CHAPTER 1 GENERAL INTRODUCTION

1.1 Introduction

The research reported in this thesis investigated lateralization of behaviour in feral, domestic (*Equus caballus*) and Przewalski horses (*Equus ferus przewalski*) to gain insight into lateralization in horses and to see whether it might have been influenced by domestication and/or handling and management practices. Horses, more so than other domestic animals, have been intensely handled over centuries (Hall, 2005; Levine, 2005). They have been ridden in competitions of dressage, in races and been used as transport and carrier animals (Hall, 2005). Any such activities have been accompanied by intense training and handling by humans. Lateralization in horses is interesting in its own right, especially in the context of studies of other vertebrates. It is also important to see whether any existing lateralization in domestic horses today has little or no link to that in horses that may still be described as 'wild' horses (Przewalski) or to horses that have become feral and have been roaming wild for a number of generations. If horses living without human intervention show no lateralization or lateralization contrary to that shown in domestic horses, there may be welfare implications or, indeed, implications for more effective or better training methods in future, as will be explained further in Chapter 2.

Lateralization refers to differential processing of information and control of behaviour by the left and right hemispheres of the brain (Hellige, 2001; Andrew and Rogers, 2002; Vallortigara and Rogers, 2005). Such asymmetries can occur at the individual and/or the population level. At the individual level, but not at the population level, the majority of animals in a population or species display preferences but roughly equal numbers of individuals are left and right-biased. In such a case, the population distribution is bimodal or platykurtoic; examples are pawedness in some strains of mice (Collins, 1968) and foreleg preferences in sheep (Versace et al., 2007).

Lateralization at the population level means the majority of animals are lateralized in the same direction (a directional bias is present) and the distribution is skewed to the left or right. The most well known example is 90% right handedness in humans for writing (Perelle and Ehrman, 1994). In addition, several species of cockatoos (*Callocephalon fimbriatum, Calyptorhynchus banksii, Cacatua galerita*) show population biases of 84 to100% in the use of the left foot to manipulate food (Rogers, 1980; Magat and Brown, 2009).

A similar pattern of lateralization exists in many vertebrate species (reviewed by Rogers, 2002; Andrew and Rogers, 2002). Table 1.1 summarises these lateralized functions and cites the research showing them.

Ghirlanda and Vallortigara (2004) used game-theory analysis to show population biases can exist as an evolutionary stable strategy. This model takes into account the existence of a minority of individuals that are lateralized in the direction opposite to that of the majority (i.e. they show reversed lateralization of brain functions). The percentages of the majority vary and can differ within and between species (Vallortigara and Rogers, 2005). According to the model, during predator-prey interactions the frequencies of both majority and minority groups are dependent on probability of escape. If the majority of the herd responds in a similar manner and thus turns to escape in the same direction when confronted by a predator, the group will benefit by staying together (i.e. ‘dilution effect’, Ghirlanda and Vallortigara, 2004). On the other hand, individuals belonging to the minority group (i.e. those that turn in the opposite direction) may benefit by being unpredictable to predators.

Ghirlanda et al. (2009) applied the mathematical model from the earlier paper by Ghirlanda and Vallortigara (2004) to predict the strength of population biases based on intra-species interactions. Population biases were shown to be weaker when there is a competitive advantage and stronger when cooperation was essential. It could be said that social hierarchies, although giving a competitive advantage, once formed, serve to maintain cooperation between individuals. Consistent with this, Rogers and Workman (1989) demonstrated a population asymmetry for visual functions in chicks resulted in the formation of more stable hierarchies compared to chicks non-lateralized for visual functions.

1.2 Hemispheric specialisation

Substantial research into lateralization has led to establishing some general principles of function. Generally, it is said that the right hemisphere is involved in attending to global and spatial geometric cues, detection of novelty, viewing of conspecifics, rapid species-typical responses in emergency situations and the expression of intense emotions (reviews, Rogers, 2002; Rogers and Andrew, 2002; MacNeilage et al., 2009). The left hemisphere is involved in attending to object-specific and local cues, object discrimination, routine learned behaviour patterns and responses that require inhibition of an immediate response while a decision is made (review, Rogers and Andrew, 2002).

Table 1.1 Summary of the main functions found to be lateralized in vertebrates.

