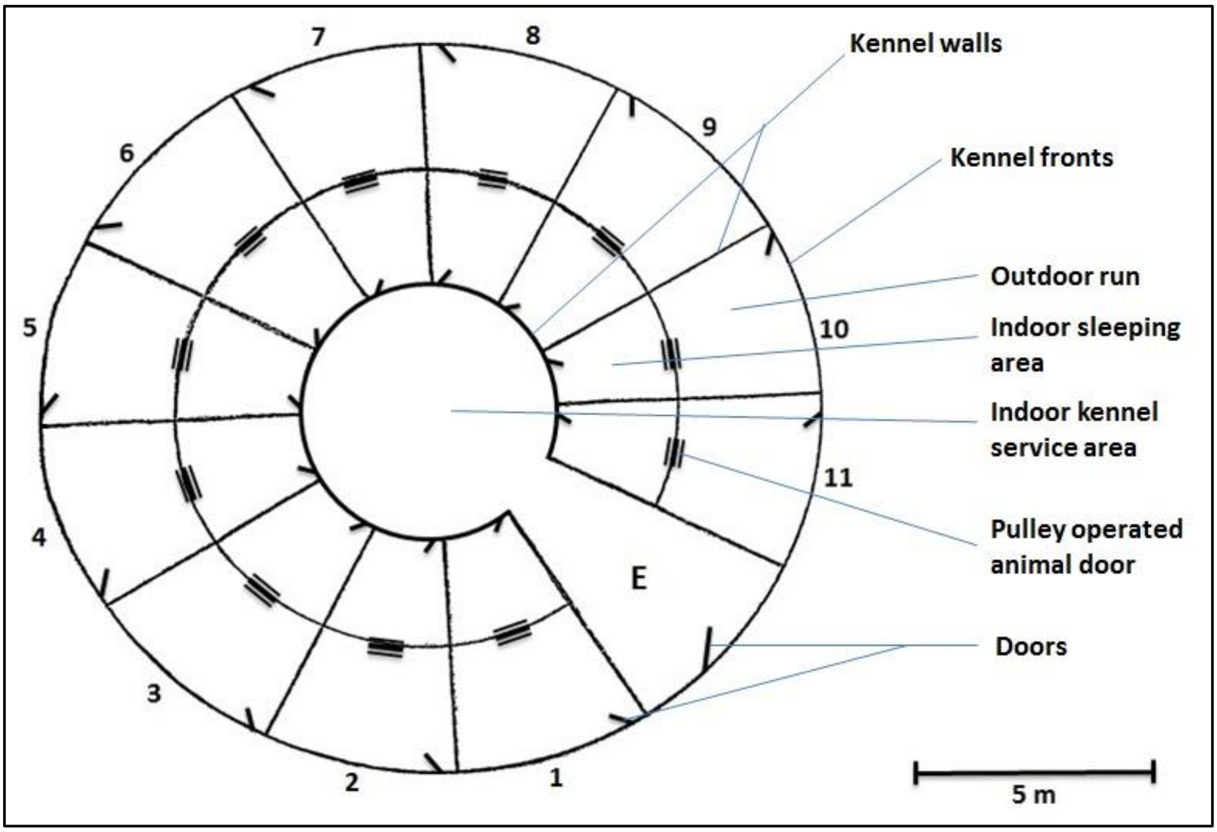

Figure 2.4. Shows the internal design (drawn to scale) of the parasol kennel blocks (in this case kennel block K3, Figure 2.3), again, ‘E’ represents the entrance to the kennel block, and the individual kennels are numbered one to 11.

Each morning (before 1100 hours, BST) all kennel runs were deep cleaned by staff, with dog waste removed (though, this was removed *ad-hoc* throughout the day also), kennel floors and walls scrubbed with disinfectant, bedding replaced, and water bowls replenished. Dogs were kept within their indoor sleeping areas during cleaning to minimise stress. Staff members fed individual dogs commercial dog food twice per day at specified times (1100 and 1500 hours, BST), consequently, dogs associated specific time periods with food (however, this is standard practise for domestic dogs). On occasion some visitors also supplied commercial dog ‘treats’ to individual dogs.

2.3: Ethical statement

All data collection for this research consisted of non-invasive behavioural observations, therefore, no special requirements to handle study subjects or to enter wolf enclosures or dog kennels was obtained from the UKWCT or Dogs Trust. All observational protocols were approved by Durham University’s Animal Welfare Ethical Review Board (AWERB), and all procedures complied with BIAZA and Dogs Trust ethical guidelines.

2.4: Data collection and experimental protocol.

Previous studies have focused on the use of static photos for the quantification of facial expressions in non-human animals (Bloom & Friedmand 2013; Costa et al. 2016; Leach et al. 2012; McLennan et al. 2016; Miller et al. 2016; Wingenbach et al. 2016). In fact, very few studies have utilised video footage, which is perplexing as facial expressions are dynamic and video has the advantage of capturing all facial movements that may become visible during a specific affective facial expression (Cohn et al. 2007; Waller et al. 2013), therefore, allowing for individual differences to be studied (Cohn et al. 2002). Video footage also allows the context of social interactions to be studied in more detail (via video playback), providing a better understanding of the true affective states being conveyed by the study subjects in question. Therefore, for the purposes of this research video footage of both wolf and dog facial expressions was obtained and processed using standardised approaches previously used in similar research. Data collection was conducted using the same methodology for both wolves and dogs, so, for concision, wolves and dogs will be collectively referred to as canids (unless stated otherwise). In addition, wolf enclosures and dog runs will be collectively referred to as enclosures (again, unless stated otherwise), and the methodology will be described as a whole.

2.4.1: Video collection.

The aim of video collection was to obtain footage of focal canids engaging in social interactions with one or more conspecifics and/or humans (collectively referred to as social interactants), and when focal canids reacted to novel, external stimuli. These social interactions and reactions to stimuli prompted the occurrence of an affective response in the observed focal canids. Affective responses pertained to one of the primary (basic) affective states as described in previous literature (Table 2.3, Arapakis et al. 2008; Izard 1992; Oatley & Johnson-Laird 2014; Plutchik 2001; Rutherford et al. 2012). These primary affective states have been previously described and studied in non-human animals (Abrantes 2005; Bekoff 2007; Briefer EF 2018; Haber & Holleman 2013; King & Landau 2003; Mech & Boitani 2003; Mech 2007; Miele M 2011; Schenkel 1947), including dogs (Albuquerque et al. 2016; Bloom & Friedmand 2013; Catia et al. 2017; Morris et al. 2008), therefore these same affective states were selected to be investigated in this research. However, throughout the literature the descriptions of these primary affective states have varied, therefore, for the purposes of this research, objective descriptors of each affective state were produced. These descriptors were derived from the contextual information gathered from the obtained video footage (of social interactions and reactions to stimuli) and included information such as canid ‘body language’ and social proximity (Table 2.3). These descriptors are considered to be objective, as they provide the motivation (the functional response) of the focal canid for each affective state and aided their identification during data collection. In addition to these primary affective states, ‘Friendly’ was included as this affective state has been reported and qualitatively described for both wolves and dogs in previous literature (Mech 2007; Mech & Boitani 2003; Schenkel 1967; Vas et al. 2005). However, to date, Friendly has not been studied in depth or quantified for either wolves or dogs.

It should be noted that varying intensities of the aforementioned primary affective states (Table 2.3) exist, known as secondary and tertiary affective states (Morris et al. 2008; Scheutz 2002; Sloman 2001). For example, the primary state ‘Anger’ could become ‘Irritability’ (secondary) and ‘Frustration’ (tertiary), though they are still categorised under the province of their primary affective state, ‘Anger’. However, secondary and tertiary affective states have seldom been reported or described for non-human animals. Therefore, secondary and tertiary affective states were not considered in this research.

Much of the literature that exists for non-human animal affective states tend to focus upon negative states (states that generally cause harm, such as pain, to the study subject), as opposed to positive states (states that do not usually cause harm to the study subject, Beerda et al. 1997; Briefer et al. 2015; Boissy et al. 2007; Burman et al. 2011; Desire et al. 2002; Hummel et al. 2008; Reefman et al. 2009; Reimert et al. 2013; Wiepkema 1984; Yeates & Main 2008). This is partly due to negative affective states existing in larger numbers than positive affective states (Fredrickson 1998); for every single positive affective state that exists, there generally exists four negative affective states (Fredrickson 1998). Identifying and assessing positive affective states as well as negative affective states in non-human animals is necessary to fully evaluate the psychological well-being and health of an individual animal (Yeates & Main 2008). Therefore, each primary affective state investigated in this research was also classified as either positive or negative (Table 2.3).

The affective state Surprise (alert) can be classified as both negative and positive (Noordewier & Breugelmans 2013). However, for this research Surprise was classified as positive only, as none of the focal canids were harmed in any instance that Surprise was induced in them. Focal canids were not harmed when surprised as only auditory stimuli were used to evoke this affective state, and these did not pose any real threat. In addition, the affective state Friendly is associated with submissive behaviour towards higher ranking individuals and can occur during both positive and negative social interactions (Mech 2007; Mech & Boitani 2003; Schenkel 1967; Vas et al. 2005). However, within stable wolf and dog packs submission exists in two forms, ‘active’ and ‘passive’ (as described by Mech & Boitani 2003; Mech 1999; Mech 2007; Schenkel 1967). During positive interactions (such as greetings) Friendly takes the form of active submission (Schenkel 1967), while during negative interactions (such as conflict) Friendly takes the form of passive submission (Schenkel 1967). Within stable wolf and dog packs, active submission is used to reinforce social bonds (Mech 2007; Mech & Boitani 2003; Schenkel 1967; Vas et al. 2005) and passive submission is used to avoid conflict (Mech 2007; Mech & Boitani 2003; Schenkel 1967; Vas et al. 2005). The wolf and dog packs used for this research were deemed stable and therefore, for the purposes of this research, Friendly was classified as a positive affective state only as focal canids were not harmed during social interactions.

Table 2.3. Primary affective states with corresponding objective descriptors. Descriptors are derived from contextual information gathered from video footage of canid social interactions and reactions to external, novel stimuli.

Primary affective state and positive (+ve) or negative (-ve) classifications	**Descriptors**	**Previous literature of affective states**
Anger (-ve)	Aggressive interactions; can be offensive or defensive; often results in decreased social proximity, unless full conflict occurs.	Oatley & Johnson-Laird 2014; Plutchik 2001.
Anxious (-ve)	Focal canid displays signs of distress (e.g. vocalisations such as whimpering), often in response to uncertain anticipation; social proximity is neither increased nor decreased.	Arapakis et al. 2008; Oatley & Johnson-Laird 2014.
Curiosity (+ve)	Focal canid fully approaches 'emotive' stimuli (e.g. familiar sounds, such as dog squeak toy) and becomes fixated on it for an extended period (> five seconds).	Rutherford et al. 2012.
Disgust (Distasteful, **-ve**)	Focal canid tries to expel something unpalatable from mouth; often results in pharyngeal-reflex being triggered.	Oatley & Johnson-Laird 2014.
Disgust (Revulsion, **-ve**)	Focal canid is repelled by a social interactant (conspecific or heterospecific) or inanimate object (e.g. faeces); focal canid displays avoidance behaviour, moving away from repellent.	Oatley & Johnson-Laird 2014.
Fear (-ve)	Associated with aggressive interactions and sudden shocks by novel stimuli, e.g. the approach of an unfamiliar social interactant; social proximity is often decreased as focal canid attempts to escape from social interactant or novel stimuli.	Plutchik 2001.
Friendly (+ve)	Associated with submissive behaviour (e.g. lowered body posture) toward social interactant (who often has a higher social rank); social interactant may be familiar or unfamiliar (human social interactants only); social proximity is increased. This affective state can occur during positive interactions (as active submission) and negative interactions (as passive submission).	Schenkel 1967.
Happy (Peaceful happiness/content, **+ve**)	Focal canid is receiving tactile attention from social interactant, e.g. grooming or petting; social proximity is increased.	Oatley & Johnson-Laird 2014.
Interest (+ve)	Focal canid approaches social interactant or inanimate object, though makes no attempt to fully interact; social proximity is initially increased but maintained at approximately one body length from social interactant/inanimate object. **Note:** Focal canid may increase or decrease social proximity if social interactant attempts to interact with focal canid.	Izard 1978.
Joy (Great happiness/pleasure, **+ve**)	Excitable interactions, e.g. play or copulation; social proximity is increased.	Arapakis et al. 2008; Plutchik 2001.
Sadness (-ve)	Social interactant decreases social proximity from focal canid; results in focal canid displaying signs of distress (e.g. vocalisations such as wines, yaps and barks), while focal canid attempts to increase social proximity.	Oatley & Johnson-Laird 2014.
Surprise (Alert, **+ve**)	Focal canid reacts to sudden shocks to the sensory system, in particular auditory, visual and tactile stimuli; focal canid is momentarily fixated on stimuli (< five seconds) and often becomes immobile ('freezes'); proximity to stimuli is neither increased nor decreased.	Plutchik 2001.

Note: 'emotions' coined for each affective state are used to provide a concise way to comprehend the context of the affective response described.

All social interactions occurred spontaneously when conspecifics or humans socially engaged with focal canids. However, reactions to stimuli were both spontaneous and planned. Spontaneous reactions occurred when focal canids reacted to easily identifiable novel, external auditory and visual stimuli such as wind creating sounds, overhead aircraft, and novel objects placed around the study sites (by staff for various public engagement events). Obtaining footage of canids spontaneously reacting to olfactory stimuli was not possible as the exact source of the stimulus was not identifiable during data collection. Planned reactions were induced using audio stimuli played to canids by ERH, on a Nokia Lumia 820 mobile phone. The audio stimuli used in planned reactions included one of four naturally occurring animal vocalisations (rabbit distress call, fawn distress call, squirrel alarm call, and domestic dog puppy whines) and one familiar sound (dog squeak toy; wolves were familiar with dog squeak toy sounds as they were provided with dog toys as pups by human handlers). These audio stimuli were used to evoke ‘Curiosity’, ‘Interest’ and ‘Surprise’ (Table 2.3) affective states in focal canids. Similar methods using standardised audio stimuli have been successfully used in previous studies to evoke affective states in non-human animals (Twiss et al. 2012). Planned audio stimuli were obtained via non-copyrighted YouTube videos (freely available for game-hunting and dog enrichment purposes) and converted online to mp3 formatted audio recordings (for non-commercial purposes) to be used solely in this research. Different audio recordings were used to ensure that canids did not become habituated to anyone recording. Furthermore, recordings were played at random to different canids, at non-specific times, again preventing habituation to recordings and maintaining stimulus arousal. Although reactions to stimuli generally occurred in a non-social context, the aforementioned affective states (Curiosity, Interest and Surprise) that were evoked are transferable to different social contexts. For example, Surprise can be induced by a sudden, loud novel sound, but can also be induced via the sudden, unexpected approach of a conspecific.

Videos were recorded *ad-hoc* (as and when social interactions or reactions to stimuli occurred) using a hand-held Canon Legria HFR36-D video camera (51x zoom). It was concluded from preliminary trials that the use of a camera tripod was unsuccessful due to the rapid movements of canids and the unpredictability of when and where social interactions or reactions to stimuli would occur. ERH (the researcher) had to anticipate the occurrence of interactions and reactions; as a consequence, video recordings were initiated within one minute (at least) prior to interactions and reactions commencing and terminated within one minute after interactions and reactions ceased. Social interactions commenced when eye-contact was made between a focal canid and one or more social interactants, with the focal canid becoming immediately focussed upon the interactant(s). Interactions ceased when the focal canid and social interactant(s) dispersed, and eye-contact was lost. Reactions to stimuli commenced when a focal canid reacted immediately (within less than one second) to an external stimulus and became focussed upon the direction of the stimulus origin (the focal canid attempted to make eye-contact with the stimulus). Reactions ceased when the external stimulus ceased, and the focal canid averted its gaze from the stimulus origin. All videos were recorded from the outside of enclosures, at a minimum distance of one metre from enclosure boundaries, however maximum recording distance to focal canids varied due to the ranging of canids within their enclosures.

2.4.2: Video data extraction.

Many facial expressions in canids (and many other non-human animals) are extremely subtle (Waller et al. 2013), and challenging for human observers to quantify and classify. However, it is known that both wolves and dogs see the world ‘faster’ than humans (Mech & Boitani 2003), in that they have a greater sensitivity to motion than humans do, and are thus able to make finer temporal use of visual information (Mech & Boitani 2003).

To maximise the potential to detect subtle movements of wolf and dog facial expressions (and to do so rigorously), the Dog Facial Action Coding System (DogFACS, Waller et al. 2013) was used (ERH is a certified DogFACS coder). This anatomical coding-based system allows the standardised quantification of dog facial expressions from video footage, frame by frame and in slow motion (Cohn et al. 2007; Waller et al. 2013), thus allowing coders to manually detect and record nearly every possible facial movement a dog may produce (Cohn et al. 2007; Waller et al. 2013). DogFACS was used to code wolf facial expressions as well as domestic dogs’, based on the theory that domestic dogs descend from a wolf lineage (Brauer et al. 2013; Clutton-Brock 1995; Cooper et al. 2003; Goodwin et al. 1997; Morey 1992; 1994; Olsen 1985; Scott 1967; Serpell 2016; Vila et al. 1997) and are therefore likely to have a phylogenetically conserved mimic muscle blueprint, similar to the conserved mimic muscles found across primate species (Parr et al. 2010).

Additionally, the DogFAC system has the potential to become an Emotional DogFACS (Meridda et al. 2014); a single pilot study suggested that the six basic affective states investigated (anger, disgust, fear, happiness, sadness, surprise) were conveyed by domestic dogs using specific mimic muscles (Meridda et al. 2014). This is an important concept, as linking facial expressions and affective states could (as previously stated) provide a useful tool for monitoring animal welfare (Paul et al. 2005). In addition, this pilot study provides a ‘proof of principle’ for this research, to compare and contrast the functions (with respect to affective states) of facial signalling in wolves and domestic dogs.

DogFACS comprises a list of 43 codes (Table 2.4) that correspond to specific facial landmarks (Figure 2.5) that move in association with the underlying mimic muscles (Figure 2.6, Waller et al. 2013). Codes consist of Action Units (AUs), Action Descriptors (ADs), and Ear Action Descriptors (EADs), each with a unique number, corresponding to a singular, specific facial movement (Table 2.4). To apply DogFACS to the collated video footage, the codes were converted into a ‘control panel’ format (Figure 2.7), using Visual Basic for Applications (VBA) in Microsoft Excel (2010). In addition to the DogFACS codes, the following additional facial movements (AFMs, comprising various mimic muscle movements) were included in data extraction; ‘jaw snapping’ (JSNAP, Figure 2.7), defined here as non-vocal auditory communication, resulting from the sudden, rapid closing of the jaws of a focal canid. ‘Tongue flicking’ (TONGUE, Figure 2.7), defined here as when a focal canid protrudes its tongue from its mouth, and immediately retracted back into the mouth without licking the lips or nose, a social interactant or an inanimate object. Finally, ‘Whites of eyes visible’ (WHITES, Figure 2.7), defined here as when the whites (sclera) of a canids eye(s) become clearly visible. JSNAP and TONGUE were included as these phenomena have been occasionally reported to occur in wolves (Fox 1970; Mech & Boitani 2003), and, were both observed several times during the collection of wolf video footage. Therefore, because JSNAP and TONGUE strictly involve the movement of the face, they were included in data extraction for analyses. WHITES was included as sclera visibility during facial communication was once thought to be unique to humans until recent years (Mayhew & Gomez 2015), however, during video collection canid sclera visibility was observed frequently and so was also included in data extraction for analyses.

Table 2.4. The Dog Facial Action Coding System (DogFACS) codes and corresponding names for quantifying specific facial movements in domestic dogs.

Action Units (AUs)	**Name of movement**	**ADs continued**	**Name of movement**
AU0	No movement	**Head**	
Upper face		AD51	Head Turn Left
AU101	Inner brow raiser	AD52	Head Turn Right
AU143	Eye closure	AD53	Head Up
AU145	Blink	AD54	Head Down
Lower face		AD55	Head Tilt Left
AU109+110	Nose wrinkler and upper lip raiser	AD56	Head Tilt Right
AU110	Upper lip raiser	**Eyes**	
AU12	Lip corner puller	AD61	Eyes Turn Left
AU116	Lower lip depressor	AD62	Eyes Turn Right
AU118	Lip pucker	AD63	Eyes Up
AU25	Lips part	AD64	Eyes Down
AU26	Jaw drop	**Gross behaviour ADs**	
AU27	Mouth stretch	AD40	Sniff
Ear Action Descriptors (EADs)		AD50	Vocalisations
EAD101	Ears Forward	AD81	Chewing
EAD102	Ears Adductor	AD126	Panting
EAD103	Ears Flattener	AD119	Lick
EAD104	Ears Rotator	AD160	Body Shake
EAD105	Ears Downward	**Other ADs**	
Action Descriptors (ADs)		AD70	Frontal Region Not Visible
Muzzle		AD71	Eyes Not Visible
AD19	Tongue Show	AD72	Lower Face Not Visible
AD33	Blow	AD73	Entire Face Not Visible
AD35	Suck		
AD37	Lip Wipe		
AD137	Nose Lick		

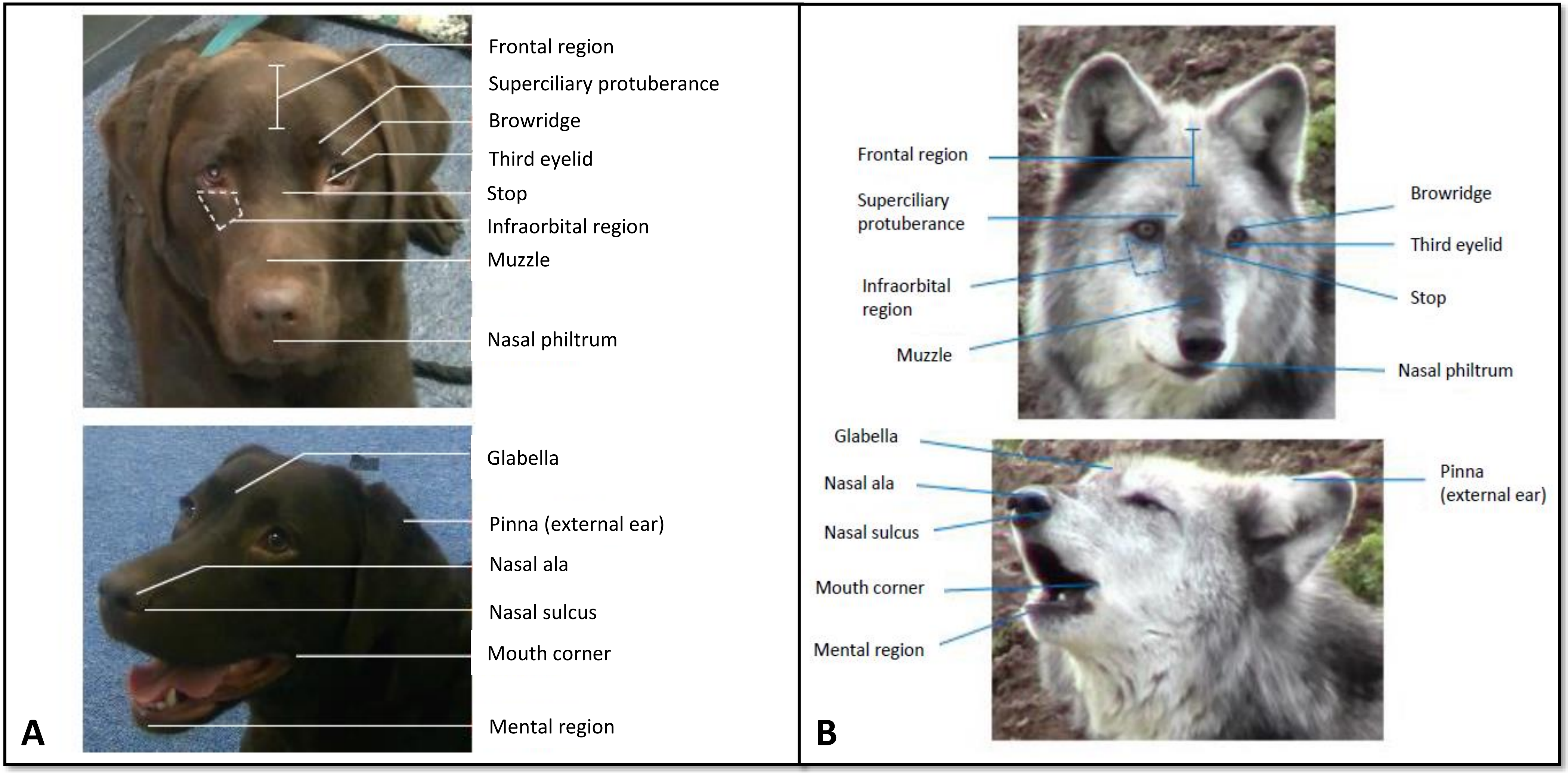

Figure 2.5. A) Domestic dog facial feature landmarks, upper image: Rostral view; lower image: Profile view. (Adapted from Waller et al. 2013). **B)** Wolf facial feature landmarks, upper image: Rostral view; lower image: Profile view (photographs by ER Hobkirk).