Left Hemisphere	Right Hemisphere	References
Approach	Withdrawal	humans, Davidson 1992, 1995; Davidson et al. 1985, 1990, 2000; dogs, Quaranta et al. 2007; Australian magpies, Koboroff et al. 2008
Considered responses	Rapid responses	fish, Miklosi et al. 1998; Miklosi and Andrew 1999; Bisazza and deSanti 2003; toads, Robins and Rogers 2004
Object specific cues/ Local characteristics	Geometric cues/ Global characteristics	humans, Hellige 2001; Volberg and Hübner 2006; Hübner and Studer 2009; chicks, Tommasi et al. 2000, 2003; Tommasi and Vallortigara 2004; Chiandetti et al. 2005; Chiesa et al. 2006; pigeons, Yamazaki et al. 2007
	Reactivity/fear	chicks, Andrew et al. 1982; Dharmaretnam and Rogers 2005; hens, Evans et al. 1993; fish, Cantalupo et al. 1995; toads, Lippolis et al. 2002; dunnarts, Lippolis et al. 2005; Australian magpies, Hoffman et al. 2006; lizards, Bonati et al. 2010; domestic horses, Larose et al. 2006; Austin and Rogers 2007; dogs, Siniscalchi et al. 2008;
	Novelty	chicks, Vallortigara and Andrew 1991, Andrew and Dharmaretnam, 1991; Vallortigara et al. 1999a, 2001; toads, Robins and Rogers 2006a; cows, Robins and Phillips 2009; dogs, Siniscalchi et al. 2010; magpies, Koboroff et al. 2008; domestic horses, Basile et al. 2009a
Categorisation of stimuli		chicks, Rogers and Anson 1979; Mench and Andrew 1986; Dharmaretnam and Rogers 2005; humans, Marsolek 1995, 1999; Zwaan and Yaxley 2004; Marsolek and Burgund 2008; pigeons, Güntürkün and Kesch 1987; Yamazaki et al. 2007; quails, Valenti et al. 2003; zebra finch, Alonso 1998; cows, Robins and Phillips 2009
Processing vocalisations of conspecifics		rhesus monkeys, Hauser and Andersson 1994; sea lions, Boye et al. 2005; dogs, Siniscalchi et al. 2008; mangabeys, Basile et al. 2009b; domestic horses, Basile et al. 2009a
	Viewing of conspecifics	chicks, Vallortigara and Andrew 1994; fish, Bisazza et al. 1999; Deng and Rogers 2002; tadpoles, Bisazza et al. 2002; Sovrano et al. 2001; dolphins Sakai et al. 2006; sheep, Peirce et al. 2000, 2001; da Costa et al. 2004; Versace et al. 2007
	Copulation	stilts, Ventolini et al. 2005; chicks, Rogers 1982; sparrows, Nyland et al. 2003; newts, Green 1997
	Aggression	rats, Denenberg 1981; chicks, Howard et al.,1980; Zappia and Rogers 1983; Rogers et al. 1985; Vallortigara et al. 1998, 2001; lizards, Deckel 1995; Hews and Worthington 2001; Hews et al. 2004; Gelada baboons, Casperd and Dunbar 1996; toads, Robins et al. 1998; frogs, Robins and Rogers 2006b
Routine behaviour		chicks, Rogers and Anson 1979; Mench and Andrew 1986; Dharmaretnam and Rogers 2005; pigeons, Güntürkün and Hoferichter 1985; Güntürkün and Kesch 1987; zebra finch, Alonso 1998; quails, Valenti et al. 2003
	Emergency behaviour	chicks, Andrew et al. 1982; Dharmaretnam and Rogers 2005; primates, Hatta and Koike, 1991; hens, Evans et al. 1993; fish, Cantalupo et al. 1995; toads, Lippolis et al. 2002; dunnarts, Lippolis et al. 2005; Australian magpies, Hoffman et al. 2006; domestic horses, Larose et al. (2006), Austin and Rogers 2007; lizards, Bonati et al. 2010

The suite of right-hemisphere functions includes a superior ability to process global cues (humans, Volberg and Hübner 2006; Hübner and Studer 2009; chicks, Tommasi and Vallortigara, 2004), as used in spatial ability (chicks, Tommasi et al*.,* 2003; humans, Hellige 2001), attending to novelty (toads, Robins and Rogers, 2006a) and in vigilance (humans, Warm et al*.,* 2009; magpies, Koboroff et al*.,* 2008). All of these functions play a role in detecting and attending to potential threats. According MacNeilage et al. (2009), early in the evolution of vertebrates the right hemisphere controlled responses to unexpected, potentially dangerous stimuli. Later, such responses may have become accompanied by high arousal and intense emotions, such as fear, which are also controlled by the right hemisphere.

As MacNeilage (1998, 2007) has pointed out, the right hemisphere is used in functions related to survival risk (MacNeilage, 1998) and the left hemisphere is used in behaviour involving routine motor actions (MacNeilage, 2007). The right hemisphere processes variance, hence attending to novelty, which makes it good at responding to potential threats by predators or conspecific rivals (MacNeilage et al., 2009). In contrast, the left hemisphere attends to invariant properties of stimuli and analyses abstract qualities, thereby enabling categorisation of stimuli based on experience (MacNeilage et al., 2009). As a general distinction, as MacNeilage et al. (2009) suggested, one might think of the two hemispheres as different in type of processing: the left hemisphere primarily uses top-down (directed by higher cognitive circuits) processing and the right hemisphere uses bottom-up (directed by sensory input) processing.

Numerous studies have shown that, as a stimulus becomes familiar, processing of it shifts from the right to left hemisphere (e.g. Cantalupo et al., 1995). As the animal learns about the properties of an object it assigns the object to a category. Information held in memory about that category is then used in decision making. Such categorisation has been shown to be a primary function of the left hemisphere in humans (e.g. Zwaan and Yaxley, 2004) and in other animals (e.g. chicks, Rogers and Anson 1979). It has been found that abstract visual object recognition is a function of the left hemisphere (humans, Marsolek and Burgund, 2008; Marsolek, 1995, 1999; pigeons, Yamazaki et al., 2007). This requires the use of top-down processing, whereby invariant features of an object are matched to mental templates, thus enabling categorisation (MacNeilage et al*.,* 2009).

The expression of emotion appears to involve the hemispheres differentially. One theory proposes that the right hemisphere only processes all intense emotions (review Harrington, 1995; Wager et al., 2003), the other that negatively and positively valenced emotions are processed by the right and left hemispheres, respectively (Bogen, 1985; Davidson, 1992, 1995). In humans, Alpers (2008) found a right-hemisphere bias for orientating to emotion-eliciting cues, irrespective of emotional valence, lending support to the ‘right hemisphere theory’. In a meta-analysis of neuroimaging research of the human brain, however, Wager et al. (2003) found little evidence in support of the ‘right hemisphere theory’. Support for the valence hypothesis in humans has been demonstrated by Canli et al. (1998) and Wheeler et al. (1993) using functional magnetic resonance imaging (fMRI) and electroencephalography (EEG), whereas a meta-analysis of neuroimaging study by Murphy et al. (2003) found little evidence for right-hemispheric processing of negative emotion but a left bias for positive emotion. Based on research on humans, Davidson (1992) proposed that the left hemisphere controls approach and the right avoidance. Using facial expressions indicative of emotional withdrawal (disgust) and approach (happiness), Davidson et al. (1990) found greater anterior temporal activation in the right hemisphere when participants responded with disgust, whereas the same area of the left hemisphere became activated when participants responded with happiness. Evidence supporting one or the other of these two theories is inconclusive in humans.

Research on horses using behavioural responses to objects differing in emotional value (de Boyer des Roches et al., 2008) and on chimpanzees measuring tympanic membrane temperature (Parr and Hopkins, 2000) has provided partial support for the valence hypothesis in that negative emotions are regulated by the right hemisphere, whereas positive emotions are regulated by both hemispheres (i.e. no bias). The tympanic membrane temperature, measured by placing a thermometer in the ear (left or right), and so measuring blood flow to the brain and, hence, relative activity of the hemispheres has shown higher temperature in the right ear is linked to greater autonomic activity and cerebral arousal (Parr and Hopkins, 2000). Research on rhesus monkeys suggested a right-hemisphere bias for control of expression of facial emotions (fear, Hauser, 1993), and such right-hemisphere control occurred irrespective of emotional valence (Hauser and Akre, 1999). In contrast, Hook-Costigan and Rogers (1998) found in marmosets a left-hemisphere bias for controlling the facial expression of negative emotion (i.e. fear) and a right-hemisphere bias for positive emotion (i.e. when producing a contact call). In red-capped mangabeys, the more palatable a food stimulus the greater the left-eye preference for viewing that food item (Latude et al., 2009). Consistent with these findings, Quaranta et al. (2007) observed that, when dogs saw a stimulus likely to elicit approach (i.e. cat in a non-aggressive posture or their owner), tail wagging amplitude was greater to the right side (controlled by the left hemisphere), whereas a stimulus regarded to elicit withdrawal (i.e. unfamiliar dog) resulted in a left-side (right-hemisphere) bias in tail wagging. Hence, there appears to be support for the 'right hemisphere theory' and partial support for the 'valence theory' in behavioural studies on animals. At the very least, negative intense emotion is under control of the right hemisphere in most cases.