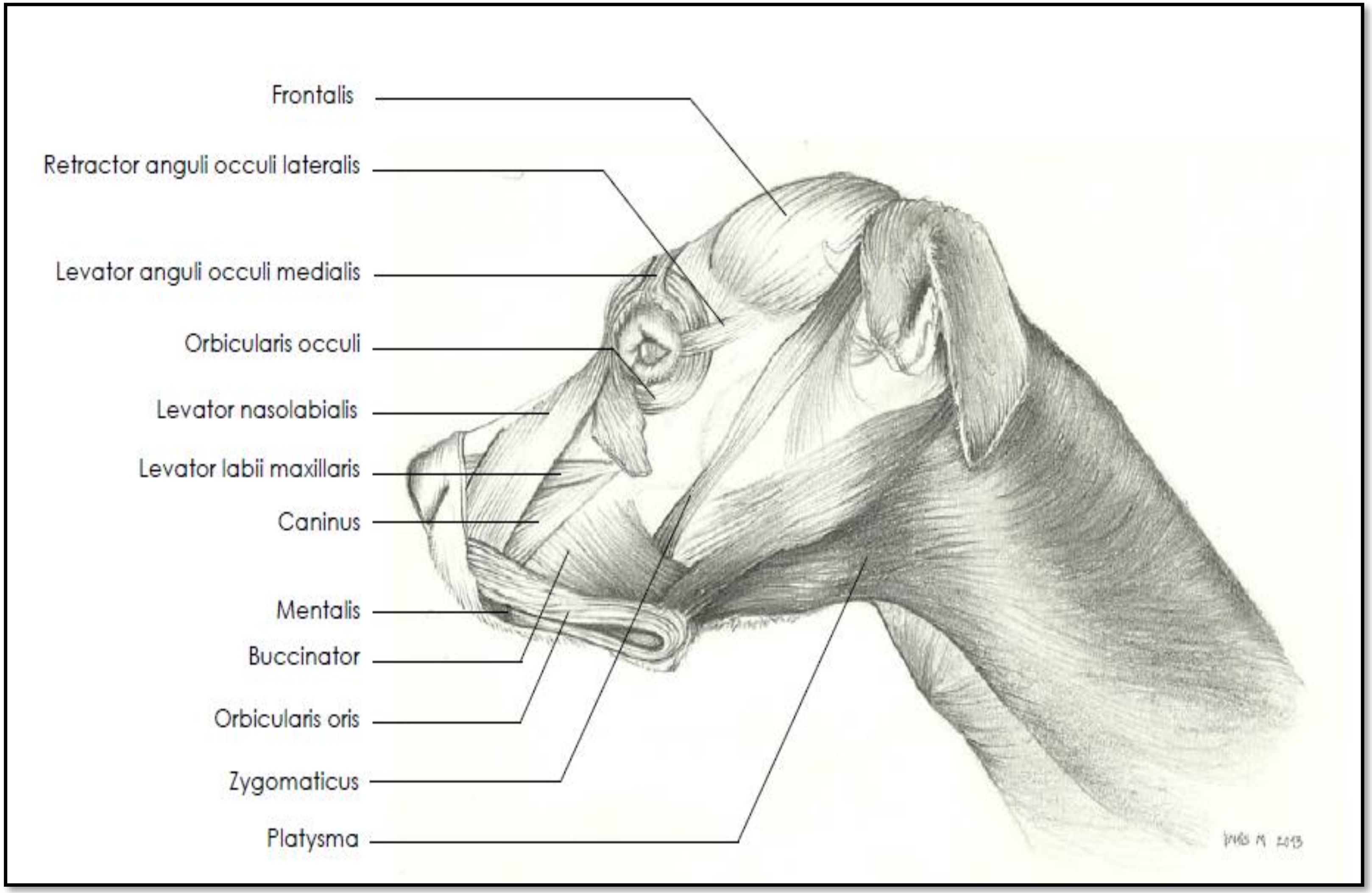

Figure 2.6. Domestic dog mimic muscles used to move facial feature landmarks (Adapted from Waller at al. 2013).

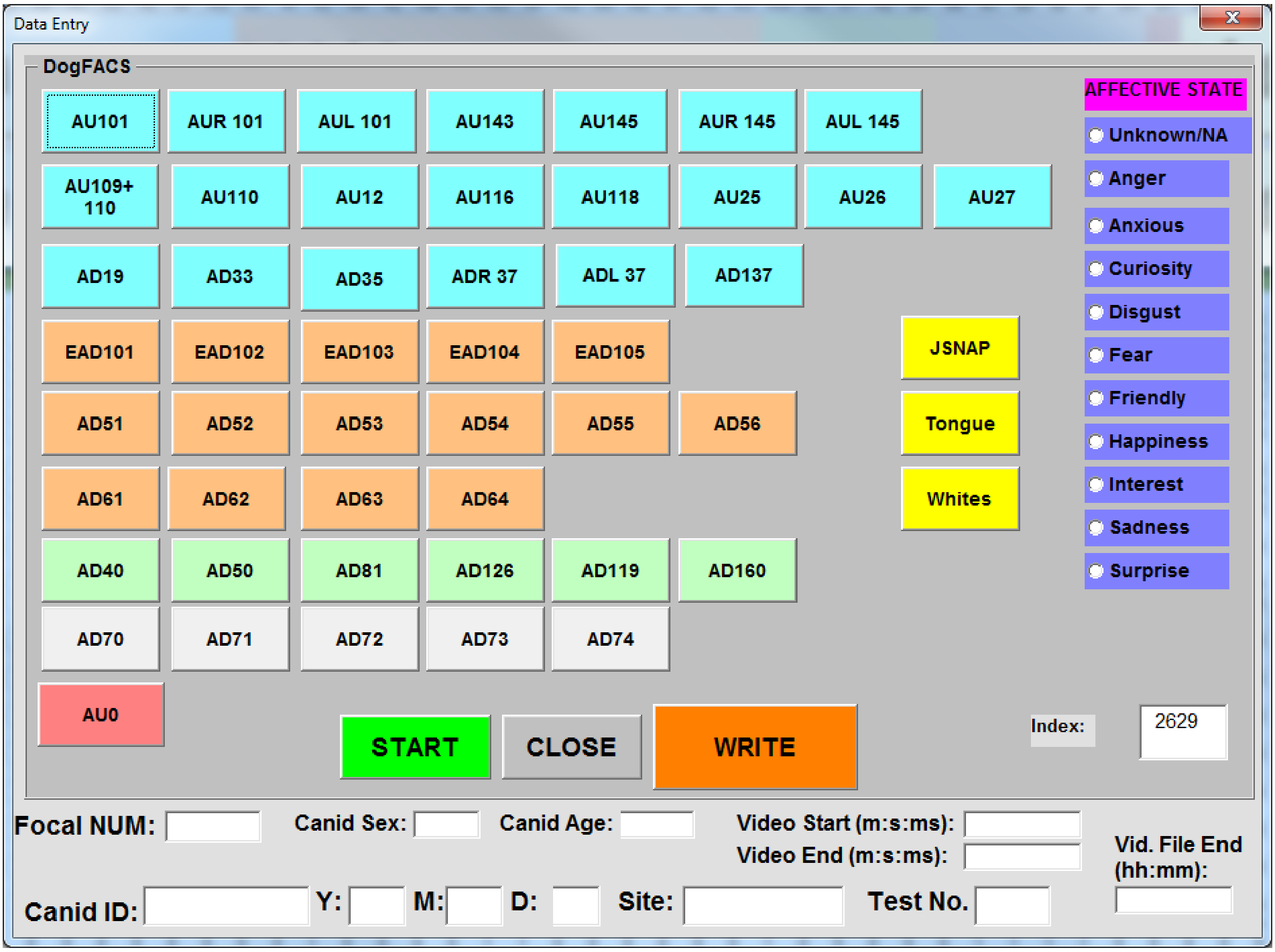

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Figure 2.7. Microsoft Excel Visual Basic for Applications (VBA) 'control panel' window used to decode wolf and dog facial expressions from collected video footage. DogFACS codes (Table 2.4) are shown here as the pastel colours, blue, orange, green, red, and light grey buttons. AFM codes (JSNAP, TONGUE and WHITES) are shown as yellow buttons. Affective states are shown as purple-blue selection tabs. Video decoding was initiated using the 'START' button (green), and data were written to the Excel database using the 'WRITE' button (orange). The 'control panel' was closed using the 'CLOSE' button (grey). Video and focal canid information was inputted manually into the white text boxes in the light grey panel below the buttons/tabs. 'Focal NUM' is the unique number assigned to the video collected. 'Y'/'M'/'D' are the year, month and day the video was recorded. 'Site' is the study site where the video was collected. 'Test No.' is the number assigned to each audio stimuli (section 2.4.1) used to evoke affective states. 'Video Start and End' are the start and end times (in minutes, seconds and milliseconds) within the video that a social interaction or reaction to stimuli occurred. 'Vid. File End' is the time of day the video was recorded during video collection (in hours and minutes). 'Canid ID' is the unique identification name assigned to the focal canid. 'Canid Sex' is the sex of the focal canid. 'Canid age' is the age in years of the focal canid. Designed by ER Hobkirk and SD Twiss.

The collated video footage was decoded (by ERH, a trained DogFACS coder) in slow-motion (0.25x playback speed) using AVS video editor 7.2. Decoding of facial expressions frame by frame proved to be too time consuming, thus this method was rejected in favour of slow-motion. Decoding commenced at the exact moment focal canids engaged in a social interaction (as described above, section 2.4.1) or reaction to stimuli (as described above, section 2.4.1), and ceased at the exact moment focal canids disengaged from interactions and reactions. Therefore, decoding duration varied depending on how long a single interaction or reaction lasted. Videos also tended to comprise of more than one focal canid, in which cases, these videos were decoded separately for each focal canid. All DogFACS codes and AFMs that occurred within a single interaction or reaction were recorded once per video as either 'on' or 'off' (producing binary data) using the VBA control panel (Figure 2.7). For example, if AU101 occurred three times in a focal canid, in a single interaction, then it was only recorded once (as 'on') in the VBA for that focal canid in that interaction. This method was employed to categorise the range of facial movements in each event, and to allow for individual canid facial expressions to be quantified, therefore producing a database of all facial movements per affective state, per individual.

Affective states were objectively recorded by ERH (using the aforementioned descriptors, Table 2.3), in the VBA control panel as categorical data, one affective state for each focal canid, for each interaction and reaction. To test the reliability of these recorded affective state classifications, a sample of video clips, one per affective state, per wolf and dog (amounting to 18 videos in total, chosen for their high quality and maximum clarity of facial expressions, see Appendix B, Table B1) were played at random to a group of independent observers (*n* = 8). Sample clips of 'Sadness' in wolves and 'Disgust (distasteful)' in dogs were not included as these were not observed in their respective canids during data collection. Each independent observer had prior experience with dogs, though no prior experience with wolves. Independent observers were tasked to record which primary affective state (Table 2.3) they perceived the focal canid, from each video clip, to be conveying.

Whether the affective states (recorded by ERH) were positive or negative was not recorded using the VBA control panel as this information was not needed for the main data analyses (section 2.4.3). Instead this information was later recorded manually for each of the 18 video clips described above, to determine the level of agreement between positive and negative classifications of the recorded affective states. To test the level of agreement, the same 18 video clips described above were played at random to a second group of independent observers (*n* = 9). These observers were tasked to record whether they perceived the affective state to be positive or negative.

2.4.3: Data analyses.

During social interactions and reactions to stimuli canids would alter their gaze, and head orientation (in the sagittal plane), only to maintain eye contact with social interactants and reaction stimuli (as described for the initiation and cessation of interactions and reactions, section 2.4.2). Consequently, all eye movements (Table 2.4), and left and right head movements (Table 2.4) coded were considered unusable, and therefore removed from data analyses. 'Body shake' (Table 2.4) was also removed from data analyses as this code is predominantly a body movement with involuntary head and facial movements. Furthermore, on occasion social interactants or inanimate objects (such as enclosure fencing) would cause involuntary head and facial movements in focal canids (by tactile manipulation). These involuntary head and facial movements were also excluded from data analyses. In addition, 'other' ADs (Table 2.4) are associated with the visibility of focal canid faces in video footage, and not with facial movements. As a consequence, 'other' ADs were removed from analyses, as videos lacking canid face visibility were not decoded. Finally, Mia1 and Milo1 (appendix A) underwent surgery to be sterilised during data collection. All events for Mia1 and Milo1 were omitted from data analyses after they underwent surgery due to the influences of anaesthetic and discomfort on their behaviour. All DogFACS codes and additional facial movements were processed the same for data analyses.

The affective state 'Disgust' predominantly consisted of 'Distasteful', in which facial expressions generally consisted of a pharyngeal reflex in response to consuming something unpalatable. As a result, Distasteful is not considered transferable to a social communicative context, and thus, was removed from data analyses. Disgust in a 'revulsion' context was also removed from data analyses due to a lack of sample size for both wolves (*n* = 1) and dogs (*n* = 2). Likewise, the sample size for 'Sadness' in dogs was too small (*n* = 1) for data analyses, and was not observed in wolves during data collection, thus was also removed.

Analyses were conducted for wolf and dog data separately, using the free, open-source R programming language (version 3.4.3), in the R studio environment (version 1.0.143). DogFACS coding was not tested for reliability due to a lack of additional certified DogFACS coders. To examine the level of agreement between ERH affective state classifications and those of the independent classifications acquired during data collection (see section 2.4.2), inter-observer concordance analyses (Caro et al. 1979; Garcia et al 2010; Phythian et al. 2013; Whitham & Wielebnowski 2009) was performed using the R package 'raters', showing substantial agreement at 70% (according to the Kappa statistic, Table 2.5, see Appendix B, Table B2 for independent observer classifications). Concordance analyses were also performed to examine the agreement between ERH positive and negative classifications of affective states, showing almost perfect agreement at 82% (according to the Kappa statistic, Table 2.5, see Appendix B, Table B3 for independent observer classifications).

Table 2.5. Agreement measures for categorical data, according to Landis & Koch 1977. Kappa statistics have been converted to percentages for unambiguous interpretations.

Kappa statistic (%)	Strength of Agreement
< 0	Poor
0 - 20	Slight
21 - 40	Fair
41 - 60	Moderate
61 - 80	Substantial
81 - 100	Almost perfect

The main data analyses of this research were conducted to determine if specific, unique groupings (clusters) of DogFACS codes and AFM codes existed for each affective state observed in this research. To determine these clusters, binary data (DogFACS and AFM codes) were separated from categorical data (observed affective state classifications) and subjected to unsupervised hierarchical cluster analyses. The optimum number of clusters was determined via a Bayesian Information Criterion (BIC) model selection, using the 'ward.D2' method, in the R package 'mclust' (Scrucca et al. 2016). Using combined binary and categorical data, a confusion matrix was then generated, to see how well the BIC model fitted the observed affective states. Linear discriminant analysis, using the Cross-Validation (CV) function in the R package 'MASS' (Venables & Ripley 2002), was used to determine the percentage of correctly predicted (true positives) and incorrectly predicted (false negatives and false positives) affective states for the confusion matrix (based upon the clustering of DogsFACS and AFM codes). Using positive predictive values (precision, see equation 1), the linear discriminant analyses and resulting confusion matrices provided an overall success of the clustering of DogFACS and AFM codes per affective state.

Precision = True Positives / (True Positives + False Positives) [Eqn. 1]

Resulting confusion matrices for wolves and dogs were then examined separately for main points of confusion (false negatives and false positives), to determine which affective states were being incorrectly predicted, and with which states they were being confused with (for example, was Anger being confused with Happy?). Wolf and dog matrices were then compared to determine differences and similarities in confusion, and to determine if dogs showed more confusion than wolves, thus providing evidence for potential limitations in dogs to produce facial expressions like their wolf ancestors. Where confusion did occur for dogs, these data were separated from the confusion matrix to examine the morphologies of the dogs whose affective states were incorrectly predicted, thus providing evidence for which morphological divergences (from wolves) are the limiting factors in dogs for producing affective facial expressions. Finally, using a 10% rule of thumb as a measure of acceptance (used to remove facial movements that do not feature heavily for different affective states, as used by Bennett et al. 2017), the DogFACS and AFM codes per affective state were examined to determine which codes pertained to each affective facial expression for both wolves and dogs.

It has already been suggested that wolf facial expressions convey social status as well as affective states (Fox 1970; Mech 2007; Schenkel 1947). Therefore, in addition to the main analyses of this research, further analyses were conducted to determine if wolf facial expressions differed according to distinct social ranks, as a means to investigate the potential causes of confusion within the matrix. Distinct social ranks of wolves were defined here as those that were the same sex (as separate social rank orders exist for both females and males within wolf packs, Mech 1974, 1999, 2007; Sheldon 1992), housed within the same pack, and were either an obvious 'alpha' (α, highest ranking member) or an obvious 'omega' (Ω, lowest ranking member). It should be noted that the terms alpha and omega are archaic terms that imply forceful dominance (α) and fearful submission (Ω), which have their origins in dated research conducted on non-stable captive wolf packs (those not born of mated pairs or family units such as siblings, Mech LD 1999). Here, these terms are used only to describe social ranks and not the general behaviour of individual wolves. The wolves selected for comparative purposes of social rank orders, were α-male Massak and Ω-male Pukak of the Arctic pack, and α-female Tundra, and Ω-female Tala of the Beenham pack (Table 2.1). Furthermore, during data collection several separate vocalisations (AD50, Table 2.4) were observed to occur with both wolf and dog facial expressions (Table 2.6). Therefore, in addition to the main analyses of this research, vocalisations were examined in more detail to determine what type of vocalisation (Table 2.6) accompanied which affective facial expressions, and if these showed similarities with Fox's (1970) study (Table 1.1, section 1.3).

Table 2.6. Vocalisations observed for wolves and domestic dogs during data collection, with corresponding descriptors, including frequently observed DogFACS codes for each vocal.

Vocals	**Canid**	**Descriptors**
Bark	Wolf & Dog	Explosive, short, sharp, deep cry. Mouth is stretched (AU27) wide to achieve vocal.
Chuff	Wolf & Dog	Short, low sounding 'chuff' sound created by the rapid expulsion of air through the lips (AD33). Mouth is usually closed, or only partially opens via a jaw drop (AU26).
Growl	Wolf & Dog	Long, low guttural rumbling. Mouth is often closed, or partially open (AU26).
Growl-Bark	Wolf & Dog	Long, low rumbling followed immediately by explosive, sharp cry. Mouth often closed initially, then opens wide (AU27) towards end of vocal.
Grunt	Dog	Short, low, guttural vocal originating from the throat. Mouth often partially open (AU26).
Howl	Wolf	Long, doleful, drawn-out cry. Mouth opens wide (AU27) with puckering (AU118).
Whimper	Wolf & Dog	Short, high-pitched, repetitive calls. Air appears to be sucked in through lips (AD35) with mouth closed.
Whine	Wolf & Dog	Long, high-pitched, repetitive calls. Mouth initially closed, but often opens (AU27) between whines.
Yelp	Wolf	Short, sharp, high-pitched cry. Mouth opens wide (AU27).
Yelp-Bark	Wolf	Initial short, sharp high-pitched cry followed immediately by short, sharp deep cry. Mouth is wide (AU27).

Chapter 3: Results.

Data collection provided 92 hours of video footage and behavioural observations (34 hours for wolves and 58 hours for domestic dogs), in which 559 events for wolves and 753 events for domestic dogs were obtained. The average duration of events for wolves was four seconds and the average duration of events for domestic dogs was nine seconds. This chapter details the results obtained from unsupervised hierarchical cluster analyses, linear discriminant analyses and confusion matrices, for both wolf and domestic dog affective facial expressions. The main facial movements and vocalisations that occur per affective state for both wolves and dogs are then considered, along with the potential limitations of domestic dog head and facial morphologies.

3.1: Hierarchical cluster analyses of DogFACS and AFM codes (binary data).

Hierarchical cluster analyses gave an optimum number of four distinct clusters (based on BIC model selection) for wolf facial expressions (as described by Scrucca et al. 2016, Table 3.1A, n_{events} = 559), but an optimum number of just two distinct clusters for domestic dog facial expressions (again, as described by Scrucca et al. 2016, Table 3.1B, n_{events} = 753). The geometric characteristics of the clusters produced from the best models for wolf and dog facial expressions consist of ellipsoidal distributions, with equal volume, shape and orientation (EEE, Table 3.1A and 3.1B, Scrucca et al. 2016). The geometric characteristics of the clusters produced represent three dimensions, therefore dimensionality reduction was performed to visualise these clusters in two dimensions (Figure 3.1 and 3.2).

Dimensionality reduction of the four wolf clusters (Figure 3.1) shows that clear spatial separation exists between clusters three and four, though clusters one and two are in close proximity to each other (but, both are still distinct from clusters three and four). However, dimensionality reduction of the two dog clusters (Figure 3.2) show that they are within very close proximity, even somewhat overlapping.

Table 3.1A. Top three models based on Bayesian Information Criterion (BIC, as described by Scrucca et al. 2016) for wolf facial expressions, with corresponding optimum numbers of clusters, BIC values and BIC differences.

Model name	**EEE**	**EEE**	**EEE***
Optimum number of clusters	6	5	4
BIC	-5534.13	-5549.45	-6069.30
BIC difference	0.00	-15.33	-535.17

* Best model (though five and six clusters are possible too).

Each model consists of clusters that have unique combinations of geometric characteristics; EEE clusters correspond to ellipsoidal distributions, equal volumes, shapes and orientations.

Table 3.1B. Top three models based on Bayesian Information Criterion (BIC, as described by Scrucca et al. 2016) for dog facial expressions, with corresponding optimum numbers of clusters, BIC values and BIC differences.

Model name	**VEV**	**VEI**	**EEE***
Optimum number of clusters	2	2	2
BIC	-7053.75	-10304.80	-13718.47
BIC difference	0.00	-3251.04	-6664.72

* Best model.

Each model consists of clusters that have unique combinations of geometric characteristics; VEV clusters correspond to ellipsoidal distributions, equal shapes, and variable volumes and orientations. VEI clusters correspond to diagonal distributions, variable volumes, equal shapes and coordinate axes orientations. EEE clusters correspond to ellipsoidal distribution, equal volume, shape and orientation.