1.2.1 Limb preferences

Limb use may be discovered either as an individual or as a population bias. As an example of population biases, some parrots show a population bias in the left foot to manipulate food as already mentioned (Rogers, 1980) and toads (*Bufo bufo*) preferentially use their right forelimb to remove an item from their snout (Bisazza et al., 1997).

Research results on limb preference in primates were first marred by methodology and by identifying differences of postures and tasks. Olson et al. (1990) tested orang-utans on three different retrieval tasks, finding that most of the individuals exhibited hand preferences but there was no group bias for left or right handedness. The authors attributed this apparent lack of handedness in orang-utans to their limited performance of bipedal locomotion and hence their lesser need to adopt an unstable posture, which might lead to handedness. Indeed, specifically since the 1990s, it has been reported that limb preference may vary with task. For instance, Hopkins (1993) showed that orang-utans exhibit hand preferences when they reach for food in a standing posture (six out of the nine were right-hand preferent) but not when they reach from a quadrupedal posture. This result is consistent with results for prosimians (Ward et al., 1993). Furthermore, Rogers and Kaplan (1996) found a significant left-hand preference in rehabilitant orang-utans for fine manipulation of parts of the face, the strength of this preference increasing with age. No group bias for either a left- or right-hand preference was found for hand use in feeding, although some individuals displayed significant left- or right-hand preferences for unimanual holding of food when in either the hanging or propped position. Hopkins et al. (2003) also found left-hand preference in captive orang-utans. Captive rhesus macaques were also shown to have a left-hand preference for quadrupedal reaching (Westergaard et al., 2002; 2004).

Some researchers have argued that handedness in chimpanzees has been demonstrated only in captive chimpanzees and it was, therefore, not a characteristic of the species (e.g. McGrew and Marchant, 1997) but it has now been shown that chimpanzees display right-hand preferences for tool use in the wild (Lonsdorf and Hopkins, 2005). Right handedness for a bimanual task requiring reaching into a tube has also been shown in captive baboons (Vauclair et al., 2005) and captive chimpanzees (Hopkins et al., 2003). Tufted capuchin monkeys show right-hand preference in bipedal reaching and two bimanual tasks but not in quadrupedal reaching (Spinozzi et al., 1998). These and other studies indicate that handedness is not a trait that evolved only in humans.

After a thorough analysis of the data on hand preferences in primates, MacNeilage et al. (1987) proposed the *Postural Origins Theory of Handedness* to account for the evolution of handedness in primates (also MacNeilage, 1991, 2007). The theory stated that early primates evolved handedness because of asymmetrical postural constraints in an arboreal habitat and unimanual, prehensile capture of prey. Prosimians, which use clinging and leaping locomotion, show left-hand preference to capture prey because the right side of the body provides postural support while the left hand captures prey (right hemisphere ballistic specialisation). Primates which are more quadrupedal and have a more varied diet retain some left-hand preference for reaching but have evolved right-hand preference in tasks that require manipulation of food. As primates adopted bipedal locomotion right-hand use for fine manipulation strengthened. This is especially evident in tasks requiring bimanual coordination (e.g. Vauclair et al., 2005).

Some evidence suggests that limb preference may indicate the active or dominant hemisphere, either in general or for specific tasks (Vallortigara and Rogers, 2005; Rogers, 2009; Gordon and Rogers, 2010a). Motor responses on one side of the body are controlled by the motor cortex of the contralateral hemisphere and there is evidence that motor preferences are associated with increased activation of the hemisphere contralateral to the limb being used, not only in the motor cortex, but also in a wider area of the cortex (e.g. rats, Vyazovskiy and Tobler, 2008; humans, Harmon-Jones, 2006).