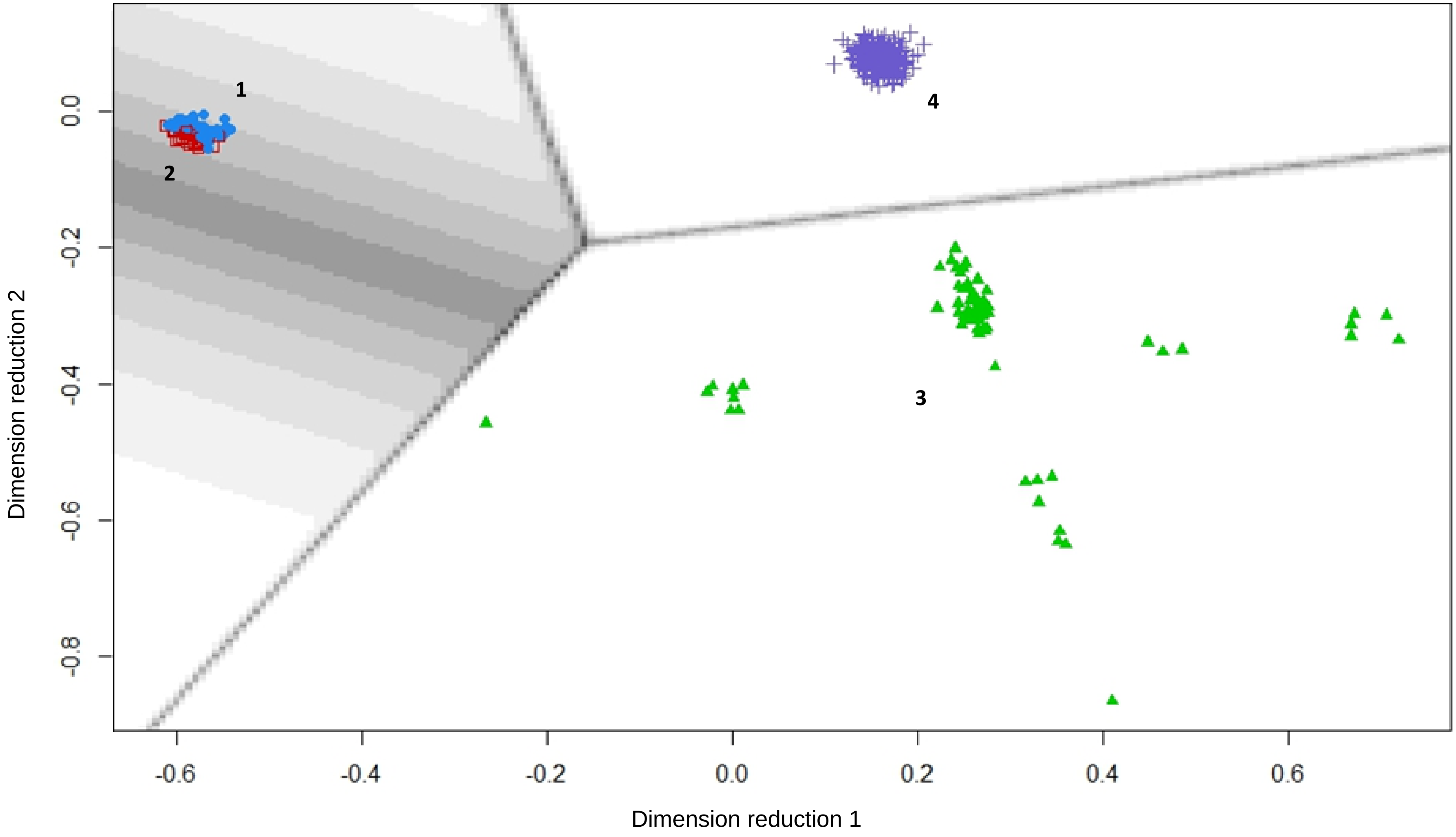


Figure 3.1. Dimensionality reduction scatter plot showing the four EEE clusters (Table 3.1A) obtained from hierarchical cluster analysis (based on BIC model selection) for wolf facial expressions, *n* = 559. Solid dark blue dots represent cluster one, open red squares represent cluster two, solid green triangles represent cluster three, purple crosses represent cluster four. Grey lines indicate where cluster separations occur within three dimensions.

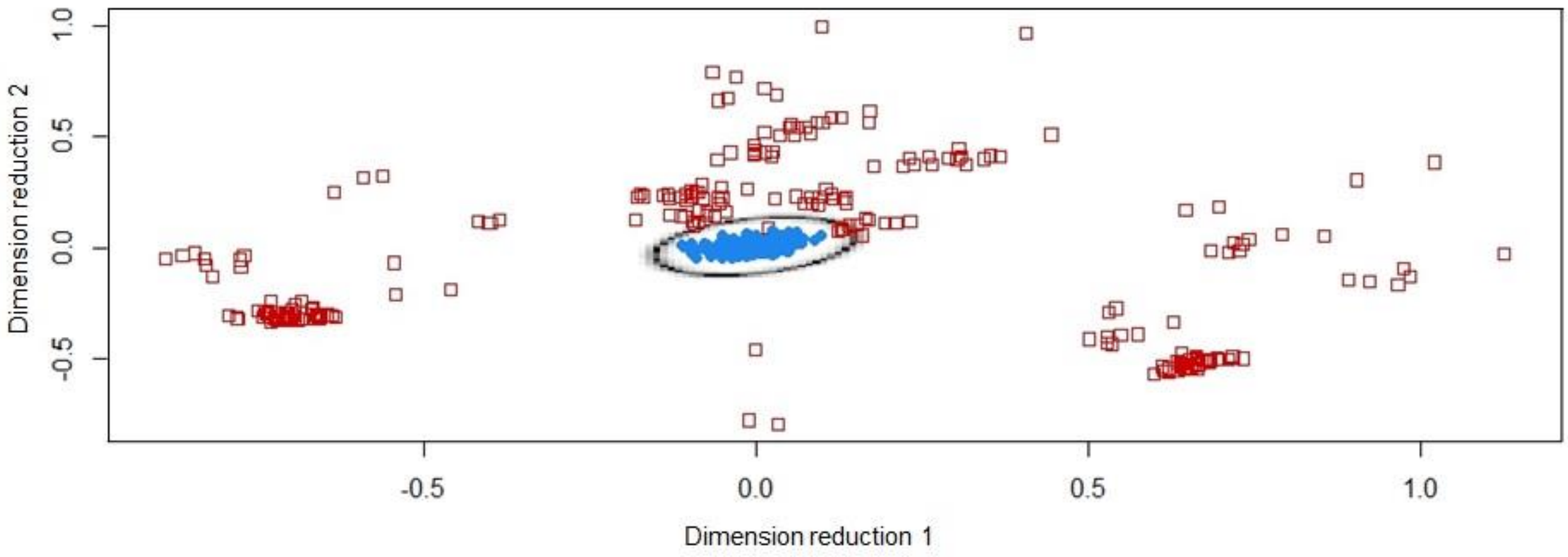


Figure 3.2. Dimensionality reduction scatter plot showing the two EEE clusters (Table 3.1B) obtained from hierarchical cluster analysis (based on BIC model selection) for domestic dog facial expressions, *n* = 753. Solid dark blue dots represent cluster one, open red squares represent cluster two, and the grey circle indicates where cluster separation occurs within three dimensions.

Figure 3.3 displays the same four wolf clusters but, in a dendrogram formation with linear alignment of clusters, showing separation at the same height for each cluster. Each cluster consists of groupings of DogFACS and AFM codes per 'event' (*n* = 559), where each event is allocated an affective state. The distribution of affective states per cluster is shown in Table 3.2A, which shows a degree of mixing across affective states within the same clusters. For example, cluster one mostly represents Curiosity (69%) and Surprise (75%) yet, Curiosity has a lower overall frequency of occurrence (11) compared to Surprise (33). Anger is mostly represented in cluster four (84%) as is Fear (59%), Friendly (83%), Happy (63%) and Joy (69%) however, the frequency of occurrence for Happy and Joy (20 and 27 respectively) are much lower than those of Anger, Fear, and Friendly (133, 41 and 113 respectively). This mixing is interesting as Figure 3.3 implies that clear separation exists between the four clusters yet, looking strictly at affective states, these same clusters appear to be largely shared by different affective states, especially cluster four.

Table 3.2A. Frequency and percentage (in brackets) of events for affective states within each wolf cluster.

Affective states	**Clusters**			
	1	**2**	**3**	**4**
Anger	4 (3)	0 (0)	21 (13)	133 (84)
Anxious	7 (32)	6 (27)	3 (14)	6 (27)
Curiosity	11 (69)	1 (6)	3 (19)	1 (6)
Fear	21 (30)	5 (7)	2 (3)	41 (59)
Friendly	6 (4)	2 (1)	15 (11)	113 (83)
Happy	4 (13)	2 (6)	6 (19)	20 (63)
Interest	1 (2)	25 (58)	6 (14)	11 (26)
Joy	1 (3)	0 (0)	11 (28)	27 (69)
Surprise	33 (75)	3 (7)	4 (9)	4 (9)

Figure 3.4 displays a dendrogram of the two dog clusters obtained, showing separation at the same height (but higher than that of the wolf dendrogram) for each cluster. Again, these two clusters consist of groupings of DogFACS codes and AFM codes per event. The distribution of affective states per cluster is shown in Table 3.2B, and considerable mixing of affective states can be seen within cluster one and two, in fact both clusters are proportionally the same, despite Figure 3.4 suggesting otherwise.

Table 3.2B. Frequency and percentage (in brackets) of events for affective states within each dog cluster.

Affective states	**Clusters**	
	1	**2**
Anger	46 (49)	48 (51)
Anxious	16 (73)	6 (27)
Curiosity	20 (91)	2 (9)
Fear	15 (94)	1 (6)
Friendly	199 (69)	90 (31)
Happy	9 (60)	6 (40)
Interest	46 (98)	1 (2)
Joy	129 (61)	83 (39)
Surprise	33 (92)	3 (8)

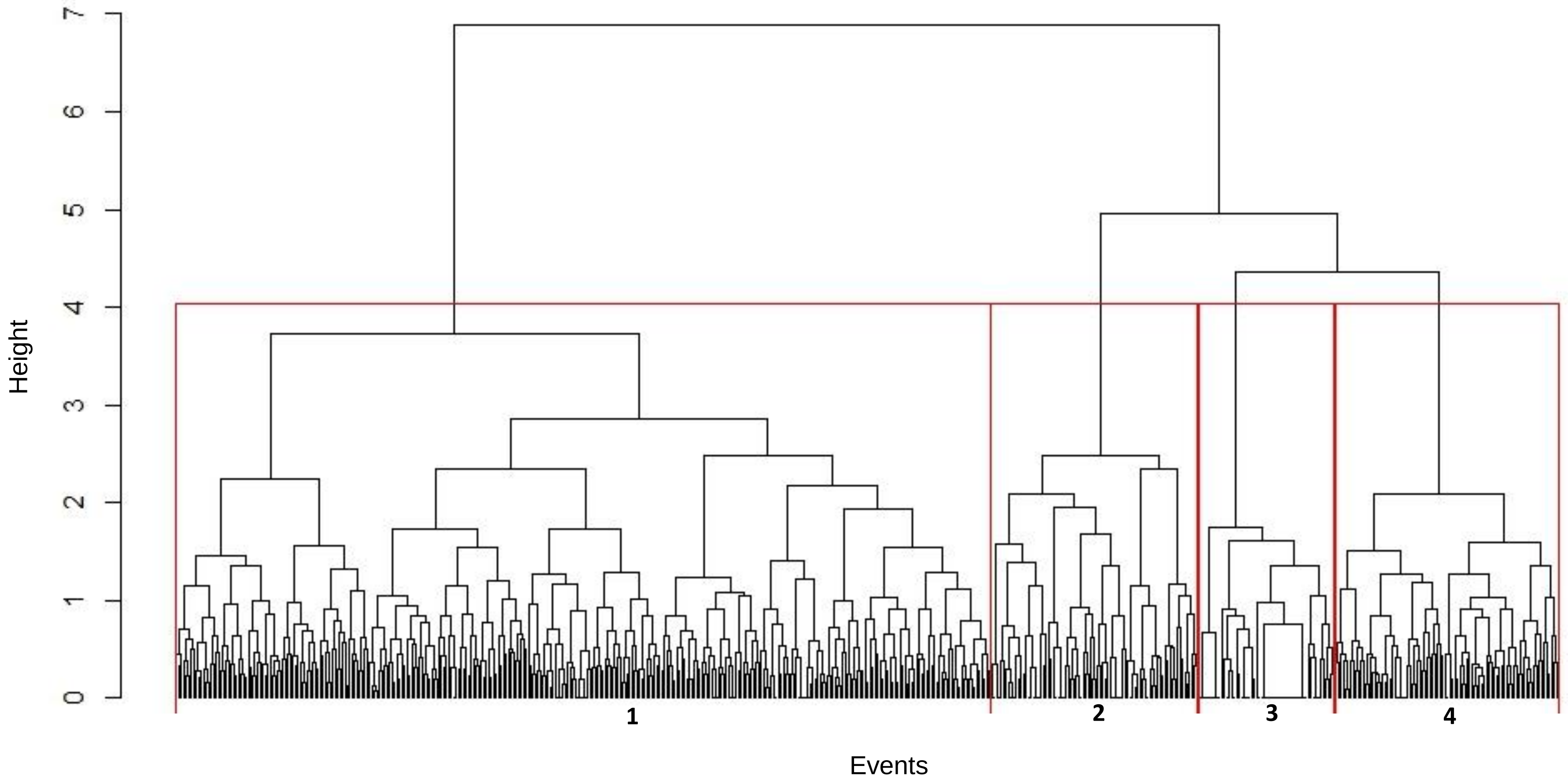


Figure 3.3. Dendrogram showing four clusters (separated by red lines; clusters one to four are left to right) for wolf facial expression obtained from hierarchical cluster analysis (based on BIC model selection). Each branch end is one 'event', and each event is allocated an affective state, *n* = 559. Clusters are groups of DogFACS codes and additional facial movement (AFM) codes per event.

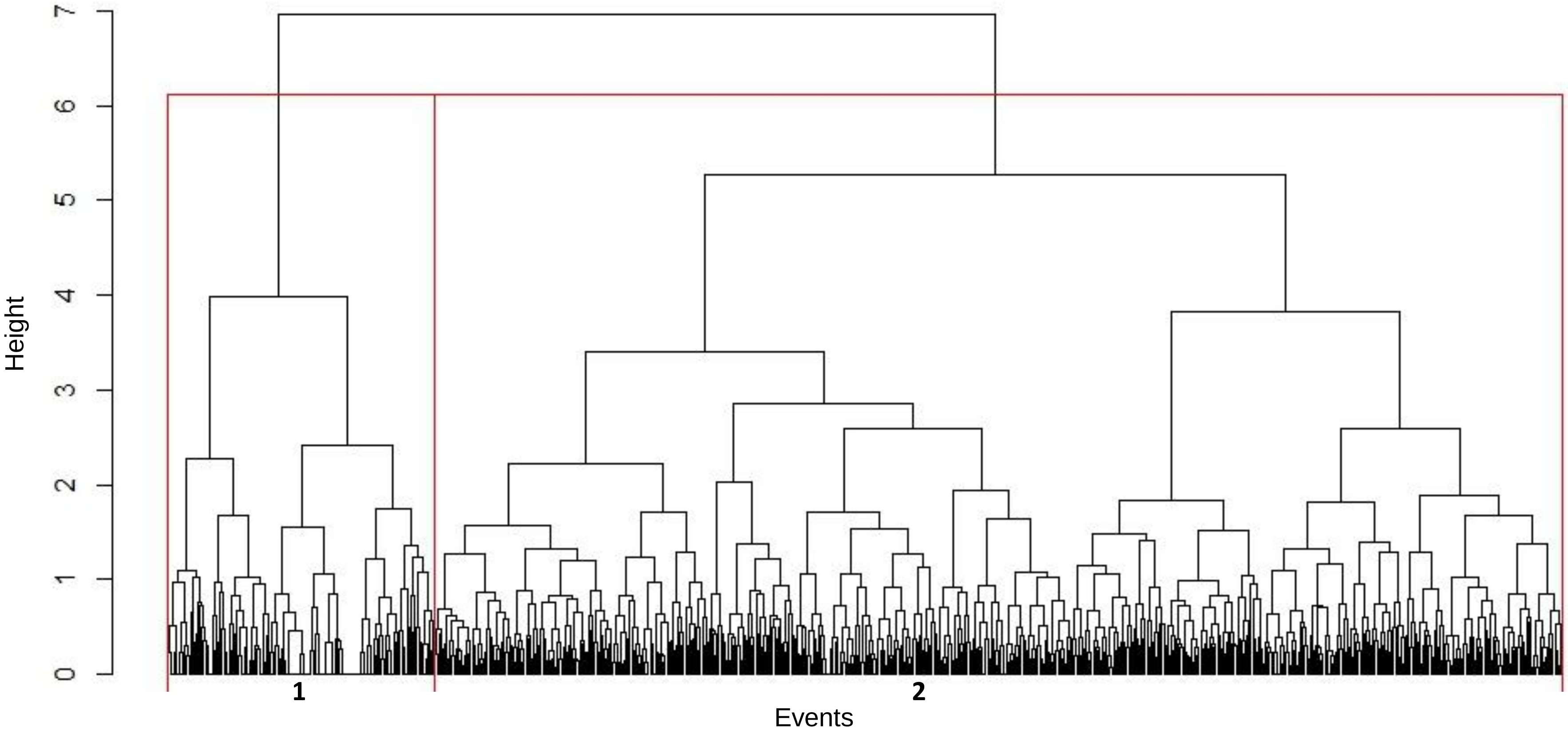


Figure 3.4. Dendrogram showing two clusters (separated by red lines; left to right are clusters one and two) for domestic dog facial expressions obtained from hierarchical cluster analysis (based on BIC model selection). Each branch end is one 'event', and each event is allocated an affective state, *n* = 753. Clusters are groups of DogFACS codes and additional facial movement (AFM) codes per event.

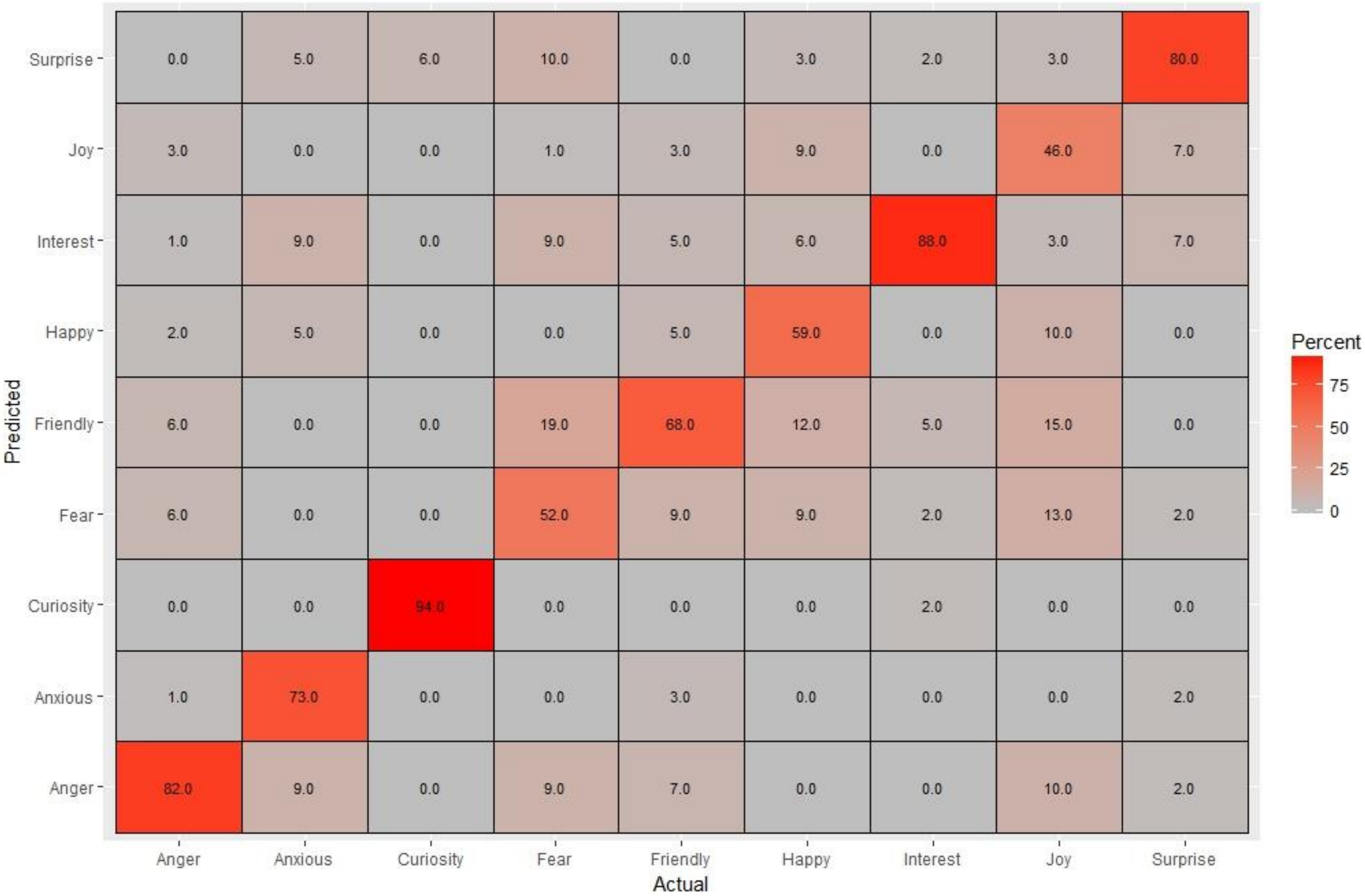
3.2: Linear discriminant analyses of binary data and affective state classifications.

Despite investigating nine affective states, neither of the hierarchical cluster analyses for wolf or dog facial expressions gave nine distinct clusters aligned to each affective state. Therefore, to test how well the hierarchical cluster analyses actually predicted affective states based upon DogFACS and AFM decoding, linear discriminant analyses was performed to generate confusion matrices and allow for examination of the precision of affective state predictions.

Figure 3.5 shows the confusion matrix for wolf affective states based upon the clustering of facial movements (DogFACS and AFM codes). To determine the number of instances each affective state was correctly predicted by this confusion matrix, the overall precision of the matrix, and the individual precisions for each affective state within the matrix were calculated. Overall precision indicated substantial agreement (71%, Table 2.5, section 2.4.3) between the actual and predicted affective states. However, individual precision values (shown within the tiles of Figure 3.5) range between moderate and almost perfect agreement, with the lowest precision being that of Joy at only 46%, while the highest precision is that of Curiosity at 94%. In addition, where confusion does occur within the matrix (values other than True Positives), the values do not exceed slight agreement (according to the Kappa statistic, Table 2.5, section 2.4.3). However, the level of confusion is not necessarily the main concern in this instance, but rather where the confusion occurs. For example, Joy is a positive affective state (Table 2.3, section 2.4.1) and is confused with Happy (10%) and with Friendly (15%), which are also positive affective states. However, Joy is also confused with Fear (13%) and with Anger (10%), which are negative affective states. In fact, confusion between positive and negative affective states occurs throughout the matrix. However, all of these positive and negative confusions are typically 10% or less, with the exception of that between Fear and Friendly, amounting to no more than slight agreement (according to the Kappa statistic, Table 2.5, section 2.4.3). Therefore, due to the low values of confusion seen throughout the matrix and high value of overall precision of the matrix, it can be concluded that wolf affective states can be predicted via facial movements.

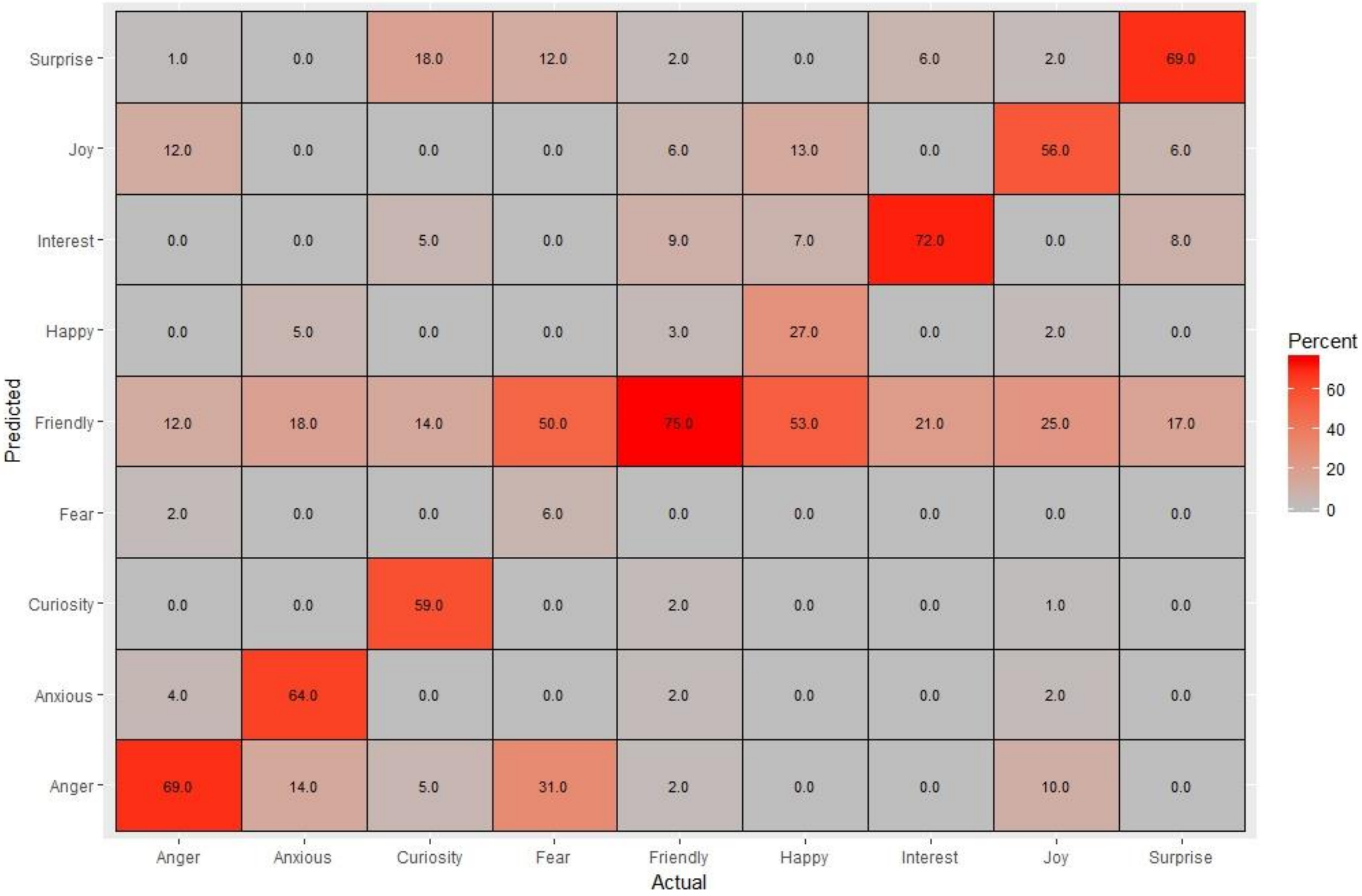
Figure 3.6 shows the confusion matrix of dog affective states. The overall precision of this matrix showed substantial agreement (65%), though this is reduced to that of wolves. In fact, individual precision within the matrix is also reduced, compared to that of wolves, ranging between slight and substantial, with the lowest precision being with Fear at 6%, and the highest being with Friendly at 75%. Some of the confusion within the matrix appears to be similar to that of wolves, though more extreme. For example, Joy is also confused with

Friendly, though to a much greater degree at 25%. In addition, Happy is confused with Friendly at 53%, and Fear is confused with Anger at 31%. These are either positive affective states that are confused with other positive affective states, or negative affective states confused with other negative affective states. However (as with wolves), throughout the matrix positive affective states are also confused with negative affective states. Examples of these (>5%) include, Joy confused with Anger (11%). Fear confused with Friendly (50%). Curiosity confused with Anger (5%). Anxious confused with Friendly (18%), and with Happy (5%), and Anger confused with Friendly (12%), and with Joy (12%). These confusion values are typically much greater than that of wolves, which suggests that dog affective states are less predictable via facial movements, compared to wolves. Some affective states such as Curiosity, Fear, Happy, and Joy have quite low precision (<60%, Table 2.5, section 2.4.3), which suggests that these affective states are less predictable than others. Furthermore, within the matrix it appears that Friendly is the affective state that all other affective states (positive and negative) are greatly confused with, ranging between 12% and 53% of confusion. Despite this, Friendly still has substantial precision and the highest level of precision within the dog confusion matrix, which suggests that Friendly may be too subjective for classification in domestic dogs. However, this will be considered later in the discussion.



	Anger	Anxious	Curiosity	Fear	Friendly	Happy	Interest	Joy	Surprise
Surprise	0.0	5.0	6.0	10.0	0.0	3.0	2.0	3.0	80.0
Joy	3.0	0.0	0.0	1.0	3.0	9.0	0.0	46.0	7.0
Interest	1.0	9.0	0.0	9.0	5.0	6.0	88.0	3.0	7.0
Happy	2.0	5.0	0.0	0.0	5.0	59.0	0.0	10.0	0.0
Friendly	6.0	0.0	0.0	19.0	68.0	12.0	5.0	15.0	0.0
Fear	6.0	0.0	0.0	52.0	9.0	9.0	2.0	13.0	2.0
Curiosity	0.0	0.0	94.0	0.0	0.0	0.0	2.0	0.0	0.0
Anxious	1.0	73.0	0.0	0.0	3.0	0.0	0.0	0.0	2.0
Anger	82.0	9.0	0.0	9.0	7.0	0.0	0.0	10.0	2.0

Figure 3.5. Confusion matrix, showing the actual (observed) versus predicted affective states for wolf facial expressions (*n* = 559). Values within each true positive tile (diagonal) display the precision percentages per affective state. Overall precision = 71%.



	Anger	Anxious	Curiosity	Fear	Friendly	Happy	Interest	Joy	Surprise
Surprise	1.0	0.0	18.0	12.0	2.0	0.0	6.0	2.0	69.0
Joy	12.0	0.0	0.0	0.0	6.0	13.0	0.0	56.0	6.0
Interest	0.0	0.0	5.0	0.0	9.0	7.0	72.0	0.0	8.0
Happy	0.0	5.0	0.0	0.0	3.0	27.0	0.0	2.0	0.0
Friendly	12.0	18.0	14.0	50.0	75.0	53.0	21.0	25.0	17.0
Fear	2.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0
Curiosity	0.0	0.0	59.0	0.0	2.0	0.0	0.0	1.0	0.0
Anxious	4.0	64.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0
Anger	69.0	14.0	5.0	31.0	2.0	0.0	0.0	10.0	0.0

Figure 3.6. Confusion matrix, showing the actual (observed) versus predicted affective states for dog facial expressions (*n* = 753). Values within each true positive tile (diagonal) display the precision percentages per affective state. Overall precision = 65%.

3.3: Which facial movements are important in each affective state?

So far, it has been found that facial movements can predict the affective states of wolves, and to an extent, dogs too. However, it has not been shown yet, exactly which facial movements (DogFACS codes and AFM codes) correspond to, and which are the most important for, each affective state. To determine this, the occurrence of each facial movement per affective state was extracted from the raw data (n_{events} = 559 for wolves and n_{events} = 753 for dogs), summed (regardless of individual canids), and tabulated to inspect for differences and similarities in facial movements across separate affective states.

Table 3.3 shows the frequency and percentage of occurrence for each facial movement per affective state for wolves. Using the 10% rule of thumb (Bennett et al. 2017), any occurrence of 10% or more was considered important for their respective affective states. At first glance this table shows that Anxious, Curiosity, Fear, Happy, Interest, Joy and Surprise constitute relatively unique combinations of key facial movements (ranging between four movements for Joy and 12 movements for Fear) with little overlap, whereas Anger and Friendly constitute a wide range of facial movements (29 for Anger and 27 for Friendly) with a large degree of overlap. However, no two of these affective states share the same combinations of facial movements, though they do sometimes use some of the same movements, especially for Anger and Friendly. However, despite the large degree of overlap seen between Anger and Friendly some key differences still exist. These include the absence of AU118 (Fisher's exact test p = <0.001), EAD101 (Fisher's exact test p = <0.001), EAD102 (Fisher's exact test p = 0.07, marginally significant) and JSNAP (Fisher's exact test p = <0.001, see Table 2.4, section 2.4.2) for Friendly, and the absence of AD55 (Fisher's exact test p = <0.001) and AD40 (Fisher's exact test p = 0.07, marginally significant) for Anger.

The affective state Friendly (associated with submissive behaviour towards higher ranking individuals, see section Table 2.3, section 2.4.1) uses some facial movements that one would associate with agonistic behaviour. These movements include AU109+110 (45%), AU110 (36%), AU116 (15%) and AD81 (15%), all of which involve displaying teeth or using teeth (AD81) to communicate. When some mammals, such as spotted hyenas (*C. crocuta)*, foxes (*Vulpes spp.*), coyotes (*C. latrans*) and many simian primates display their teeth (their weapons) towards another individual it signals a threat (Fox 1969, 1970; McGraw et al. 2002; White 2008), so it is perplexing that in this instance some wolves are conveying Friendly affective states via seemingly threatening facial expressions. Conversely, Anger (associated with aggressive interactions, see Table 2.3, section 2.4.1) uses some facial movements that one would associate with submissive behaviour, which include AU143 (13%), AU145 (19%), EAD103 (22%) and AD54 (23%). These movements involve closing of the eyes (thus, aversion of eye contact with interactants), making the head look smaller (EAD103), and lowering of the head, thus lowered body posture. Generally, when non-human animals try to make themselves appear smaller, it is in an attempt to signal submission to avoid agonistic behaviour (Fatjo et al. 2007; Seaman et al. 2002; van der Borg et al. 2015). However, this thesis research shows that some wolves are conveying Anger via seemingly submissive facial expressions.

Table 3.3. Frequency and percentage (in brackets) of occurrence of DogFACS and additional facial movement (AFM) codes (see Table 2.4, section 2.4.2) per wolf affective state. Accepted percentages (10% and above, Bennett et al. 2017) are highlighted in grey, *n* = 559.

DogsFACS and AFM codes	**Affective states**								
	Anger	**Anxious**	**Curiosity**	**Fear**	**Friendly**	**Happy**	**Interest**	**Joy**	**Surprise**
AU101	87 (28)	13 (4)	15 (5)	52 (17)	33 (11)	22 (7)	20 (6)	29 (9)	41 (13)
AU143	5 (13)	1 (3)	0 (0)	0 (0)	11 (29)	17 (45)	1 (3)	3 (8)	0 (0)
AU145	42 (19)	15 (7)	9 (4)	29 (13)	60 (27)	28 (13)	21 (9)	11 (5)	8 (4)
AU109+110	89 (80)	0 (0)	0 (0)	4 (4)	13 (12)	0 (0)	0 (0)	5 (5)	0 (0)
AU110	33 (33)	2 (2)	0 (0)	13 (13)	36 (36)	5 (5)	2 (2)	8 (8)	2 (2)
AU12	46 (30)	1 (1)	0 (0)	13 (8)	66 (43)	8 (5)	1 (1)	20 (13)	0 (0)
AU116	52 (71)	1 (1)	0 (0)	2 (3)	11 (15)	2 (3)	0 (0)	5 (7)	0 (0)
AU118	42 (86)	1 (2)	0 (0)	0 (0)	3 (6)	0 (0)	0 (0)	2 (4)	1 (2)
AU25	154 (37)	9 (2)	2 (0)	43 (10)	128 (31)	26 (6)	14 (3)	38 (9)	5 (1)
AU26	95 (29)	9 (3)	2 (1)	36 (11)	114 (35)	24 (7)	14 (4)	31 (9)	5 (2)
AU27	80 (51)	1 (1)	0 (0)	8 (5)	33 (21)	9 (6)	0 (0)	24 (15)	1 (1)
AD19	46 (29)	1 (1)	1 (1)	15 (10)	57 (36)	21 (13)	2 (1)	13 (8)	1 (1)
AD33	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	0 (0)	0 (0)	1 (25)
AD35	1 (5)	15 (71)	0 (0)	0 (0)	4 (19)	0 (0)	0 (0)	0 (0)	1 (5)
ADR37	2 (15)	0 (0)	0 (0)	0 (0)	8 (62)	1 (8)	1 (8)	1 (8)	0 (0)
ADL37	3 (18)	0 (0)	0 (0)	0 (0)	6 (35)	4 (24)	2 (12)	1 (6)	1 (6)
AD137	28 (18)	2 (1)	1 (1)	25 (16)	62 (39)	15 (9)	10 (6)	13 (8)	2 (1)
EAD101	57 (30)	10 (5)	16 (8)	13 (7)	5 (3)	7 (4)	19 (10)	19 (10)	44 (23)
EAD102	7 (15)	1 (2)	15 (31)	3 (6)	1 (2)	3 (6)	7 (15)	4 (8)	7 (15)
EAD103	68 (22)	12 (4)	3 (1)	54 (17)	112 (36)	21 (7)	23 (7)	21 (7)	0 (0)
EAD104	1 (8)	2 (17)	3 (25)	1 (8)	0 (0)	0 (0)	3 (25)	0 (0)	2 (17)
EAD105	42 (46)	0 (0)	0 (0)	15 (16)	21 (23)	4 (4)	6 (7)	3 (3)	0 (0)
AD53	29 (21)	4 (3)	3 (2)	6 (4)	40 (29)	14 (10)	29 (21)	13 (9)	2 (1)
AD54	51 (23)	1 (0)	4 (2)	32 (14)	82 (36)	16 (7)	22 (10)	18 (8)	0 (0)
AD55	3 (7)	0 (0)	12 (27)	3 (7)	19 (43)	1 (2)	2 (5)	4 (9)	0 (0)
AD56	9 (16)	0 (0)	12 (22)	4 (7)	23 (42)	3 (5)	1 (2)	3 (5)	0 (0)
AD40	2 (2)	9 (10)	9 (10)	6 (7)	9 (10)	6 (7)	40 (46)	2 (2)	4 (5)
AD50	75 (59)	19 (15)	0 (0)	6 (5)	20 (16)	0 (0)	2 (2)	3 (2)	2 (2)
AD81	8 (40)	0 (0)	0 (0)	0 (0)	3 (15)	0 (0)	0 (0)	9 (45)	0 (0)
AD126	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AD119	6 (13)	0 (0)	0 (0)	0 (0)	36 (80)	0 (0)	1 (2)	2 (4)	0 (0)
WHITES	26 (26)	4 (4)	1 (1)	20 (20)	19 (19)	16 (16)	4 (4)	9 (9)	2 (2)
TONGUE	36 (37)	0 (0)	1 (1)	9 (9)	33 (34)	7 (7)	2 (2)	9 (9)	0 (0)
JSNAP	11 (85)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)

3.3.1: The influence of social rank on the affective facial expressions of wolves.

A closer look at Anger and Friendly shows that the high degree of overlap between these two affective states may be due to differences in facial expressions, expressed by individuals of differing social status. As previously stated, wolf facial expressions have been purported to convey social status as well as affective states, namely dominant versus submissive social ranks (Fox 1970; Schenkel 1947). However, again these forms of facial expressions have not been quantified or examined in any detail. Here, an opportunity to perform a preliminary investigation into the similarities and differences in affective facial expressions between wolves of distinct social ranks was possible.

Table 3.4A shows the frequency and percentage of occurrence for each facial movement per individual wolf for the affective state Anger. The data shown represent the cumulative ‘events’ for Anger for α- and Ω-wolves, extracted from the raw data (*n* = 109). Again, using the 10% rule of thumb (Bennett et al. 2017), any occurrence of 10% or more for a single facial movement was considered an acceptable occurrence and important in usage for the individual wolf. The table shows that despite the high usage of an array of facial movements, there are several notable similarities and differences between α- and Ω-wolves. Similarities between α- and Ω-wolves include facial movements AU109+110 (16% for Massak, 55% for Pukak, 11% for Tala and 18% for Tundra), AU116 (12% for Massak, 48% for Pukak, 18% for Tala, 21% for Tundra), AD19 (13% for Massak, 47% for Pukak, 13% for Tala, 28% for Tundra), EAD103 (27% for Massak, 45% for Pukak, 12% for Tala, 15% for Tundra), TONGUE (12% for Massak, 48% for Pukak, 16% for Tala, 24% for Tundra), and AU143 that is equally used by both α and Ω wolves at 25%. However, the frequency of AU143 is very low for each wolf (1 for both α- and Ω-wolves), which suggests that this facial movement may not be important for Anger in both α- and Ω-wolves, and its occurrence in may just be coincidental.

Several notable differences that occur between α- and Ω-wolves for ‘angry’ facial expressions include the acceptable occurrence for α-wolves (but not for Ω-wolves) of AU118 (71% for Massak and 17% for Tundra), EAD101 (74% for Massak and 15% for Tundra), and AD50 (61% for Massak and 25% for Tundra). Other differences include the acceptable occurrence for Ω-wolves (but not α-wolves) of AD55 (33% for Pukak and 67% for Tala) and AD81 (13% for Pukak and 38% for Tala). However, the frequency of AD55 is very low for both Pukak and Tala (1, and 2 respectively), as is the frequency for AD81 (1 for Pukak and 3 for Tala). These low frequencies suggest that AD55 and AD81 may not be important for Anger in Ω-wolves and may be coincidental. Furthermore, Pukak (Ω-wolf) produces the highest occurrence of JSNAPs at 78%. JSNAP and AD81 are associated with agonistic behaviour, and one would expect agonistic behaviours to be associated with dominant individuals (α-wolves) asserting themselves over other group individuals, as observed across mammals (Miller 1981; Muller & Wrangham 2004; Newton-Fisher 2004; Rutberg & Greenberg 1990; Schenkel 1967; Twiss et al. 1998; van der Borg et al. 2015; Yadav 2000), but this is not the case here. These intriguing patterns will be considered further in the discussion.

Table 3.4B shows the frequency and percentage of occurrence for each facial movement per individual wolf for the affective state Friendly. Again, the data shown represent the cumulative 'events' for Friendly for α- and Ω-wolves, extracted from the raw data (*n* = 73). This table shows that despite the high usage of an array of facial movements, several similarities and differences still exist between α- and Ω-wolves. Similarities between α- and Ω-wolves include AU101 (12% for Massak, 53 for Pukak, 18% for Tala, 18% for Tundra) and AD40 (11% for Massak, 11% for Pukak, 22% for Tala and 33% for Tundra). However again, the frequency of occurrence for AD40 (1 for Massak and Pukak, 2 for Tala and 3 for Tundra) is low, which suggests that the use of this facial movement for 'friendly' facial expressions may just be coincidental. Table 3.4B also shows several differences that exist for friendly facial expressions between α- and Ω-wolves, which include the acceptable occurrence for α-wolves (but not for Ω-wolves) of AU118 at 50% for both Massak and Tundra, though this facial movement again has a very low frequency (1 for Massak and Tundra), thus again, its occurrence may be purely coincidental. It should be noted that Ω-wolves do not seem to use AU118 for either Anger or Friendly, therefore, perhaps this is a facial movement used strictly by higher ranking wolves. But this will be considered later in the discussion. Other differences between α- and Ω-wolves include the acceptable occurrence for Ω-wolves (but not for α-wolves) of EAD103 (63% for Pukak and 22% for Tala), AD55 (62% for Pukak and 31% for Tala), AD50 (73% for Pukak and 27% for Tala), and unexpectedly AU109+110 (69% for Pukak and 23% for Tala), which, as previously explained, is associated with agonistic facial expressions, generally used by mammals to signal threat (Fox 1969, 1970; McGraw et al. 2002; White 2008). Again, these intriguing patterns will be considered further in the discussion.

Table 3.4A. Frequency and percentage occurrence (in brackets) of DogFACS and additional facial movement (AFM) codes (see Table 2.4, section 2.4.2) per individual 'alpha' (α) and 'omega' (Ω) wolf for the affective state Anger. Accepted percentages (10% and above, Bennett et al. 2017) are highlighted in grey, *n* = 109.

Facial movements	**Canid ID**			
	MASSAK α	**TUNDRA α**	**PUKAK Ω**	**TALA Ω**
AU101	32 (56)	12 (21)	12 (21)	1 (2)
AU143	1 (25)	1 (25)	1 (25)	1 (25)
AU145	15 (50)	4 (13)	9 (30)	2 (7)
AU109+110	9 (16)	10 (18)	31 (55)	6 (11)
AU110	12 (55)	5 (23)	4 (18)	1 (5)
AU12	5 (17)	1 (3)	20 (69)	3 (10)
AU116	4 (12)	7 (21)	16 (48)	6 (18)
AU118	25 (71)	6 (17)	3 (9)	1 (3)
AU25	46 (43)	18 (17)	35 (33)	7 (7)
AU26	32 (48)	10 (15)	23 (34)	2 (3)
AU27	21 (38)	10 (18)	19 (35)	5 (9)
AD19	4 (13)	9 (28)	15 (47)	4 (13)
AD33	0 (0)	0 (0)	0 (0)	0 (0)
AD35	1 (100)	0 (0)	0 (0)	0 (0)
ADR37	0 (0)	0 (0)	0 (0)	0 (0)
ADL37	0 (0)	0 (0)	1 (100)	0 (0)
AD137	8 (53)	3 (20)	4 (27)	0 (0)
EAD101	35 (74)	7 (15)	4 (9)	1 (2)
EAD102	3 (43)	3 (43)	0 (0)	1 (14)
EAD103	9 (27)	5 (15)	15 (45)	4 (12)
EAD104	0 (0)	0 (0)	0 (0)	0 (0)
EAD105	3 (10)	7 (23)	20 (65)	1 (3)
AD53	11 (58)	1 (5)	6 (32)	1 (5)
AD54	9 (28)	8 (25)	12 (38)	3 (9)
AD55	0 (0)	0 (0)	1 (33)	2 (67)
AD56	1 (25)	0 (0)	2 (50)	1 (25)
AD40	0 (0)	0 (0)	0 (0)	0 (0)
AD50	36 (61)	15 (25)	4 (7)	4 (7)
AD81	0 (0)	0 (0)	1 (25)	3 (75)
AD126	0 (0)	0 (0)	0 (0)	0 (0)
AD119	1 (17)	4 (67)	0 (0)	1 (17)
WHITES	0 (0)	9 (82)	2 (18)	0 (0)
TONGUE	3 (12)	6 (24)	12 (48)	4 (16)
JSNAP	0 (0)	1 (11)	7 (78)	1 (11)

Table 3.4B. Frequency and percentage occurrence (in brackets) of DogFACS and additional facial movement (AFM) codes (see Table 2.4, section 2.4.2) per individual ‘alpha’ (α) and ‘omega’ (Ω) wolf for the affective state Friendly. Accepted percentages (10% and above, Bennett et al. 2017) are highlighted in grey, *n* = 73.