Rogers (2010) proposed that limb preference may be associated with an animal’s temperament and perhaps its cognitive bias. If so, left-limbed individuals should score high on temperament traits such as anxiousness and aggressiveness, as already found, whereas right-limbed individuals should be explorative and sociable. In fact, right-handed marmosets are more responsive to social facilitation than left-handed marmosets, as measured by performance of capturing crickets when alone and when with a social companion: right-handed marmosets were more influenced by the presence of a companion than were left-handed ones (Gordon and Rogers, 2010a). The authors concluded right-handed marmosets were more proactive and explorative, as shown by more vocalisations in the presence of crickets and production of more mobbing calls and head movements when a fear-inducing object was presented, than found in left-handed marmosets. Likewise, right-handed (left hemisphere more active) marmosets displayed shorter latencies to enter a novel room and touched more novel objects than their left-handed counterparts (Cameron and Rogers, 1999). Left-handed marmosets displayed behaviour consistent with functions, such as fear and withdrawal, which are under the control of the right hemisphere (Table 1.1). The same association has been found between hand preference and latency to touch novel toys in chimpanzees (Hopkins and Bennett, 1994) and, in Geoffroy's marmosets, behaviour indicative of fear, such as duration of freezing on hearing the call of a hawk and latency to sniff novel food, is more pronounced in left-handed than right-handed individuals (Bracinni and Caine, 2009).

1.2.2 Lateralization of eye use in viewing

Lateralization can also be observed as a side bias in attending to a stimulus. Animals with laterally placed eyes and large monocular fields of vision are particularly suitable for the study of such lateralization because input received from one eye is processed predominantly by the contralateral side of the brain. By applying an eye patch to one eye and then the other it can be determined which functions are characteristic of each hemisphere. Extensive research conducted using birds in monocular testing has revealed asymmetries in the types of information processed according to which eye is being used. Some early research found that chicks made more distress calls in response to seeing a novel stimulus with the left-eye (use of right hemisphere; Andrew et al., 1982) and they were able to discriminate food from non-food items when using the right eye but not when using the left-eye (Mench and Andrew, 1986). Chicks recognise strangers from familiar conspecifics better when using the left eye/right hemisphere than they do when using the right eye/left hemisphere (Vallortigara and Andrew, 1995). A number of studies have found a left-eye/right-hemisphere bias for processing geometric spatial cues compared to a right-eye/left-hemisphere bias for processing local cues (e.g. Tommasi et al., 2000; Chiesa et al., 2006): the chicks were trained to find hidden food and, when tested using the left eye, they located the food using local cues (landmarks) and, when using the right eye, they located it using geometric spatial cues.

In horses, each monocular field is 95º and the binocular field is approximately 65º (reviewed by Roberts, 1992; Murphy et al., 2009). Cummings and De Lahunta (1969) estimated the amount of decussation of the optic fibres at the optic chiasm is 90%. This is important because visual information received by one eye may be processed differently from information from the other eye. Moreover, even in the binocular field input travels to the contralateral hemisphere more rapidly than input to the ipsilateral hemisphere (Bishop et al., 1953; Maddess, 1975). Hence, horses may respond to a stimulus differently depending on which side it is seen.

Social functions, with the exception of some aspects of communication, appear to be processed primarily by the left eye/right hemisphere. For example, recognition of conspecifics is performed better when chicks use the left rather than right eye (Vallortigara et al., 2001) and a number of species display a left-eye bias for looking at their own reflection in a mirror and for viewing a conspecific (tadpoles, Bisazza et al., 2002; fish, Bisazza et al., 1999; Sovrano et al., 2001; dolphins, Sakai et al., 2006). Some evidence suggests these biases may be the result of familiarity since once the conspecific becomes familiar the bias disappears (Dadda et al*.,* 2003; Sovrano, 2004). A recent study by Rosa Salva et al. (2009) found a left-eye/right-hemisphere bias in social learning. Chicks were required to learn not to peck at a red bead, which was coated in a bitter substance, by observing the response of a demonstrator. Observer chicks tested monocularly using the right eye performed more poorly at avoiding the red bead than those tested using the left eye. The researchers suggested this is due to right-hemisphere use in social viewing and processing rather than a lesser ability of the left hemisphere to learn the task.

Courtship also appears to be under the control of the right hemisphere. For example, male black winged stilts make more courtship displays and copulatory attempts with females seen in their left-monocular visual field than in their right-monocular visual field (Ventolini et al. 2005). In addition, both male and female chicks exhibit more copulatory responses following treatment of the left hemisphere with glutamate than following the same treatment of the right hemisphere (Rogers, 1982), probably because the left hemisphere usually has an inhibitory effect on the right-hemispheric specialisation for copulation and the glutamate treatment removes this.