Facial movements	**Canid ID**			
	MASSAK α	**TUNDRA α**	**PUKAK Ω**	**TALA Ω**
AU101	2 (12)	3 (18)	9 (53)	3 (18)
AU143	0 (0)	3 (33)	5 (56)	1 (11)
AU145	1 (3)	9 (26)	17 (49)	8 (23)
AU109+110	0 (0)	1 (8)	9 (69)	3 (23)
AU110	0 (0)	3 (15)	13 (65)	4 (20)
AU12	1 (2)	5 (12)	29 (71)	6 (15)
AU116	0 (0)	3 (38)	4 (50)	1 (13)
AU118	1 (50)	1 (50)	0 (0)	0 (0)
AU25	3 (4)	11 (16)	39 (57)	15 (22)
AU26	3 (5)	9 (15)	33 (56)	14 (24)
AU27	0 (0)	2 (10)	14 (67)	5 (24)
AD19	1 (4)	3 (11)	15 (56)	8 (30)
AD33	0 (0)	0 (0)	0 (0)	0 (0)
AD35	0 (0)	2 (67)	0 (0)	1 (33)
ADR37	0 (0)	1 (33)	2 (67)	0 (0)
ADL37	0 (0)	3 (75)	0 (0)	1 (25)
AD137	2 (5)	7 (18)	18 (47)	11 (29)
EAD101	0 (0)	2 (67)	1 (33)	0 (0)
EAD102	0 (0)	0 (0)	1 (100)	0 (0)
EAD103	4 (7)	5 (8)	37 (63)	13 (22)
EAD104	0 (0)	0 (0)	0 (0)	0 (0)
EAD105	0 (0)	7 (39)	9 (50)	2 (11)
AD53	1 (5)	7 (37)	6 (32)	5 (26)
AD54	3 (7)	6 (13)	32 (71)	4 (9)
AD55	0 (0)	1 (8)	8 (62)	4 (31)
AD56	1 (6)	2 (13)	7 (44)	6 (38)
AD40	1 (14)	3 (43)	1 (14)	2 (29)
AD50	0 (0)	0 (0)	8 (73)	3 (27)
AD81	0 (0)	0 (0)	0 (0)	0 (0)
AD126	0 (0)	0 (0)	0 (0)	0 (0)
AD119	0 (0)	5 (28)	4 (22)	9 (50)
WHITES	1 (8)	5 (42)	2 (17)	4 (33)
TONGUE	1 (6)	3 (19)	7 (44)	5 (31)
JSNAP	0 (0)	0 (0)	0 (0)	0 (0)

Table 3.5A shows the frequency and percentage of occurrence for each facial movement per affective state for domestic dogs. Again, using the 10% rule of thumb (Bennett et al. 2017), any occurrence of 10% or more was considered an acceptable occurrence and important for their respective affective states. At first glance this table shows that extensive overlap with Anger, Friendly and Joy exists. In fact, Joy uses all facial movements except AD119, and even includes JSNAP (as previously mentioned, this movement is considered to be associated with agonistic behaviour), though the frequency of occurrence for JSNAP is very low at 1, which suggests a coincidental occurrence. Fear appears to have no facial movements of an acceptable occurrence and the remaining affective states use very few movements, ranging between just one (for Happy and Interest) and three (for Curiosity and Surprise) combinations of movements. This suggests that dogs are limited in their ability to produce facial expressions for a wide range of affective states.

In addition, Table 3.5B shows the same data as Table 3.5A, but is highlighted to show 55 similarities (grey highlighted), and 82 differences (orange highlighted) for dogs compared to wolves, in their use of facial movements per affective state. This suggests that domestic dogs only in part, produce facial expressions like wolves, and for a limited range of affective states. Furthermore, like wolves, Anger and Friendly comprise the greatest range of facial movements, but for dogs Joy also has a great range of facial movements, in fact all but one facial movement (AD119, licking) are used for Joy. Additionally (unlike wolves), the use of facial movements in the context of Anger, Friendly and Joy were not found to be influenced by social status, sex or age of the dogs.

Table 3.5A. Frequency and percentage (in brackets) of occurrence of DogFACS and additional facial movement (AFM) codes (see Table 2.4, section 2.4.2) per domestic dog affective state. Accepted percentages (10% and above, Bennett et al. 2017) are highlighted in grey, n = 753.

DogsFACS and AFM codes	**Affective states**								
	Anger	**Anxious**	**Curiosity**	**Fear**	**Friendly**	**Happy**	**Interest**	**Joy**	**Surprise**
AU101	54 (13)	7 (2)	17 (4)	11 (3)	155 (38)	11 (3)	20 (5)	111 (27)	25 (6)
AU143	0 (0)	1 (3)	0 (0)	0 (0)	8 (26)	5 (16)	0 (0)	17 (55)	0 (0)
AU145	34 (11)	17 (5)	10 (3)	7 (2)	126 (40)	9 (3)	16 (5)	88 (28)	6 (2)
AU109+110	25 (34)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48 (66)	0 (0)
AU110	27 (18)	6 (4)	3 (2)	2 (1)	46 (31)	1 (1)	0 (0)	62 (42)	0 (0)
AU12	14 (5)	5 (2)	1 (0)	2 (1)	126 (43)	7 (2)	1 (0)	131 (45)	3 (1)
AU116	32 (15)	0 (0)	2 (1)	0 (0)	90 (43)	3 (1)	3 (1)	77 (36)	4 (2)
AU118	59 (44)	3 (2)	1 (1)	5 (4)	8 (6)	0 (0)	0 (0)	57 (43)	1 (1)
AU25	91 (15)	21 (3)	12 (2)	11 (2)	242 (40)	13 (2)	10 (2)	198 (33)	11 (2)
AU26	34 (7)	19 (4)	12 (2)	8 (2)	233 (48)	13 (3)	8 (2)	153 (31)	8 (2)
AU27	71 (26)	8 (3)	1 (0)	4 (1)	48 (17)	3 (1)	0 (0)	140 (51)	1 (0)
AD19	13 (5)	2 (1)	9 (4)	3 (1)	138 (57)	10 (4)	0 (0)	64 (27)	2 (1)
AD33	15 (60)	3 (12)	1 (4)	0 (0)	2 (8)	0 (0)	0 (0)	4 (16)	0 (0)
AD35	7 (33)	4 (19)	0 (0)	0 (0)	5 (24)	0 (0)	0 (0)	5 (24)	0 (0)
ADR37	0 (0)	1 (3)	0 (0)	0 (0)	23 (72)	2 (6)	0 (0)	6 (19)	0 (0)
ADL37	1 (4)	0 (0)	0 (0)	0 (0)	19 (68)	0 (0)	0 (0)	8 (29)	0 (0)
AD137	11 (5)	3 (1)	8 (3)	1 (0)	131 (57)	5 (2)	6 (3)	63 (28)	1 (0)
EAD101	28 (12)	3 (1)	17 (7)	6 (3)	41 (17)	2 (1)	10 (4)	99 (42)	29 (12)
EAD102	25 (13)	2 (1)	22 (12)	4 (2)	35 (18)	2 (1)	7 (4)	67 (35)	27 (14)
EAD103	81 (14)	19 (3)	4 (1)	15 (3)	256 (43)	14 (2)	39 (7)	158 (27)	4 (1)
EAD104	3 (17)	0 (0)	2 (11)	1 (6)	2 (11)	0 (0)	0 (0)	8 (44)	2 (11)
EAD105	4 (15)	0 (0)	0 (0)	0 (0)	7 (26)	0 (0)	0 (0)	15 (56)	1 (4)
AD53	68 (13)	14 (3)	15 (3)	7 (1)	228 (43)	12 (2)	36 (7)	147 (27)	8 (1)
AD54	30 (9)	8 (2)	6 (2)	8 (2)	130 (37)	8 (2)	7 (2)	151 (43)	1 (0)
AD55	7 (6)	1 (1)	8 (7)	0 (0)	22 (18)	3 (2)	0 (0)	79 (65)	1 (1)
AD56	6 (5)	0 (0)	14 (11)	5 (4)	37 (29)	1 (1)	0 (0)	66 (51)	0 (0)
AD40	16 (6)	7 (3)	14 (5)	3 (1)	134 (48)	4 (1)	47 (17)	35 (13)	18 (6)
AD50	76 (32)	21 (9)	2 (1)	7 (3)	57 (24)	0 (0)	0 (0)	70 (30)	1 (0)
AD81	10 (11)	1 (1)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	79 (87)	0 (0)
AD126	8 (6)	5 (4)	0 (0)	0 (0)	53 (41)	6 (5)	1 (1)	55 (42)	2 (2)
AD119	0 (0)	1 (2)	1 (2)	0 (0)	56 (86)	0 (0)	1 (2)	6 (9)	0 (0)
WHITES	72 (13)	8 (1)	9 (2)	14 (3)	208 (38)	11 (2)	30 (6)	176 (32)	16 (3)
TONGUE	10 (7)	1 (1)	4 (3)	2 (1)	98 (65)	8 (5)	0 (0)	28 (19)	0 (0)
JSNAP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)

Table 3.5B. Comparison of similarities and differences in domestic dog and wolf affective facial expressions. Grey highlighted represents similarities of dogs with wolves in the use of facial movements for each affective state. Orange highlighted represents differences of dogs with wolves in the use of facial movements for each affective state. Numbers represent the same as those seen in Table 3.5A, *n* = 753.

DogsFACS and AFM codes	Affective states								
	Anger	**Anxious**	**Curiosity**	**Fear**	**Friendly**	**Happy**	**Interest**	**Joy**	**Surprise**
AU101	54 (13)	7 (2)	17 (4)	11 (3)	155 (38)	11 (3)	20 (5)	111 (27)	25 (6)
AU143	0 (0)	1 (3)	0 (0)	0 (0)	8 (26)	5 (16)	0 (0)	17 (55)	0 (0)
AU145	34 (11)	17 (5)	10 (3)	7 (2)	126 (40)	9 (3)	16 (5)	88 (28)	6 (2)
AU109+110	25 (34)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48 (66)	0 (0)
AU110	27 (18)	6 (4)	3 (2)	2 (1)	46 (31)	1 (1)	0 (0)	62 (42)	0 (0)
AU12	14 (5)	5 (2)	1 (0)	2 (1)	126 (43)	7 (2)	1 (0)	131 (45)	3 (1)
AU116	32 (15)	0 (0)	2 (1)	0 (0)	90 (43)	3 (1)	3 (1)	77 (36)	4 (2)
AU118	59 (44)	3 (2)	1 (1)	5 (4)	8 (6)	0 (0)	0 (0)	57 (43)	1 (1)
AU25	91 (15)	21 (3)	12 (2)	11 (2)	242 (40)	13 (2)	10 (2)	198 (33)	11 (2)
AU26	34 (7)	19 (4)	12 (2)	8 (2)	233 (48)	13 (3)	8 (2)	153 (31)	8 (2)
AU27	71 (26)	8 (3)	1 (0)	4 (1)	48 (17)	3 (1)	0 (0)	140 (51)	1 (0)
AD19	13 (5)	2 (1)	9 (4)	3 (1)	138 (57)	10 (4)	0 (0)	64 (27)	2 (1)
AD33	15 (60)	3 (12)	1 (4)	0 (0)	2 (8)	0 (0)	0 (0)	4 (16)	0 (0)
AD35	7 (33)	4 (19)	0 (0)	0 (0)	5 (24)	0 (0)	0 (0)	5 (24)	0 (0)
ADR37	0 (0)	1 (3)	0 (0)	0 (0)	23 (72)	2 (6)	0 (0)	6 (19)	0 (0)
ADL37	1 (4)	0 (0)	0 (0)	0 (0)	19 (68)	0 (0)	0 (0)	8 (29)	0 (0)
AD137	11 (5)	3 (1)	8 (3)	1 (0)	131 (57)	5 (2)	6 (3)	63 (28)	1 (0)
EAD101	28 (12)	3 (1)	17 (7)	6 (3)	41 (17)	2 (1)	10 (4)	99 (42)	29 (12)
EAD102	25 (13)	2 (1)	22 (12)	4 (2)	35 (18)	2 (1)	7 (4)	67 (35)	27 (14)
EAD103	81 (14)	19 (3)	4 (1)	15 (3)	256 (43)	14 (2)	39 (7)	158 (27)	4 (1)
EAD104	3 (17)	0 (0)	2 (11)	1 (6)	2 (11)	0 (0)	0 (0)	8 (44)	2 (11)
EAD105	4 (15)	0 (0)	0 (0)	0 (0)	7 (26)	0 (0)	0 (0)	15 (56)	1 (4)
AD53	68 (13)	14 (3)	15 (3)	7 (1)	228 (43)	12 (2)	36 (7)	147 (27)	8 (1)
AD54	30 (9)	8 (2)	6 (2)	8 (2)	130 (37)	8 (2)	7 (2)	151 (43)	1 (0)
AD55	7 (6)	1 (1)	8 (7)	0 (0)	22 (18)	3 (2)	0 (0)	79 (65)	1 (1)
AD56	6 (5)	0 (0)	14 (11)	5 (4)	37 (29)	1 (1)	0 (0)	66 (51)	0 (0)
AD40	16 (6)	7 (3)	14 (5)	3 (1)	134 (48)	4 (1)	47 (17)	35 (13)	18 (6)
AD50	76 (32)	21 (9)	2 (1)	7 (3)	57 (24)	0 (0)	0 (0)	70 (30)	1 (0)
AD81	10 (11)	1 (1)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	79 (87)	0 (0)
AD126	8 (6)	5 (4)	0 (0)	0 (0)	53 (41)	6 (5)	1 (1)	55 (42)	2 (2)
AD119	0 (0)	1 (2)	1 (2)	0 (0)	56 (86)	0 (0)	1 (2)	6 (9)	0 (0)
WHITES	72 (13)	8 (1)	9 (2)	14 (3)	208 (38)	11 (2)	30 (6)	176 (32)	16 (3)
TONGUE	10 (7)	1 (1)	4 (3)	2 (1)	98 (65)	8 (5)	0 (0)	28 (19)	0 (0)
JSNAP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)

3.4: Domestic dog limitations in facial expression production and potential compensatory mechanisms.

The confusion seen in Figure 3.6 consisted of 262 (35%) incorrectly predicted affective states within the entire dataset of 753 events for dog facial expressions analysed. Table 3.6 shows how these 262 incorrect classifications distribute across dogs with varying morphological facial features. This table shows that brachycephalic and mesocephalic (non-wolf-like) heads make-up a combined 78% (equally 39% each) of the confusion seen Figure 3.6, while dolichocephalic (wolf-like) heads only make-up 22% of the confusion. This suggests that head shape may be a limiting factor for dogs to produce affective facial expressions. It can also be seen that flopped and semi-flopped (non-wolf-like) ears make-up 57% and 27% (respectively) of the confusion, while erect (wolf-like) ears only make-up 16%. These findings again, suggest that ear position may be a limiting factor for dogs to produce affective facial expressions. Table 3.6 also shows that flews (pendulous upper lips) are responsible for 22% of the confusion seen in Figure 3.6, ectropion (drooping lower eye-lids) is responsible for 15% and neutral abnormalities are only responsible for 7% of the confusion. This suggests that to an extent facial features may also be limiting factors for dogs to produce affective facial expressions, but they are certainly not as limiting as head shape and ear position.

Table 3.6. Domestic dog morphological facial features and their corresponding number of dogs and percentage of entries within the incorrectly predicted (confused, *n* = 262) affective states seen in Figure 3.6 (dog confusion matrix).

Morphological features	***n*, number of dogs**	**% of entries**
Head shape		
Brachycephalic	103	39
Mesocephalic	102	39
Dolichocephalic*	57	22
Ear position		
Flopped	149	57
Semi-flopped	70	27
Erect*	43	16
Face		
Flews	104	40
Ectropion	40	15
Neutral abnormalities	18	7

*wolf-like morphological facial features

During data collection, several different vocalisations (AD50, Table 2.4, section 2.4.2) were observed to accompany different affective facial expressions in both wolves and dogs. These vocalisations were categorised and their cumulative frequencies (and percentages) were recorded for each affective state (Table 3.7A and Table 3.7B). Table 3.7A shows that wolves use bark vocalisations in the context of Anxious (67%) and Surprise (33%), though the frequency of occurrence of these bark vocalisations are very low at just 2 for Anxious and 1 for Surprise. Chuff vocalisations occurred mostly in the context of Anger (88%), as did growl vocalisations (89%), and growl-bark (100%), though the frequency of growl-bark is very low at just 1. Howl (the most well-known and studied of all wolf vocalisations, Dungol et al. 2008; Harrington and Mech 1978; Harrington and Mech 1979; Harrington and Mech 1982; Nowak et al. 2007; Theberge and Falls 1967; Tooze et al. 1990) occurred only once (100%) in the context of Anxious. Whimper mostly occurred in situations where wolves were Anxious (61%) and Friendly (29%), and whine vocalisations appear to be quite spread among Joy (12%), Fear (18%), Anger (24%) and Friendly (41%), though Joy and Fear have very low frequencies of 2 and 3, respectively. Yelp mostly occurred in the context of Anger (67%) and yelp-bark occurs equally (50%) for both Anger and Fear, though yelp-bark vocalisations have very low frequencies for Anger and Fear at just 1 each. The low frequencies seen in Table 3.7A may indicate that the vocalisations used were purely coincidental, but perhaps they are vocalisations that are used to emphasise the intensity of the affective state being conveyed (Le Roux 2002).

Table 3.7B shows that dogs use bark vocalisations mostly in the context of Anger (58%), Joy (33%) and Friendly (16%), which are the affective states that have the most facial movements associated with them (Table 3.5A). However, bark vocalisations are also used to an extent in all affective states (except Interest). Chuff vocals (like wolves) occurred mostly in the context of Anger (100%) though with a somewhat low frequency of occurrence of 6. Growl also occurred mostly (like wolves) in the context of Anger (60%), and also with Joy (37%). Growl-bark (again, like wolves) mostly occurred with Anger (75%), though it does have a low frequency of 3. Grunt mostly occurred in the context of Friendly (50%) and Joy (38%), though both have low frequencies of 4 and 3 respectively. Whimper mostly occurred (just like wolves) in situations where dogs were Anxious (43%) and Friendly (35%), and to a lesser extent in the context of Joy (16%), which has a somewhat low frequency of 6. Whine vocalisations mostly occurred (again, just like wolves) in the context of Friendly (64%), and again, to a lesser extent with Anxious and Joy at 18% each. No howl vocalisations in dogs were recorded for data analyses, but they were observed to occur during video collection in Mia1 and Milo1 (appendix A) after they underwent surgery to be sterilised and were still under the influence of anaesthetic. Similarly, yelp and yelp-bark vocalisations were not observed in dogs during data collection, though they have been reported (Yeon 2007) and observed to occur (Pers. Obs.) prior to this research. Furthermore, the low frequencies seen in Table 3.7B may indicate coincidental occurrence of their corresponding vocalisations per affective state, or they may be reserved to emphasis varying affective state intensities (Le Roux 2002).

No vocalisations were observed in the context of Curiosity in wolves (yet, they were for dogs, at very low frequencies, not exceeding 1), and no vocalisations were observed in the context of Interest in dogs (yet, they were for wolves, again at very low frequencies not exceeding 1). This may be due to the fact that Curiosity and Interest are essentially investigatory affective states and so, there is no need to vocalise when inspecting an object of curiosity or interest, as a vocal could alarm and cause evasion of that object (for example, if that object was potential prey). However, more importantly it was found that dogs vocalise more than wolves do when socially interacting and reacting to stimuli (n_{dogs} = 298, n_{wolves} = 137). This suggests that using vocalisations may be more important than using facial expressions for intra-pack communication in domestic dogs. In fact, within the events of the correctly predicted affective states of Figure 3.6 (n = 491) dogs were found to use vocalisations 29% of the time, but there was a slight increase to 35% in the use of vocalisations by dogs seen within the events that were not classified correctly according to the predicted affective state (*n* = 262). This provides some preliminary evidence that dogs who may be limited in their ability to produce affective facial expressions like wolves, may compensate by using more vocalisations to convey their affective states.

Table 3.7A. Frequency and percentage (in brackets) of occurrence of vocalisations per affective state observed in wolves, *n* = 137.

Vocals	**Affective State**								
	Anger	**Anxious**	**Curiosity**	**Fear**	**Friendly**	**Happy**	**Interest**	**Joy**	**Surprise**
Bark	0 (0)	2 (67)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)
Chuff	7 (88)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (13)
Growl	57 (89)	0 (0)	0 (0)	1 (2)	5 (8)	0 (0)	0 (0)	1 (2)	0 (0)
Growl-Bark	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Howl	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Whimper	0 (0)	17 (61)	0 (0)	1 (4)	8 (29)	0 (0)	1 (4)	0 (0)	1 (4)
Whine	4 (24)	0 (0)	0 (0)	3 (18)	7 (41)	0 (0)	1 (6)	2 (12)	0 (0)
Yelp	6 (67)	0 (0)	0 (0)	1 (11)	2 (22)	0 (0)	0 (0)	0 (0)	0 (0)
Yelp-Bark	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 3.7B. Frequency and percentage (in brackets) of occurrence of vocalisations per affective state observed in domestic dogs, *n* = 298.

Vocals	**Affective State**								
	Anger	**Anxious**	**Curiosity**	**Fear**	**Friendly**	**Happy**	**Interest**	**Joy**	**Surprise**
Bark	58 (41)	5 (4)	1 (1)	6 (4)	23 (16)	0 (0)	0 (0)	47 (33)	1 (1)
Chuff	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Growl	34 (60)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	21 (37)	0 (0)
Growl-Bark	3 (75)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)
Grunt	0 (0)	0 (0)	1 (13)	0 (0)	4 (50)	0 (0)	0 (0)	3 (38)	0 (0)
Whimper	0 (0)	16 (43)	1 (3)	1 (3)	13 (35)	0 (0)	0 (0)	6 (16)	0 (0)
Whine	0 (0)	8 (18)	0 (0)	0 (0)	29 (64)	0 (0)	0 (0)	8 (18)	0 (0)

Chapter 4: Discussion.

Overall, this research clearly shows that wolves are capable of producing a wide range of consistent facial expressions to visually convey affective states when communicating. This research also shows that wolves are capable of some plasticity within these facial expressions owing to social status influencing the facial expressions of dominant and submissive wolves. Domestic dogs, however, appear limited in their range of facial expressions due to facial morphological divergences from their wolf ancestry. Yet, dogs still seem to be capable of conveying affective states, which may be due to their use of vocalisations when communicating.

4.1: Wolf facial expressions convey affective states.

This research provides the first comprehensive, quantitative account of affective facial expressions in a non-primate species, the wolf. Four clusters of facial movements were statistically defined for wolf affective states (Table 3.1A, section 3.1), which show clear separation, indicating that facial movements (DogFACS and AFM codes) do map onto affective states well in wolves. Moreover, linear discriminant analyses and the subsequent confusion matrix show that wolf affective states can be predicted, with substantial agreement (according to the Kappa statistic, Table 2.5, section 2.4.3) using facial movements. The results reveal several unique combinations of facial movements that correspond to the nine affective states investigated in this research (Table 3.3, section 3.3), thus providing nine separate affective facial expressions.

There remains some disagreement with the mapping of facial movements onto affective states, which is represented by the existence of only four clusters instead of nine, one for each affective state. This is due to the mixing of affective states across and within these four clusters (Table 3.2A, section 3.1), which in turn, is due to the overlapping of various facial movements across the affective states themselves (Table 3.3, section 3.3). One explanation for the overlap is that facial movements were only recorded as either ‘on’ or ‘off’ during any one social interaction or reaction to stimuli. However, including the total frequencies and durations of each facial movement per interaction or reaction per affective state could provide better distinction between clusters and therefore, affective states (Caeiro et al. 2017). For example, the facial movement AD19 (tongue show) occurs in the context of Anger, Fear, Friendly and Happy (all found within cluster four, Table 3.2A, section 3.1). However, it was observed that AD19 occurred more frequently during singular interactions of Friendly affective states than it did in Anger, Fear and Happy. Similarly, the facial movement EAD102 (ears adductor) is present in Curiosity and Surprise (both within cluster one), and Interest (cluster two, Table 3.3, section 3.3). However, it was observed that the durations of EAD102 were longer in the context of Curiosity, as opposed to Surprise and Interest. This is due to Surprise and (to an extent) Interest facial expressions being brief compared to those of Curiosity. Nonetheless, even without the inclusion of total frequencies and durations, as previously mentioned, the confusion matrix confirms substantial agreement (according to the Kappa statistic), based solely upon the use of 'on/off' occurrences of facial movements. That being said, the confusion matrix also contains some disagreement with a lack of distinctiveness seen between some affective states, in particular between positive and negative affective states (Figure 3.5, section 3.2). Though, it should be noted that all of the lack of distinctiveness within the wolf confusion matrix is below that of slight agreement (according to the Kappa statistic).

Figure 3.5 (section 3.2) shows a regular lack of distinctiveness (confusion within the matrix) between the affective states Joy, Happy, Anger, Fear and Friendly. Confusing Joy with Happy is not a major disadvantage for our human understanding or wolf communication, as these are both positive affective states, and one could argue that Joy is simply an increase in the intensity of Happy, and therefore a secondary affective state (Neviarouskaya et al. 2007, 2009). If Joy is merely considered an increase in the intensity of Happy, then this research demonstrates for the first time, the existence of a quantified and defined secondary affective state in a non-primate species, the wolf. However, a lack of distinctiveness between positive and negative affective states is potentially detrimental for human understanding and for wolf communication (with regards to the inappropriate transfer of information, which would increase the chances of conflict arising, Arnold & Whiten 2001; Aureli 1997; Mech 2007; Smith et al. 2011).

In general, facial expressions of Joy were produced during the contexts of play (both social and lone play with inanimate objects) and mating. Depending upon the context of Joy, it was observed that rapid changes in facial expressions, and therefore affective states would occur, which was marked during social play. When wolves socially play, they often engage in 'play fighting' (Bekoff 1974a; Cordoni 2009; Sommerville et al. 2017), which was observed frequently during this research. Play fighting, can appear very aggressive as wolves will often wrestle, bite and pull on each other (Bekoff 1974a; Cordoni 2009), which results in many, varying facial movements. Indeed, in this research it was found that facial movements such as wide-mouthed (AU12 and AU27) biting and chewing (AD81) of conspecifics were often produced during facial expressions of Joy (Table 3.3, section 3.3). These facial movements used in the context of Joy are also used in the context of Anger, which provides an explanation for the lack of distinctiveness seen between Joy and Anger within the wolf confusion matrix

(Table 3.3, section 3.3). However, biting associated with Joy is inhibited and does not cause harm to conspecifics, whereas biting associated with Anger is not inhibited and does cause harm to conspecifics (Bauer & Smuts 2007; Bekoff 1974a, 1974b; Schenkel 1967). When one wolf is bitten by another wolf and pain is inflicted it often results in the bitten wolf producing a 'yelp' vocalisation (Table 2.6, section 2.4.3, Mech & Boitani 2003; Tembrock 1976). Therefore, the inclusion of factors such as whether the receiving wolf yelps or produces a pain grimace (figure 4.1) typical of that seen in other mammalian species (Dalla Costa et al. 2014; Keating et al. 2012; Matsumiya et al. 2012; Sotocinal et al. 2011), would improve the distinctiveness between Joy and Anger of the signalling wolf.

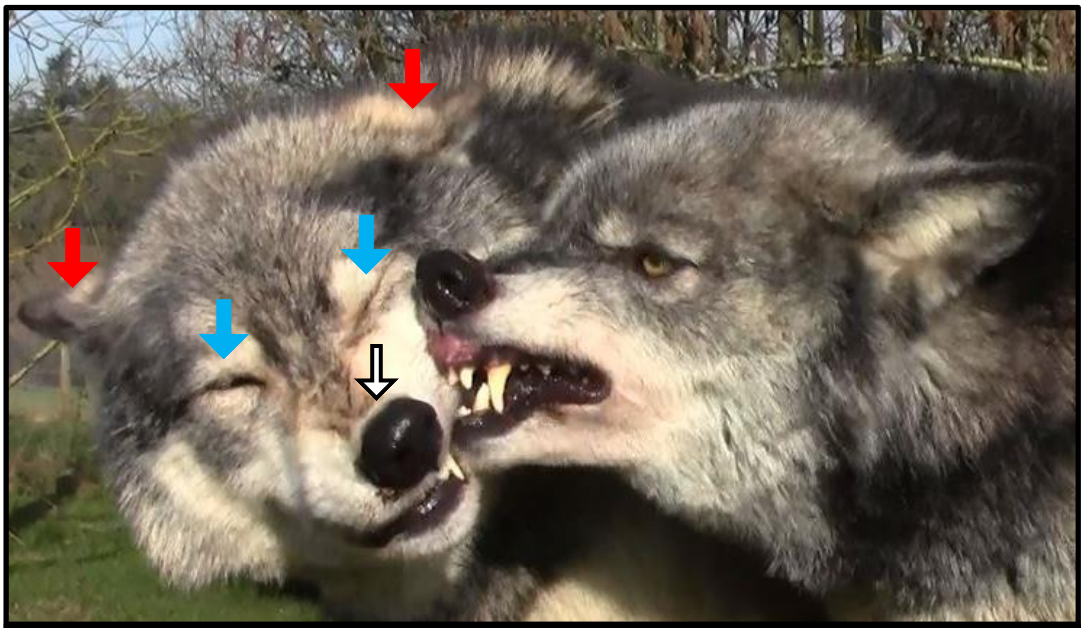


Figure 4.1. Example of a pain grimace observed in the grey wolf (*Canis lupus*) observed during this research. The wolf on the left is bitten by the wolf on right, and displays a facial expression typical of a mammalian pain grimace with ears pulled back (red arrows), orbital tightening (blue arrows) and nose bulging (black outlined white arrow). Photograph by ER Hobkirk.

The affective state Friendly is associated with submissive behaviour towards higher ranking individuals (Table 2.3, section 2.4.1). However (as previously mentioned), submission in wolves exists in two forms, 'active' and 'passive' (Mech & Boitani 2003; Mech 1999; Mech 2007; Schenkel 1967). Active submission is generally expressed in an excitable manner with pronounced facial signals of friendliness such as, repeated licking (AD119), mouthing, chewing (AD81) and grasping hold of the muzzle of the dominant wolf (Mech 2007; Schenkel 1967), which agrees with the findings of this research (Table 3.3, section 3.3). However, both licking and chewing are also used during facial expressions of Anger (Table 3.3, section 3.3).

Moreover, it was observed that the submissive grin of a Friendly wolf (which can occur in both active and passive forms of submission) can become very elaborate (suggesting an increase in its intensity) where the wolf will also bare its teeth (which uses the facial movements, AU109+110, AU12, AU116 and AU25), as if 'smiling' like a human (see appendix C for an example). The facial movements observed with an elaborate submissive grin are also used in the context of Anger (Table 3.3, section 3.3), which offers an explanation for the lack of distinctiveness observed between Friendly and Anger in the wolf confusion matrix. Passive submission on the other hand, is generally expressed with timidity, mimicking that of a fearful demeanour (Schenkel 1967), with the head down (AD54) and the ears lying back, flat against the head (EAD103, Schenkel 1967). These movements agree with this research, with the addition of the whites (sclera) of the eyes being shown, for both Friendly and Fear (Table 3.3, section 3.3). This overlap in the use of facial movements explains the lack of distinctiveness observed between Friendly and Fear within the wolf confusion matrix. Therefore, the inclusion of contextual information as to whether the Friendly affective state is occurring during active or passive submission, or indeed sub-classifying Friendly into both positive (for active submission) and negative (for passive submission) affective states, would increase the distinctiveness between Friendly, Anger and Fear.

All of the facial movements observed in the context of Joy, were also observed in the context of Fear or Friendly facial expressions (Table 3.3, section 3.3), hence some confusion exists between these affective states too. Similarly, the facial movements used in the context of Happy are also used in the context of Anger, Fear and Friendly (Table 3.3, section 3.3). However, facial movements corresponding to Happy are not used in the context of Joy, which is perplexing when there remains a lack of distinctiveness between Joy and Happy within the wolf confusion matrix (Table 3.3, section 3.3). However, this lack of distinctiveness may be the result of human error in the classification of affective states. For example, when independent classifiers were shown videos of wolf facial expressions conveying Joy, some classifiers recorded the expressions as Happy instead of Joy. However, as previously mentioned, the lack of distinctiveness within the wolf confusion matrix is below that of slight agreement (according the Kappa statistic), indicating that any human error is minimal in this research.

4.1.1: The intensities of wolf facial expressions.

For each facial expression decoded in this research, only one primary affective state was allocated to each of them. However, it was observed that wolf facial expressions ranged in their intensities (the number of facial movements per expression, with more movements indicting an increase in intensity and vice versa) for some affective states. For example, some facial expressions of Anger were observed to be quite subtle (consisting of fewer facial movements, appendix D), while others appeared to be more elaborate (consisting of many more facial movements, appendix B, video 1). These varying intensities of facial expressions offer another explanation for the overlap seen in the use of facial movements, and therefore, the lack of distinctiveness seen within the wolf confusion matrix (Table 3.3, section 3.3).

To date, facial expression intensities have only been quantitatively studied in primates in the form of graded facial expressions (Parr et al. 2005a). Parr et al. (2005a) defined graded facial expressions as those that consist of variations in their intensity and completeness, in comparison to their peak, ritualised expressions (Marler 1976; Parr et al. 2005a). For example, Figure 4.2 shows the grading between two ritualised facial expressions (A and B) seen in the common chimpanzee (*P. troglodytes*), with their transitional expressions showing the changes in intensity and completeness between the two ritualised expressions. Indeed, Zimen (1981) made qualitative observations on the grading of wolf facial expressions in the context of Anger and Fear, and illustrated that these gradations also produced transitional facial expressions between their peak expressions of Anger and Fear (Figure 4.3).

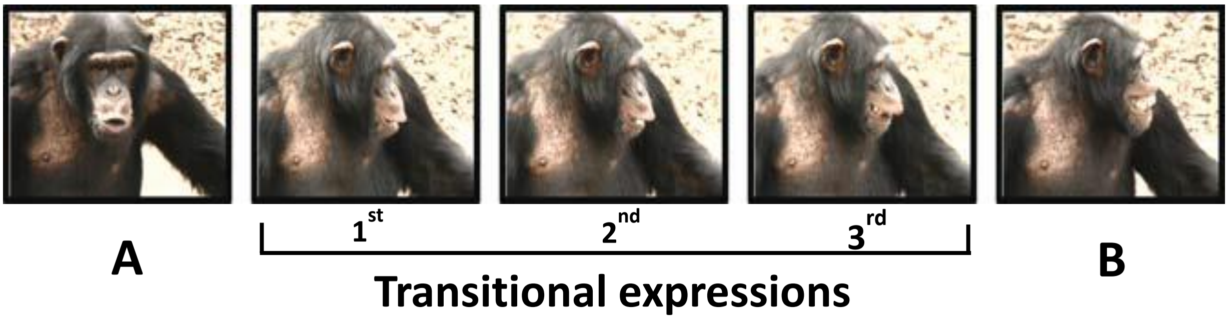


Figure 4.2. The blending of two ritualised, peak facial expressions seen in the common chimpanzee (*P. troglodytes*). Image A displays a ritualised 'pout' expression, and B displays a ritualised 'bared teeth' expression. The transitional expressions show the changes in intensity and the incompleteness that occurs when grading between expressions A and B occurs. The 3rd transitional expression displays the blended 'stretch pout whimper' expression of A and B. Adapted from Parr et al. (2005a).

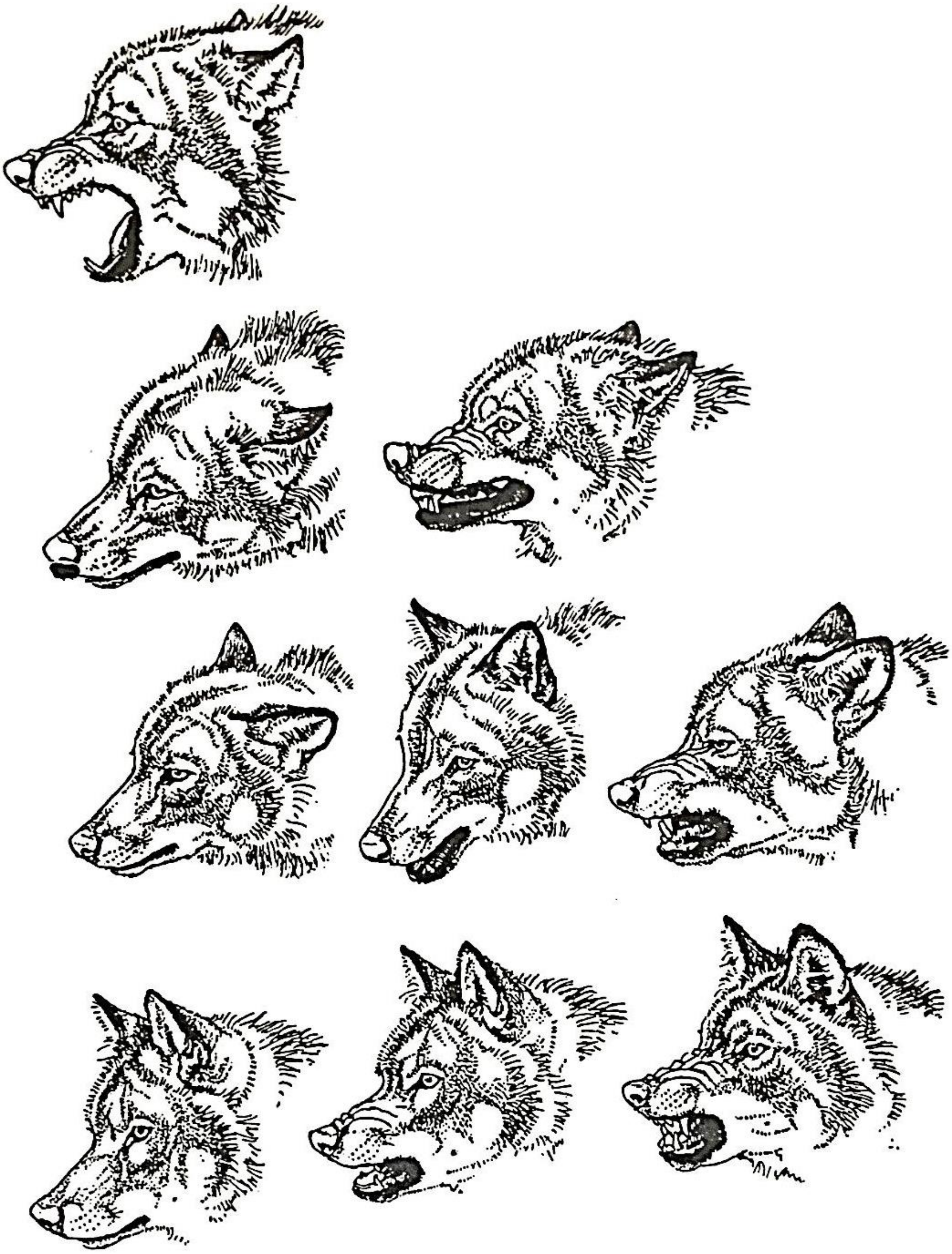


Figure 4.3. Affective facial expression intensities seen in the wolf. Bottom left corner image represents a neutral facial expression. Left to right represent transitional changes in Anger, and bottom to top represent transitional changes in Fear. Adapted from Zimen (1981).

Parr et al. (2005a) also found that the transitional expressions can produce blended facial expressions, which represent conflict within the internal motivational (affective) state of the signalling chimpanzees. For example, Figure 4.2 shows the blended 'stretch pout whimper' facial expression of a common chimpanzee, which is a combination of the 'pout' and 'bared teeth' facial expressions. Mech & Boitani (2003) also qualitatively observed that wolves use tongue flicking (section 2.4.2) to rapidly transition from Anger to Friendly, which in effect, creates a blended facial expression between Anger and Friendly (Figure 4.4). Indeed, this thesis research found that tongue shows (AD19) and tongue flicking were used proportionally the same in the context of both Anger and Friendly (Table 3.3, section 3.3), which suggests the occurrence of blending between these affective facial expressions.

Figure 4.4. Blended tongue flick facial expression of a wolf, often used to rapidly transition from Anger to Friendly affective states. The protruding tongue signals 'friendliness', while the ears forward, wide eyes, wrinkled nose, upper raise and teeth baring signals 'anger'. Adapted from Mech & Boitani (2003).

Affective state intensities have seldom been investigated in non-human animals. However, if blended facial expressions do indeed represent a conflict between affective states, then identifying the range of graded and blended affective facial expressions of wolves will allow for the quantification and classification of secondary, or even tertiary affective states in wolves. Furthermore, the inclusion of total frequencies and durations of facial movements (as described above) would provide a means to identify such graded and blended facial expressions, and thus, affective state intensities.

4.1.2: Wolf facial expressions convey social status.

It has long been suggested that wolf facial expressions convey social status (as well as affective states, Fox 1970; Schenkel 1947), yet, to date there has been no quantitative research conducted to confirm this. Here, the first preliminary, quantitative evidence for wolves conveying their social status via facial expressions is presented. This preliminary investigation is the first of its kind for any non-human animal.

The results showed that wolves use a wide range of facial movements in the context of Anger and Friendly, resulting in many facial movements overlapping between these two affective states. However, a closer look at Anger and Friendly showed some differences in the use of facial movements by dominant (α) and submissive (Ω, Table 3.4A and 3.4B, section 3.3.1) wolves. In fact, submissive wolves tended to display more elaborate (intense) facial expressions (consisting of more facial movements) compared to dominant wolves (Table 3.4A and 3.4B, section 3.3.1). In the context of Anger, dominant wolf facial expressions consisted of the ears being forward (EAD101), the lips puckering (AU118) and growling (vocalisations, AD50, Table 3.4A, section 3.3.1). Whereas submissive wolf facial expressions consisted of head tilts (AD55), mouthing and chewing (AD81) at conspecifics, and the highest occurrence of jaw snaps were produced by a single submissive wolf (Pukak, Table 3.4A, section 3.3.1). Chewing, and jaw snaps are gross movements and include other mouth and lip movements, which increases the amount of facial movement observed.

Interactions in the context of Anger were observed to be uncommon for submissive wolves, which accounts for the low frequencies of occurrence of facial movements observed Table 3.4A, section 3.3.1). Whereas interactions in the context of Anger for dominant wolves were observed to be common, as dominant wolves would regularly assert their dominance over submissive wolves. Assertion behaviour is common practice by dominant wolves to maintain social rank orders (Mech, 1974; Sands & Creel 2004; Schenkel 1967) and was observed (during this research) to be an often contactless, somewhat imposing ‘reminder’ of dominance. The dominant wolf was observed to stand rigidly tall over the submissive wolf, and therefore appearing larger than the submissive. This larger appearance seemed to be continued into the facial expression of the dominant wolf, by making its head look larger, using the ears forward movement (EAD101, much like that seen in the bottom middle and right images of Figure 4.3). Accompanying growl vocalisations (AD50) appeared to be used for intimidation and to emphasise the affective state (Anger) being conveyed. Furthermore, Anger in dominant wolves appeared to be far less intense than that of submissive wolves, appearing to be of a similar level to ‘irritated’ (a secondary affective state, Szasz et al. 2011) as opposed to 'angry' (primary state). Again, this may be due to the existence of secondary affective states in wolves.

The mouthing and chewing at the muzzles of dominant wolves displayed by submissive wolves in the context Anger may appear aggressive, but never escalates to actual biting. Biting in submissive wolves (during intra-pack social interactions) is always inhibited (Bauer & Smuts 2007; Bekoff 1974a, 1974b; Schenkel 1967), appearing to be just for 'show'. In fact, jaw snapping produced by submissive wolves in this research appeared to be just used for non-vocal auditory communication as opposed to tactile biting communication. Many non-human animals produce non-vocal auditory cues during agonistic interactions, for example, the snapping shrimp (*Alpheus armatus*) has a modified claw that it uses to produce a non-vocal 'snapping' sound to defend its territory (Knowlton & Keller 1982). Hatchling Saltwater crocodiles (*Crocodylus porosus*) engage in agonistic jaw 'clapping' when establishing dominance hierarchies (Brien et al. 2013), and gorillas (*Gorilla gorilla*) often engage in 'chest beating' during agonistic interactions to signal strength and dominance (Pika et al. 2003). These examples of non-vocal communication are used to avoid conflict. Therefore, it is possible that when submissive wolves are subjected to assertion from dominant wolves, they use jaw snapping to signal the strength of their weaponry (jaws and teeth) as a means to avoid conflict with the dominant wolf. During this research, it was observed that jaw snaps differed in the level of sound they produced, with some seemingly louder than others. Therefore, it is theorised here that the level of sound produced from the jaw snaps signals the strength of the jaws, with a louder snap indicating stronger jaws. To test this, simply measuring the decibels of jaw snaps produced by different wolves, and correlating these to measured bite forces of each of the wolves (by allowing them to bite down onto a transducer concealed within food, Kim et al. 2018) would provide an answer to this theory and greater insights into the complexity of wolf communication.

In the context of Friendly, dominant wolf facial expressions predominantly consisted of nothing more than lip puckering (AU118). Whereas submissive wolf facial expressions consisted of the ears being held back, flat against the head (EAD103), head tilting (AD55), growls and whines (AD50), and nose wrinkling and upper lip raising (AU109+110), which is essentially teeth baring. However, as previously mentioned this 'teeth baring' behaviour is associated with elaborate submissive grins and active submission, not agonistic behaviour. Interactions in the context of Friendly were observed to be common for submissive wolves but, observed to be uncommon for dominant wolves. However, this is purely because dominant wolves cannot usually display signs of submission, which is associated with Friendly (Table 2.3, section 2.4.1) else they could lose their rank position within the pack, which would lead to a loss of stable pack dynamics (Borg et al. 2015; Brainerd et al. 2008). The lack of Friendly interactions seen in dominant wolves explains the low frequencies of occurrence of facial movements seen in dominate wolves (Table 3.4B, section 3.3.1).

It should be noted that lip puckering is not used by submissive wolves for either Anger or Friendly, which suggests that this may be a facial movement used strictly by dominant wolves. This is perplexing as lip puckering hides the teeth (weaponry) of a wolf. However, the hiding of weaponry is perhaps also the reason why dominant wolves use lip puckering in the context of Friendly, to appear less threatening. But then this raises question, why do submissive wolves perform elaborate submissive grins and show their teeth? The human smile is thought to be a ritualised display that has evolved from fearful situations (from a display similar to the ‘bared-teeth’ display seen in Figure 4.2, Marsh et al. 2005) to signal a threat towards potential antagonists (Andrew 1963). Perhaps the submissive grin of wolves has evolved much in the same way as the human smile, from a readiness to attack (baring of the teeth) in fearful situations, which has evolved into a ritualised ’grin’. Moreover, submissive behaviour (active or passive) in wolves usually involves the wolf attempting to make itself look smaller than the dominant wolf (Fox 1970; Schenkel 1967), with the lowering of body posture, and making their head look smaller by way of the ears being held back, flat against the head (EAD103, Fox 1970; Mech & Boitani 2003; Schenkel 1967), which was also found in this research.

Submissive wolves are known to avert their gaze from dominant wolves (Fox 1970; Schenkel 1967), and head tilts may be used to achieve this. In the context of Anger and Friendly, it was observed that submissive wolves would avert their gaze away from the dominant wolves, and at the same time the dominant wolves would stare directly and intently at the submissive wolves. However, gaze aversion prevents the submissive wolves from seeing the face of the dominant wolves. Therefore, this may account for the less elaborate facial expressions observed in dominant wolves, as there is simply no need to facially convey their affective states when their intended receiver (submissive wolves) will not see it. Instead it was observed that the use of ‘body language’ and vocalisations (growling) seemed more important to convey Anger and Friendly in dominant wolves, which corresponds to current literature (Mech & Boitani 2003). One could argue that as submissive wolves avert their gaze, the dominant wolf cannot observe the face of the submissive wolf. But this is not the case here because submissive wolves use head tilts instead of lowering their heads vertically to avert their gaze. The head tilt means the dominant wolf can still observe the changes in the submissive wolf’s facial expressions and therefore, affective states.

4.2. Domestic dog facial expressions appear limited due to varying breed-type morphologies.

It is well known that selective breeding has led to a wealth of physical health problems in many domestic dog breeds (Asher et al. 2009; Bellumori et al. 2013; Collins et al. 2011; Dan et al. 2014; Farrell et al. 2015; Rooney 2009; Summers et al. 2010; Wood et al. 2000). However, here evidence is provided for selective breeding producing social communicative limitations across dog breeds. For the first time this research shows that different dog breed-types are limited in their ability to convey affective states via facial expressions like wolves, due to morphological divergences from wolf ancestry. Head and facial feature morphologies that alter the main conveyers of facial expressiveness (the eyes, ears, forehead, muzzle, nose and lips) are shown here to impair the production of facial expressions, and therefore, upon the conveyance of affective states. However, dogs seem to have evolved an alternative method (compared to wolves) to convey their affective states, by using vocalisations.

Only two indistinct clusters were statistically defined for domestic dog affective states (Table 3.1B, section 3.1). The mixing of affective states between these two clusters is proportionally the same, indicating a lack of separation between the clusters and implying a lack of distinctiveness between affective states. However, linear discriminant analyses and the resulting confusion matrix shows that overall, affective states can be predicted via facial movements with substantial agreement (according to the Kappa statistic, Table 2.5, section 2.4.3). That being said, the confusion matrix (Figure 3.6, section 3.2) still shows an extensive lack of distinctiveness, ranging between slight and moderate agreement (according to the Kappa statistic). In fact, the lack of distinctiveness within the dog confusion matrix is vast in comparison to that seen in wolves. For example, the lack of distinctiveness with Friendly occurs with every other affective state within the confusion matrix. As a consequence, there is also a great lack of distinctiveness between positive and negative affective states throughout the confusion matrix, which implies that domestic dogs (across breed-types) are inconsistent in the way they convey affective states via facial expressions, and clearly the linear discriminant analyses has detected these inconsistencies. Such high levels of indistinctiveness between positive and negative affective states is potentially detrimental to dog-dog and dog-human communication. For example, many dogs that are fearful can become ‘fear aggressive’ and will bite to defend themselves from potential threats (Galac & Knol 1997; Haug 2008). Consequently, if a dog or human was to mistakenly perceive that another dog was displaying a Friendly affective state, when in fact it was displaying Fear, this may lead to dog-dog conflict or the human being bitten. Therefore, it is important for dog welfare and dog bite prevention for humans to correctly identify the affective states of dogs.

The majority of the inconsistencies in the use of facial movements to convey affective states across dog breed-types are the result of head and facial feature morphologies that differ from that of wolves and alter the main conveyers of facial expressiveness. Brachycephalic (short length skull) and mesocephalic (medium length skull) heads were found to be accountable for the majority of indistinctiveness seen within the dog confusion matrix (Table 3.6, section 3.4). These head morphologies are proportionally shorter than that of wolves (dolichocephalic heads, long length skulls), and therefore have facial features that are compacted together (Packer et al. 2015). Consequently, the mimic muscles (Figure 2.6, section 2.4.2), in particular those around the muzzle, lips and nose, have much less space to develop to the same size as those seen in dolichocephalic heads. Therefore, some muscles are likely too small to produce the full range of movements needed to produce successful facial expressions. Moreover, movements involving the muzzle, nose, lips and tongue appear important for the successful production of affective facial expressions in wolves as they are often used for different affective states (Table 3.3, section 3.3), but there is a reduction in these movements seen in dogs. For example, wolves use the facial movement ‘nose wrinkler and upper lip raiser’ (AU109+110) 80% of the time in the context of Anger (89 out of 111 events, Table 3.3, section 3.3), but domestic dogs (across breed-types) only use this movement 34% of time in the same context (25 out of 73 events, Table 3.5A, section 3.3.1). Similarly, wolves sniff (AD40) 46% of the time in the context of Interest (40 out of 87 events, Table 3.3, section 3.3), but dogs sniff only 17% of the time in the context of Interest (47 out of 278 events, Table 3.5A, section 3.3.1).

Flopped and (to a degree) semi-flopped ears were also found to contribute to the majority of the indistinctiveness seen within the dog confusion matrix (Table 3.6, section 3.4). Ear movements appear to be important for the production for affective facial expressions in wolves (Table 3.3, section 3.3), but again there is a reduction in the use of ear movements across dog breed-types (Table 3.5A, section 3.3.1). For example, wolves use the movement ‘ears forward’ (EAD101), 30% of the time in the context of Anger (57 out of 190 events, Table 3.3, section 3.3), while dogs use the same ear movement a mere 12% of the time in the context of Anger (28 out of 235 events, Table 3.5A, section 3.3.1). Similarly, wolves use the movement ‘ears adductor’ (EAD102) 31% of the time in the context of Curiosity (15 out of 48 events, Table 3.3, section 3.3), and again dogs only use this movement a mere 12% in the same context (22 out of 191 events, Table 3.5A, secton 3.3.1). Indeed, Waller et al. (2013) reported that only dogs with erect (wolf-like) ears could produce the DogFACS movement ‘ears rotator’

(EAD104). This demonstrates that changes in wolf-like facial feature morphologies can impair the ability of dogs to produce certain facial movements, which is reflected in the reductions of ear movements used by dogs in this research.

Flews (pendulous lips) were also responsible for a large degree of indistinctiveness within the dog confusion matrix (Table 3.6, section 3.4), which is because flews reduce the visibility of some facial movements. For example, Waller et al. (2013) reported that flews reduced the visibility of the facial movement ‘jaw drop’ (AU26), and during this research it was observed that the movement ‘tongue show’ (AD19) was difficult to discern in dogs with flews, which is reflected in the results. For example, jaw drop is used by wolves in the context Anger and Fear, 29% (95 out of 330 events) and 11% (36 out of 330 events) of the time (respectively, Table 3.3, section 3.3), but across dog breed-types this movement is only observed 7% of the time (34 out of 488 events) in the context of Anger, and a mere 2% of the time (8 out of 488 events) in the context of Fear (Table 3.5A, section 3.3.1). Tongue show is used by wolves in the context of Anger 29% of the time (46 out of 157 events, Table 3.3, section 3.3), but dogs used this movement a mere 5% of the time in the same context (13 out of 241 events, Table 3.5A, section 3.3.1).

Some of the diverged facial features investigated in domestic dogs only accounted for a small percentage of indistinctiveness within the dog confusion matrix, such as neutral abnormalities and ectropion (drooping eyelids, Table 3.6, section 3.4). However, very few dogs in this research had neutral abnormalities (*n* = 6), which means their effect on the results when compared to all other dogs in this research is minimal. However, the inclusion of more dogs with neutral facial abnormalities would give a better indication as to whether their facial features impede upon their ability to successfully produce consistent affective facial expressions. Additionally, ectropion causes the constant exposure of the whites of the eyes (sclera), and the DogFACS system uses the exposure of the sclera to determine eye movements. Eye movements were not analysed in this research, however, had they been analysed, then this would give a better indication as to whether ectropion has a greater effect upon the successful production of affective facial expressions in domestic dogs. That being said, the results do show that in the context of Friendly and Joy, dogs (across breed-types) expose their sclera (WHITES) more than wolves (Table 3.3, section 3.3, and Table 3.5A, section 3.3.1), which suggests ectropion does indeed impede the production of affective facial expressions in domestic dogs.

It is evidenced here that different head and facial feature morphologies of dog breed-types can impair their ability to produce affective facial expressions like their wolf ancestors.

However, it must be noted that other factors may have contributed to the lack of ability of domestic dogs to convey affective states via facial expressions. For example, it has been shown that kennel environments can impact on the behaviour of domestic dogs (Kogan et al. 2012; Taylor & Mills 2007; Wells 2004), in particularly kennel environments that lack housing of dogs in groups, have a lack of dog-human contact and a lack of enrichment (such as toys, Taylor & Mills 2007; Wells 2004). However, most of the dogs used in this thesis research were housed together in groups, they were provided with regular human contact (in preparation for adoption), and they were provided with enrichment in the form of toys as per Dogs Trust policies. Nevertheless, a kennel environment can never fully mimic a human home environment that domestic dogs generally live in. Therefore, a comparison of kennel and home environments should be considered in future work.

Some of the dogs used in this research were less than 12 months of age (*n* = 3), and although they were considered to be adults physically (with fully formed facial musculature) at the time of data collection, they may not have behaved like adults socially. It was observed that dogs younger than 12 months of age would engage in play more often those dogs over the age of 12 months. It has been suggested that play among canids is an important social activity used to learn and gain social skills (Bekoff 2001; Cordoni 2009), which suggests that those dogs younger than 12 months were still learning and gaining new social skills. If these dogs did indeed lack social skills then this may have affected their abilities to produce appropriate facial signalling, however these dogs were few and so their effects on the results are minimal. Nevertheless, future work should consider using dogs no younger than 12 months of age, and preferably those who have been well-socialised with other dogs.

The past histories of the dogs used in this research are unknown, which may result in atypical behaviour of the dogs used. For example, the ontogenetic process of enculturation of the dogs used may have resulted in some dogs learning atypical social signalling (Appleby et al. 2002; Hare et al. 2002; Serpell & Jaoge 1995). Therefore, knowing the past histories of the dogs used would be beneficial to help explain unusual findings or indeed, allow one to select dogs that are well-versed in their social abilities and who display typical behaviours. Raising domestic dogs from puppies to adults would allow their past histories to be fully studied and documented and this should be considered for future work.

4.2.1: Dog domestication and communicative compensation.

Although the majority of indistinctiveness within the dog confusion matrix may be due to facial morphological divergences from wolf ancestry, the results still found that wolf-like morphologies (dolichocephalic heads and erect ears) produced a small amount of indistinctiveness (Table 3.6, section 3.4). In addition, the affective states Anger, Friendly and Joy consist of a wide diversity of facial movements. In fact, Joy consists of all facial movements, except ‘licking’ (AD119, Table 3.5A, section 3.3.1). Moreover, no differences in social status, sex, or age were found to influence the use of facial movements in the context of Anger, Friendly or Joy. The cause of the indistinctiveness observed in wolf-like dogs and the excessive use of facial movements are most likely the result of domestication.

The process of domestication has resulted in many non-human animals, such as pigs, goats, horses, cats and dogs developing specific human-directed social skills (Gaunet 2008; Langbein et al. 2018; McKinley & Sambrook 2000; Miklosi et al. 2003). But, not only are domestic dogs more skilled at understanding human social behaviour than our closest living non-human primate relatives (Hare & Tomasello 2005). In fact, Marshall-Pescini et al. (2017) showed that dogs do not cooperate with their own conspecifics (unlike wolves, which do cooperate with conspecifics), instead, dogs are far more human orientated in cooperation. Moreover, despite the facial morphological limitations that hinder the ability of dogs to produce affective facial expressions, this research showed that humans (specifically the independent classifiers, section 2.4.3) were still able to classify the affective states of dogs with substantial and near perfect agreement (according to the Kappa statistic, Table 2.5, section 2.4.3). This suggests that domestic dogs are using another method to communicate with humans.

Dogs were found to use vocalisations more than twice as much as wolves, and commonly used bark vocalisations in the context of Anger, Friendly and Joy (Table 3.7B, section 3.4). Barking was observed to produce many facial movements, which suggests that the excessive diversity of facial movements seen in the context of Anger, Friendly and Joy is a by-product of barking. Moreover, an increase in the use of vocalisations was found to occur with dogs that were present within the incorrectly predicted affective states of the dog confusion matrix (those creating the indistinctiveness). Subsequently, suggesting that dogs may be compensating for their lack of ability to produce affective facial expressions by using vocalisations. Yeon (2007) demonstrated that dogs and humans are able to communicate successfully with each other via the use of vocalisations, and also showed that humans are capable of distinguishing variances between different bark vocalisations. If humans can distinguish between different barks this offers an explanation as to why dogs were found to bark in nearly every context they are presented with (Table 3.7B, section 3.4). Other vocalisations found in this research agree with those found in Yeon's (2007) study and are used in similar context to convey similar affective states, which suggests that vocalisations may be more important for domestic dog communication than facial communication.

Dogs were once bred mainly for working purposes, to conduct tasks at a distance away from their human handlers, such as herding and guarding (Coppinger & Schneider 1995; Svartberg 2006). Working at a distance from a human handler means the use of facial expressions for primary communication is disadvantageous, instead vocalisations become more advantageous. Indeed, many herding and guard dogs are bred for their ability to bark, to drive livestock and ward off intruders, respectively, and it was once thought this barking was just a product of selective breeding and served no functional purpose (Yin 2002). However, more recently it has been suggested that barking is indeed a complex form of communication that has evolved due to thousands of years of co-existence between humans and dogs (Langbein et al. 2018; Yeon 2007; Yin & McCowan 2004). This thesis research supports this suggestion because dogs excessively use vocalisations (in comparison to wolves) and are still capable of conveying affective states (and humans are still capable of discerning them) despite their lack of ability to produce affective facial expressions. Therefore, one might not expect there to be any communication breakdown between dogs and humans. However, today dogs are mostly kept as pets and not used for work. This means that dogs are usually within close proximity to their human handlers, therefore the use of facial expressions for communication is likely more important once again. Humans are naturally drawn to faces and they use facial expressions as one of their main means of communication (Essa & Pentland 1997; Martinez & Du 2012; Sebe et al. 2007). In fact, humans who cannot communicate using facial expressions find it difficult to build and maintain social bonds (Boahene 2013; Coulson et al. 2004). Therefore, if non-wolf-like dog breeds are unable to produce appropriate facial expressions to convey affective states, then this may lead to weaker human-dog social bonding.

Although dogs do not cooperate with conspecifics, and their human-directed behaviour has a genetic basis (Marshall-Pescini et al. 2017; Perrson et al. 2015), they still seem to possess the ability to perceive affective facial expressions in other dogs. Domestic dogs have the ability to gaze follow (Albuquerque et al. 2015; Miklosi et al. 2003; Somppi et al. 2014), and demonstrate pronounced gaze durations at conspecific faces, and will often gaze at, and become fixated upon 'informative regions' of the face such as the eyes, ears, forehead, muzzle, nose and lips (the main conveyers of facial expressiveness, Figure 4.5, Somppi et al. 2012). This implies that dogs search the faces of conspecifics for information about their affective states. Gaze following of conspecifics also exists in wolves (Range & Viranyi 2011), which suggests that the ability of domestic dogs to gaze follow and hone in on the main conveyers of facial expressiveness, is an ancestral trait. Moreover, this thesis research found that wolves and dogs use some of the same facial movements for the same affective states (Table 3.5B, section 3.3.1). This suggests that despite the varying facial morphologies of dogs that hinder their ability to produce the same affective facial expressions as wolves, they still attempt to produce them, again implying an ancestral trait. These ancestral traits suggest that domestic dogs can still perceive affective states from conspecific facial expressions, but selective breeding has hindered their ability to appropriately convey affective states via facial expressions. This means a potential communication breakdown among dog breed-types exists, and perhaps this contributes to the lack of conspecific cooperation seen among dogs.

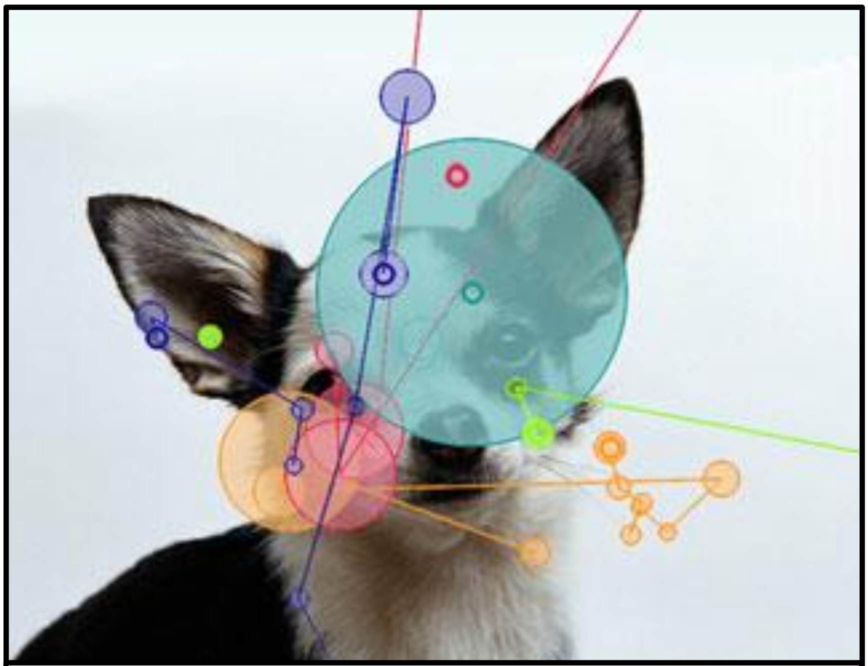


Figure 4.5. A ‘wolf-like’ dog face showing the scan paths of gaze durations by five individual dogs, represented by different colours. Lines trace the paths that the dogs’ eyes travelled across the image and circles represent fixed points of gaze (fixation). Larger circles indicate longer periods of fixation on corresponding ‘informative regions’. Adapted from Somppi et al. (2012).

4.3: Wolf communication and the Social Intelligence Hypothesis.

Several typical facial expressions (with social status differences) corresponding to the nine affective states investigated in this research have been identified in wolves (Table 4.1). Each of these facial expressions have accompanying vocalisations, which agree with the current literature (Table 4.1, Mech & Boitani 2003). The facial expressions identified illustrate how the (aforementioned) unique combinations of facial movements are used to convey each of the affective states. However, what is more intriguing is that some of these facial expressions are comparable to those seen in primates (Table 4.1), which provides insights into the complexity of wolf social communication.

Complex forms of communication have evolved in response to increased pressures to cope with complex social systems (Dunbar 2009; Freeberg et al. 2012). Complex social systems are defined as those where individuals within a social group frequently interact across varying contexts with many different individuals, and who often repeatedly interact with the same individuals within established networks, over time (Freeberg et al. 2012). Indeed, the social complexity hypothesis suggests that animals living within such complex social systems require complex communication to successfully regulate the abundance of interactions that occur among group members (Freeberg et al. 2012), which typically maintains social stability (Freeberg et al. 2012). Wolves have the most highly developed social system of all canids (Sheldon 1992), and as a result they are highly skilled in conspecific cooperation (Range & Viranyi 2014a, 2014b), which is only possible due to their ability to communicate with each other (Mech & Boitani 2003; Range & Viranyi 2014b). The ability of wolves to cooperate and communicate offers an explanation as to why wolves can produce the affective facial expressions seen in this research.

Many non-human animals that live in large, stable social groups have evolved complex ways in which they communicate with group members (Bradbury & Vehrencamp 1998; Marler 1976; Morris-Drake & Mumby 2018; Owens & Owens 1978; Theis et al. 2007), which include intricacy within singular modes of communication (such as grading and blending of facial expressions, Parr et al. 2005a), and the use of multimodal communication (such as the use of vocalisations with facial expressions, Hauser & Akre 2001; Russell et al. 2003) to emphasise the information being conveyed. At present, north American wolves live in packs averaging seven individuals (Mech & Boitani 2003; Mech 2018 Pers. Comm.), though in the past, they have been documented to live in packs of 42 individuals (Mech & Boitani 2003). This range of seven to 42 individuals implies a range of flexibility in the social organisation of wolf packs, but nonetheless, larger packs would increase social complexity (due to increased numbers of individuals, Dunbar 2009; Freeberg et al. 2012), therefore, it is probable that wolf societies were once more complex than they appear to be today. Therefore, the evolution of complex forms of communication were selected for, to cope with added social interactions. Consequently, wolves evolved a wide range of affective facial expressions, many of which are comparable to those of primates (Table 4.1).

Table 4.1. Typical affective facial expressions found in wolves including social status differences (for 'alpha' α, and 'omega' Ω wolves) with their typical accompanying vocalisations observed during data collection. Contextualised primate facial expressions are also included for comparative purposes to that of wolves.

Affective state	**Typical facial expression**	**Observed vocalisations**	**Contextualised primate facial expressions**
Anger	Eyes are wide open with inner-brows raised. Ears are up. Nose is wrinkled, upper lips raised, and lower lips depressed; teeth are bared. Jaw snapping may occur. Only α-wolves will have their ears forward, will pucker their lips, and will vocalise. Only Ω-wolves will perform head tilts and may mouth/chew at the muzzle of the dominant wolf (which can involve other lip movements and teeth baring).	Growl	Threat: Staring bared-teeth scream face* (typical facial expression comparison). Threat: Tense-mouth face (α-wolf comparison)*. Defensive threat: Frowning bared-teeth scream face (Ω-wolf comparison)*. Defence: Teeth-chattering face (jaw snap comparison)*. Defence: Chewing-smacking face (mouthing/chewing comparison)*.
Anxious	Air is sucked in through the lips creating cheek depressions. Sniffing occurs.	Whimper	No comparable facial expression.
Curiosity	Ears are up. Left and right head tilting occurs. Sniffing occurs.	None	Exploration: 'Head-cocking' in the common marmoset (Kaplan & Rogers 2006).
Fear	Eyes are wide open with inner-brows raised. Whites (sclera) of the eyes are observed. Mouth may partially open (lips may part, and slight jaw drop may occur). Nose licking occurs. Ears are back flat against the head. Head is often down/lowered.	None	Fear: Fear face in humans (Ekman & Friesen 2003; Jack et al. 2009)
Friendly	Eyes may be wide with inner-brows raised. Repeated blinking and eye closure occurs. Submissive grin is performed (lip corners are pulled back, lips may part, but jaws remain closed). Sometimes the submissive grin can be elaborate with upper lips raised, lower lips depressed, and teeth shown with closed jaws. Tongue shows and tongue flicking occurs. Only α-wolves will lip pucker. Only Ω-wolves will have their ears back, flat against the head, will head tilt, vocalise, and may wrinkle their noses and raise their upper lips to bare teeth (usually in accordance with elaborate submissive grins)	Whine	Appeasement: Silent bared-teeth face (submissive grin comparison)*. Approach: Lip-smacking face and Tongue-smacking face (tongue flicking comparison)*. Appeasement: Human smile face (Van Hooff 1972).
Happy	Repeated eye blinking and eye closure occurs. Whites (sclera) of eyes may be seen. Head is raised up. Tongue show may occur. Facial expression can appear almost neutral as movements are subtle.	None	No comparable facial expression.
Interest	Ears are up and forward. Head often bobs up and down. Sniffing occurs.	None	No comparable facial expression.
Joy	Ears are forward. Lip corners are pulled back combined with relaxed, obvious jaw drop. Mouthing/chewing anywhere on social interactant may occur.	None	Play: Relaxed open-mouth face*.
Surprise	Eyes are wide and inner-brows raised. Ears are up and forward. Mouth appears tight.	None	Surprise: Surprise face in humans (Ekman & Friesen 2003).

*As observed in catarrhine monkeys and apes in Fox's (1970) and Van Hooff's (1967) studies.

The social intelligence hypothesis (SIH) was originally proposed as an explanation for increased encephalisation (larger brain to relative body size) and social intelligence in primates compared to all other vertebrates, in response to increased social complexity (Dunbar 2009; Finarelli and Flynn 2009; Freeberg et al. 2012; Perez et al. 2007). However, the SIH is also applicable to other mammalian species that live in complex social structures such as Elephantidae, Cetacea (with increased encephalisation occurring more in Odontoceti, Montgomery et al. 2013), and Hyaenidae (Holekamp et al. 2007; Montgomery et al. 2013; Roth and Dicke 2005). However, increased encephalisation does not always correlate with increased sociality within Carnivora (as it does within Simian primates, Gittleman 1986). This is due to factors such as diet and foraging strategies, and mating systems. Carnivorous pursuit hunters (such as wolves) and omnivores have larger brains than insectivores (Gittleman 1986), and species with pair-bonded mating systems (such as wolves) also have larger brains than species without pair-bonded mating systems (Dunbar 2009). Nonetheless, many group-living carnivores and simian primates have brains that are structured along similar lines of social-cognition, and therefore have similar social communicative abilities (Dunbar and Bever 1998). These group-living comparisons between carnivores and simian primates are reflected in this research in the production of similar affective facial expressions seen in wolves and primates (Table 4.1).

The SIH does remain much contested and difficult to evaluate, however it is based upon the size of the neocortex region of the brain (not total brain size, Joffe and Dunbar 1997). The neocortex is part of the cerebral cortex of the brain and is responsible for sensory perception (Ghazanfar & Schroeder 2006; Pritchett et al. 2015), the generation of motor commands (Dudman & Krakauer 2016), spatial reasoning (Hampson et al. 1998) and consciousness (Evans 2003; Vanderwolf 2000). Essentially the neocortex is the ‘communication centre’ of the brain in higher mammals, and therefore, responsible for complex forms of communication (Darvas et al. 2009; Marino et al. 2007). It has been suggested here that wolves are capable of living in large societies, and it has been shown that they are capable of complex communication, similar to that seen in primates. Moreover, the size of the neocortex of the wolf is larger than that of most other carnivores and is the largest of all canids (84973 mm^3, Table 4.2). Therefore, it is argued here, that the SIH should be applicable to wolves too.

Table 4.2. Overall brain and neocortex size of carnivores (Dunbar & Bever 1998). Canid species are highlighted in grey.

Species	Brain volume (mm^3)	Neocortex volume (mm^3)	Species	Brain volume (mm^3)	Neocortex volume (mm^3)
Alopex lagopus	31742	17769	*Martes pennanti*	2686	18352
Canis adustus	47416	27640	*Meles meles*	6779	33705
Canis aureus	60708	36281	*Mephitis mephitis*	10998	5532
Canis latrans	85976	53217	*Mustela erminea*	4450	2043
Canis lupus	131519	84973	*Mustela nigripes*	7433	3594
Canis mesomelas	53109	31314	*Mustela nivalis*	1810	759
Chrysocyon brachyurus	113391	72173	*Mustela putorius*	8796	4326
Conepatus chinga	17843	9404	*Mustela vison*	9140	4512
Conepatus humboldti	11675	5909	*Nasua rufa*	37625	21427
Crocuta crocuta	160919	106105	*Nyc tereutes procyonoides*	28863	16004
Cuon alpinus	93900	58641	*Otocyon megalotis*	26909	14815
Eira barbara	45662	26517	*Panthera leo*	215021	145983
Galictis cuja	17919	9469	*Potos flavus*	29340	16295
Galictis vittatus	17347	9137	*Procyon lotor*	40943	23516
Helictis personata	14296	7384	*Proteles cristatus*	34650	19570
Hyaena brunnea	122040	78256	*Pieronura brasiliensis*	116080	74060
Hyaena hyaena	84889	52477	*Taxidea taxus*	59878	35736
Lutra canadensis	56799	33718	*Urocyon cineroargenteus*	5556	20134
Lutra lutra	44956	26066	*Vulpes ruppelli*	26689	14682
Lycaon pictus	128381	82744	*Vulpes velox*	31742	17769
Martes foina	20760	11133	*Vulpes vulpes*	48332	28229
Martes martes	16727	8778			

4.4: Future directions and conclusions.

This thesis has shown that humans are good at discerning the affective states of wolves and domestic dogs with substantial and near perfect agreement (section 2.4.3). However, one can never truly know the exact affective state of a non-human animal. Therefore, the application of other methods to collect physiological and neurological measures associated with varying social context would provide more evidence for the affective states classified here. Additional measures of affective states would also provide new insights into their intensities. In addition, although ERH is a certified DogFACS coder (meaning all coding of facial movements within this research are standardised) the wide range of facial movements quantified in this thesis for both wolves and domestic dogs were not checked for reliability such as that seen in Bennett et al. (2017) and Caeiro et al. (2017). Checking for the reliability of DogFACS coding should be conducted in future work to ensure standardisation is maintained. Furthermore, it should be noted that many of the results presented in this thesis are point estimates that lack accompanying estimates of uncertainty due to the difficulty in providing such information. However, future work could perhaps consider the use of non-parametric bootstrapping to provide a stronger sense of how these point estimates could vary in their uncertainty.

The measurement of heart rate variability (HRV) can be used to evaluate affective states in non-human animals (Appelhans & Luecken 2006; Borell et al. 2007). HRV can be assessed using non-invasive, externally-mounted transmitters such as Polar© heart rate monitors and telemetric or static electrocardiogram systems (Borell et al. 2007). Such transmitters have been used to measure the HRV (via inter-beat intervals) in a range of domesticated animals, such as pigs, cows, horses, sheep and goats (Borell et al. 2007). Dogs can be easily habituated to wear non-invasive, mounted heart rate monitors (McGowan et al. 2014), which means it is plausible to mount HRV transmitters to dogs and perhaps even to habituated captive wolves. Mounting such transmitters would provide greater insights into the changes of positive and negative ‘emotional’ responses in both wolves and dogs when subjected to varying social interactions and emotive stimuli (such as those used in this research). For example, a negative social interaction should result in a lower HRV in the focal canid due to stress (Schwerdtfeger & Friedrich-Mai 2009; Wu & Lee 2009; Zebunke et al. 2011). In addition, breathing rates could be measured via observations during varying social interactions and reactions to stimuli, to provide more evidence for changes in affective states. For example, it was observed during this research that wolves and dogs would momentarily hold their breath when surprised and would increase their breathing rate (pant) when fearful.

Functional magnetic resonance imaging (fMRI) is used to detect changes in activity in different regions of the brain (Andics et al. 2014; Cox & Savoy 2003; Sato et al. 2004; Weiskopf et al. 2003). Sato et al. (2004) used fMRI to study regional activity of the human brain in response to facial expressions of Happy and Fear. Sat et al. (2004) found that the amygdala was highly activated upon viewing facial expressions of Fear but, was not active when viewing facial expressions of Happy. Andics et al. (2014) trained dogs to enter fMRI scanners, to detect their brain activity in response to familiar vocalisations. Therefore, the methods used by Andics et al. (2014) could be applied again to dogs and perhaps habituated, trained wolves to measure brain activity in response to viewing video footage of wolf and dog affective facial expressions. Differences and changes in regional brain activity, in response to different affective facial expressions would provide evidence for changes in the affective states of focal canids (Anders et al. 2004; Kesler et al. 2001; Phan et al. 2002; Sato et al. 2004; Ueda et al. 2003; Vuilleumier et al. 2001) and may even offer insights into the intensities of responsive affective states, depending upon the level of activity detected (Phan et al. 2004; Yoon et al. 2007). Moreover, fMRI scanning has revealed that the activity of the brain responsible for facial processing in humans and chimpanzees (*P. trogoldytes*) are similar. Therefore, fMRI scanning could reveal if wolves and dogs share similarities in facial processing, and perhaps even reveal if wolves share similarities with primates in facial processing, in addition to sharing similarities in the production of facial expressions.

Finally, primates have a great diversity of facial patterning that they use for communication (Santana et al 2012), and the emphasis of facial expressions (Burrows 2008; Santana et al. 2012, 2013). It has been suggested that the conspicuous white coloured muzzle area of wolves enhances the perceptibility of their black lips when producing facial expressions (Fox 1970). It has also been suggested that the lighter coloured pelage patterning around the eyes of wolves serves a functional purpose related to their gaze communication with conspecifics (Ueda et al. 2014). However, there remains very little evidence to support these suggestions.

This thesis research used wolves with pure white faces (arctic wolves, Figure 2.1, section 2.2.1) and wolves that had distinct facial patterning (grey wolves, Figure 2.1, section 2.2.1). During this research it was observed that the lips of the arctic wolves were far easier to observe and detect movements, than those of the grey wolves. Similarly, the ‘eyebrows’ (supraorbital vibrissae area emphasised by paler contrasting fur, forming small bilateral structures) of the grey wolves were far easier to observe and detect movements, than those of the arctic wolves. The facial patterning of primates can be influenced by both ecological selective pressures and social communicative pressures (Santana et al 2012). Grey wolves tend to inhabit densely vegetated areas, while arctic wolves tend to inhabit wide open, snow covered areas. It could be argued that grey wolves have distinct facial patterning to aid the emphasis of facial expressions to ensure successful communication in 'darker' environments. In contrast, arctic wolves have lost their facial patterning in favour of camouflage to ensure successful hunting. The eye colour of wolves tends to range between orange and yellow, and arctic wolves (in comparison to grey wolves) have very bright yellow eyes (Pers. Obs.). Perhaps, arctic wolves evolved brighter irises (in response to losing their 'eyebrows') to emphasise pupil movement, and therefore allow their pack members to gaze follow them more efficiently (Ueda et al. 2014), thus maintaining successful communication. However, this is mere speculation of the functional purposes of the facial patterning, and iris colouration seen in wolves. Though, methods that allow the detection of reflective properties of facial patterns (as described by Stevens et al. 2007) and changes in the shapes of these patterned areas upon signalling (Allen & Higham 2013) may provide better insights into the true function of these facial patterns.

4.4.1: Conclusions.

In conclusion, this research has shown that wolves are capable of conveying a wide range of affective facial expressions, some of which are comparable to those seen in simian primates. It has also been shown that wolves are potentially capable of producing secondary and tertiary affective states via the use of graded and blended facial expressions, Moreover, it is argued that the great complexity described in wolf societies and communication warrants the application of the social intelligence hypothesis.

By contrast, domestic dogs are limited in their range of affective facial expressions, which owes to variations in their head and facial feature morphologies, produced by artificial selection. Dogs still appear to possess some ancestral (wolf) traits with regards to the perception of affective facial expressions, but many dogs have lost their ability to convey affective facial expressions. Yet, in response to thousands of years of co-existence with humans, dogs have developed a compensatory way to communicate via the use of vocalisations.

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Appendix A.

Dog ID	Age (years)	Sex	Breed	Facial fur length	Facial colour pattern	Ear position	Head shape	Flews?	Ectropion?	Neutral facial abnormalities
001M	Unknown	Male	Jack Russel Terrier	Smooth	Tan with some grey around end of muzzle.	Semi-flopped	Dolichocephalic	No	No	None
002F	Unknown	Female	Terrier X	Wire-haired	Brindle with white muzzle and white stripe from forehead down to nose.	Semi-flopped	Dolichocephalic	No	No	None
005M	Unknown	Male	Jack Russel Terrier	Smooth	Black ears and white face with black spots.	Semi-flopped	Dolichocephalic	No	No	None
006M	Unknown	Male	Chihuahua X	Smooth	Tan with some grey around end of muzzle.	Semi-flopped	Mesocephalic	No	No	None
ALFIE1	Unknown	Male	Shih Tzu	Long	Black with white around muzzle.	Flopped	Brachycephalic	No	No	None
ALFIE2	1	Male	English Cocker Spaniel	Short on face, medium on ears.	Ginger with white around muzzle and white stripe from forehead down to nose.	Flopped	Mesocephalic	Yes - light	No	None
BEAR1	3	Male	English Springer Spaniel	Short on face, medium on ears.	Black with some white around muzzle and white stripe from forehead down to nose.	Flopped	Mesocephalic	Yes - light	No	None
BEN1	3	Male	Greyhound	Smooth	Black with some white around muzzle and white stripe from forehead down to nose.	Semi-flopped	Dolichocephalic	No	No	None
BLUE1	Unknown	Male	Saluki	Short on face, long on ears.	Grey with some white around muzzle and white stripe from forehead down to nose.	Flopped	Dolichocephalic	No	No	None
BOB1	5	Male	German Pointer		Brown with some white around muzzle and white stripe from forehead down to nose.	Flopped	Mesocephalic	Yes - heavy	No	None
BOBO1	3	Female	Labrador Retriever	Short	Black.	Flopped	Mesocephalic	Yes - light	No	None
BOO1	1	Female	Staffordshire Bull Terrier	Smooth	White, brown on rightside with some black markings, right ear	Semi-flopped	Brachycephalic	No	No	None

					brown, left ear white with black tip.					
BRUNO1	5	Male	Staffordshire Bull Terrier	Smooth	Brown with some grey around end of muzzle.	Semi-flopped	Brachycephalic	No	No	None
COPPER1	11	Male	Staffordshire Bull Terrier X	Smooth	Brindle with grey around muzzle.	Flopped	Mesocephalic	Yes - light	No	None
DAISY1	2	Female	Siberian Husky	Short	Grey forehead and ears (white inside ears), white eyebrow, muzzle and cheek area (wolf-like facial patterning).	Erect	Mesocephalic	No	No	None
DEXTER1	Unknown	Male	Boxer Dog	Smooth	Brown with white muzzle and thick white stripe from forehead to nose, black around eyes doen to cheeak area, black ears.	Flopped	Brachycephalic	Yes - heavy	Yes	WHITES constantly visible due to ectropion. AU25 and AU116 constantly visible.
DOGGIE1	3	Male	Jack Russel Terrier X	Smooth	Tan with white around end of muzzle.	Erect	Mesocephalic	No	No	None
DUNSTON1	Unknown	Male	Lurcher	Wire-haired	Black and white patches, mostly black on right, mostly white on left.	Semi-flopped	Dolichocephalic	No	No	None
EDW1	0.92 (11 months)	Female	Lurcher	Smooth	Light brindle.	Semi-flopped	Dolichocephalic	No	No	None
FINN1	8	Male	Boxer Dog	Smooth	Tan with white muzzle and thick white stripe from forehead to nose, black around eyes down to cheek area, black ears.	Flopped	Brachycephalic	Yes - heavy	Yes	WHITES constantly visible due to ectropion. AU25 and AU116 constantly visible.
FINN2	1	Male	X-breed	Smooth	Black, with thin verticle white stripe on top of muzzle.	Semi-flopped	Brachycephalic	Yes - heavy	No	None
FIZZ1	1	Female	Border Collie	Short	Black, some white around muzzle and some on cheek areas.	Erect	Mesocephalic	No	No	None

HARRY1	1	Male	Whippet	Smooth	Black, some white around end of muzzle.	Semi-flopped	Dolichocephalic	No	No	None
HARVEY1	0.92 (11 months)	Male	Basset Hound	Smooth	Thick white stripe from forehead to nose, white muzzle, black around eyes with distinct tan eyebrows, tan cheek areas, black ears with some tan.	Flopped	Dolichocephalic	Yes - heavy	Yes	None
HECTOR1	10	Male	Border Collie	Short	Black with white muzzle and thick white stripe from forehead down to nose.	Semi-flopped	Mesocephalic	No	No	None
INDIE1	0	Male	Labrador Retriever	Smooth	Yellow.	Flopped	Mesocephalic	Yes - light	No	None
JACK1	3	Male	Border Collie	Short	Mostly black, some white around end of muzzle, tan cheek areas and distinct tan eyebrows.	Semi-flopped	Mesocephalic	No	No	None
JOE1	2	Male	Greyhound	Smooth	Thick white stripe from forehead to nose, white muzzle, tan forehead, tan around eyes and tan ears.	Semi-flopped	Dolichocephalic	No	No	None
JONAS1	4	Male	Lurcher	Smooth	Black some grey around end of muzzle.	Smei-flopped	Dolichocephalic	No	No	None
KEONE1	1	Male	Whippet	Smooth	Black with white around muzzle and white stripe from forehead to nose.	Semi-flopped	Dolichocephalic	No	No	None
KIERA1	5	Female	Labrador Retriever	Smooth	Black.	Flopped	Mesocephalic	Yes - light	No	None
LEXI1	1	Female	X-breed	Smooth	Dark brindle with white around muzzle and white stripe from forehead to nose.	Flopped	Mesocephalic	Yes - light	No	None
LILY2	Unknown	Female	German Shepherd Dog X	Smooth	Black and tan, black muzzle and ears, some black around eyes with visible black eyebrows.	Erect	Dolichocephalic	No	No	None

LOTTIE1	5	Female	American Bull Dog	Smooth	White with black spots on ears.	Flopped	Brachycephalic	yes	yes	WHITES constantly visible due to ectropion. AU25 and AU116 constantly visible.
LUCY1	5	Female	Jack Russel Terrier	Smooth	Black with some white on cheeak area and end of muzzle, thin white stripe from forehead to nose, some tan on ear edges.	Semi-flopped	Mesocephalic	No	No	None
MARSHAL1	Unknown	Male	Boxer Dog	Smooth	White some black spots along top lip.	Flopped	Brachycephalic	Yes - heavy	No	None
MAVI1	2	Male	Jack Russel Terrier	Smooth	Black ears and forehead, with black stripe from forehead to nose. Tan around eyes and cheek areas. Some white at end of muzzle (mostly on rightside).	Flopped	Mesocephalic	No	No	None
MAX1	Unknown	Male	Border Collie	Short	Black with white around muzzle and white stripe from forehead to nose.	Erect	Mesocephalic	No	No	None
MAX2	3	Male	Labrador Retriever	Smooth	Yellow	Flopped	Mesocephalic	Yes - light	No	None
MIA1	4	Female	German Shepherd Dog X Staffordshire Bull Terrier	Smooth	Black and tan, black forehead and ears, some black around muzzle and lips, black eyebrows.	Erect	Mesocephalic	No	No	None
MIA2	1	Female	German Shepherd Dog X Siberian Husky	Short	Golden face, dark tan on ears.	Erect	Dolichocephalic	No	No	None
MILO1	4	Male	Lurcher	Long	Golden	Flropped	Dolichocephalic	No	No	None
MILO2	5	Male	German Shepherd Dog	Short	Black, some tan in places.	Erect	Dolichocephalic	No	No	None
MINDY1	Unknown	Female	Labrador Retriever	Smooth	Black	Flopped	Mesocephalic	Yes - light	No	None
MOHA1	2	Female	Jack Russel Terrier	Smooth	Tan with some white around end of muzzle.	Erect	Mesocephalic	No	No	None

MORGAN1	10	Male	English Cocker Spaniel	Short, medium on ears	Ginger with thin white stripe from forehead to nose.	Flopped	Mesocephalic	Yes-light	No	None
MORK1	4	Male	Lurcher	Smooth	Tan with black eyebrows.	Semi-flopped	Dolichocephalic	No	No	None
NORMAN1	1	Male	Lhasa Apso	Long	Grey.	Flopped	Brachycephalic	No	No	AU116, AU25 constantly visible. AD71 often visible due to long fur.
OBI1	Unknown	Male	Daschund	Short on face, long on ears.	Black with tan muzzle (some grey at very end) and cheek areas, tan eyebrows.	Flopped	Dolichocephalic	No	No	None
PICH1	5	Male	Labrador Retriever X	Smooth	Black.	Semi-flopped	Mesocephalic	No	No	Half of right ear missing.
POPPY1	1	Female	Staffordshire Bull Terrirer	Smooth	Black with white muzzle and white stripe from forehead to nose.	Semi-flopped	Brachycephalic	No	No	None
RANG1	4	Male	German Shepherd Dog	Short	Black and tan, black on ears, forehead and muzzle.	Erect	Dolichocephalic	No	No	None
REXIE1	5	Female	Labrador Retriever	Short	Black.	Flopped	Mesocephalic	Yes - light	No	None
ROCCO1	4	Male	Akita X Rottweiler	Smooth	Black with tan eyebrows and some tan on cheek areas.	Erect	Brachycephalic	Yes - light	No	None
SALLY1	Unknown	Female	German Shepherd Dog X	Short	Tan with black ears, black on end of muzzle (with some grey), black eyebrows.	Erect	Dolichocephalic	No	No	None
SAM1	1	Male	Border Collie X	Short on face, medium on ears and cheek areas.	Ginger with white on muzzle, golden eyebrows.	Semi-flopped	Mesocephalic	No	No	None
SULLY1	Unknown	Male	Lurcher	Smooth	Black with thick white stripe from forehead down to nose, white end of muzzle.	Semi-flopped	Dolichocephalic	No	No	None
SUZIE1	5	Female	Staffordshire Bull Terrier	Smooth	Brown with some grey around end of muzzle.	Semi-flopped	Brachycephalic	No	No	None

TEDDY1	2	Male	Akita	Short on face, medium around ears and sides of head.	Black with grey at ends of fur on side of head.	Erect	Mesocephalic	No	No	None
TEDDY2	0.75 (9 months)	Male	Japanese Chin	Medium	Black, white on muzzle (mostly on rightside).	Flopped	Brachycephalic	No	No	None
TYLER1	5	Male	Staffordshire Bull Terrier	Smooth	White.	Semi-flopped	Brachycephalic	No	No	None
TYSON1	Unknown	Male	Boxer Dog X	Smooth	Black with some white around end of muzzle, thin white stripe from forehead to nose.	Semi-flopped	Mesocephalic	Yes - light	No	None
ULANI1	1	Female	Beagle	Smooth	Tan with white around muzzle and white strip from forehead to nose.	Flopped	Mesocephalic	Yes - light	No	None
VERA1	6	Female	Boxer Dog X Labrador Retreiver	Smooth	Black with some grey around end of muzzle.	Flopped	Mesocephalic	Yes - heavy	No	None

Appendix B.

Table B1. 18 sample video clips used for inter-rater reliability of affective states.

Video	**Affective state**	**Canid species**	**Focal canid**
1	Anger	*Canis lupus*	TALA
2	Anger	*Canis lupus familiaris*	MILO2
3	Anxious	*Canis lupus*	MAI
4	Anxious	*Canis lupus familiaris*	SULLY1
5	Curiosity	*Canis lupus*	TALA
6	Curiosity	*Canis lupus familiaris*	SAM1
7	Fear	*Canis lupus*	MOTOMO
8	Fear	*Canis lupus familiaris*	LUCY1
9	Friendly	*Canis lupus arctos*	SIKKO
10	Friendly	*Canis lupus familiaris*	BOO1
11	Happy	*Canis lupus*	MAI
12	Happy	*Canis lupus familiaris*	FINN1
13	Interest	*Canis lupus*	NUKA
14	Interest	*Canis lupus familiaris*	ULANI1
15	Joy	*Canis lupus*	MOTOMO
16	Joy	*Canis lupus familiaris*	LOTTIE1
17	Surprise	*Canis lupus*	NUKA
18	Surprise	*Canis lupus familiaris*	DOGGIE1

Corresponding video footage for appendix B found at: doi:10.15128/r16q182k15c

Table B2. Independent observer classifications of individual affective states used for inter-rater reliability, observed within the 18 sample videos in Table B1.

Video	Affective states classified by ERH	Affective states classified by independent observers (n = 8)										Number of matching classifications
		Anger	Anxious	Curiosity	Fear	Friendly	Happy	Interest	Joy	Surprise	Unknown	
1	Anger	7	1	0	0	0	0	0	0	0	0	7
2	Anger	8	0	0	0	0	0	0	0	0	0	8
3	Anxious	0	8	0	0	0	0	0	0	0	0	8
4	Anxious	0	8	0	0	0	0	0	0	0	0	8
5	Curiosity	0	0	8	0	0	0	0	0	0	0	8
6	Curiosity	0	0	8	0	0	0	0	0	0	0	8
7	Fear	0	3	0	5	0	0	0	0	0	0	5
8	Fear	0	1	0	7	0	0	0	0	0	0	7
9	Friendly	0	0	0	0	6	0	2	0	0	0	6
10	Friendly	0	0	0	0	5	1	1	1	0	0	5
11	Happy	0	0	0	0	3	5	0	0	0	0	5
12	Happy	0	0	0	0	0	8	0	0	0	0	8
13	Interest	0	0	1	0	0	0	6	0	1	0	6
14	Interest	0	0	5	0	0	0	3	0	0	0	3
15	Joy	0	0	0	0	0	0	0	8	0	0	8
16	Joy	0	0	0	0	0	0	0	7	1	0	7
17	Surprise	0	0	0	0	0	0	0	0	8	0	8
18	Surprise	0	0	1	0	0	0	0	0	7	0	7

Table B3. Independent observer classifications of positive and negative affective states used for inter-rater reliability, observed within the 18 sample videos in Table B1.

Video	**Affective state**	**Affective state classifications by ERH**	**Affective states classified by independent observers (*n* = 9)**	
			Positive	**Negative**
1	Anger	Negative	0	9
2	Anger	Negative	0	9
3	Anxious	Negative	0	9
4	Anxious	Negative	0	9
5	Curiosity	Positive	9	0
6	Curiosity	Positive	9	0
7	Fear	Negative	0	9
8	Fear	Negative	0	9
9	Friendly	Positive	6	3
10	Friendly	Positive	9	0
11	Happy	Positive	9	0
12	Happy	Positive	9	0
13	Interest	Positive	9	0
14	Interest	Positive	9	0
15	Joy	Positive	9	0
16	Joy	Positive	9	0
17	Surprise	Positive	8	1
18	Surprise	Positive	7	2