Agonistic responses, especially those involving a high level of aggression, have been shown also to be primarily under the control of the right hemisphere (Rogers, 1982). Aggression is displayed predominantly towards conspecifics occupying the left-visual field. For example, chicks were more likely to use their left than right eye when pecking at an unfamiliar conspecific (Vallortigara et al., 2001), gelada baboons engaged more often in agonistic interactions with conspecifics on their left rather than their right side (Casperd and Dunbar, 1996), and both male and female lizards fixated opponents with their left eye during aggressive encounters (Deckel, 1995; Deckel and Jevitts, 1997; Hews and Worthington, 2001; Hews et al., 2004). More recently, Siniscalchi et al. (2010) found dogs exhibited a bias to turn their head leftwards (and thus used the right hemisphere) in response to seeing a stimulus evoking aggression presented simultaneously on both sides of the dog (e.g. the silhouette of a cat with an arched back).

From studies of agonistic interactions, it appears that it depends on the strength of agonistic responses whether they are lateralized or not. Toads, for instance, struck with their tongue more aggressively at the eyes of conspecifics located on their left side but they did not show a bias for less aggressive tongue strikes aimed at other parts of the opponent's body (Robins et al., 1998). Lizards also showed a left-eye bias only when engaged in the most aggressive interactions, including bites, charges with bite threats, and highly aggressive head-bobbing displays, whereas no eye bias was shown for less aggressive threats (Deckel, 1995). This evidence suggests that the more aggression an animal exhibits the more it uses its right hemisphere.

Novelty is likewise processed predominantly by the right hemisphere. For example, chicks turned rightwards when detouring around a barrier through which they could see a novel stimulus, and thus fixated a novel object with the left eye (Vallortigara et al., 1999b), and toads struck preferentially at novel prey in the left-visual field (Robins and Rogers, 2006a). Attending to novelty using the left eye may be related to right-hemispheric specialisation for processing characteristics (or changes) inconsistent with previously learned categories (Vallortigara et al*.,* 1999a).

Interestingly, there are instances in which right-hemispheric control over the expression of aggression may be masked. Fish have been shown to have a right-eye preference before directing agonistic aggressive displays and attacks towards conspecific rivals or towards their own mirror reflection (Miklosi and Andrew, 1999; Bisazza and de Santi, 2003). The authors suggested this is due to inhibition of response by the left-hemisphere, which allows the fish to assess the rival's size and to decide on the appropriate response to make. Similarly, a right-eye bias was found in fish for examining a target when deciding whether to bite or not (Miklosi et al., 1998).

Right-hemispheric dominance/left-eye use has been demonstrated for the control of responses to predators. For example, toads (Lippolis et al*.,* 2002) and stripe-faced dunnarts (Lippolis et al*.,* 2005) exhibited greater reactivity when a model predator was presented in the left- compared to the right-monocular field. Adult hens also showed a right-hemispheric preference for attending to predators; in response to their species-typical alarm call signalling an aerial predator, they tilted their head to look overhead using the left eye (Evans et al*.,* 1993). Similarly, when confronted by a predator, fish (*Girardinus falcatus*) initially exhibited a rightwards bias for escape turning, enabling use of the left-eye/right-hemisphere system to process information (Cantalupo et al., 1995). However, in subsequent presentations of the predator, escapes were progressively directed more leftwards until there was an opposite direction of population bias, which the authors concluded may be due to habituation. Lizards also showed a left-eye bias for monitoring potential danger (Bonati et al., 2010). An auditory stimulus was used to evoke an escape response from a lizard that had been placed in a tube. Following exit from the tube, lizards turned rightwards (thus viewing the surroundings with their left eye) and after a distance stopped and turned their head leftwards so as to look behind with their left eye. Australian magpies with a tendency to use the left eye more frequently to view an approaching human gave more alarm calls than magpies that were more likely to use the right eye (Hoffman et al*.,* 2006), indicating an association between right-hemisphere use and the expression of fear behaviour. Recent research (Koboroff et al*.,* 2008) has revealed Australian magpies tended to use the left eye prior to withdrawal from a taxidermic specimen of a predator and they used the right eye immediately before approaching the predator. During high alert inspection of the predator, the left eye was used predominately, which may indicate fear of the predator and vigilance needed to monitor changes in behaviour of the predator, both of which are under right-hemisphere control.

On the other hand, predatory strikes by toads were predominantly evoked by prey entering the right- rather than the left-visual hemifield (Vallortigara et al., 1998; Robins and Rogers, 2004, 2006a). Ventolini et al. (2005) also observed that black winged stilts fished predominantly for prey in the right-visual field and were more successful in capturing prey fixated by the right eye than the left eye and Bonati et al. (2008) found that lizards use their left eye preferentially to view prey. These findings are consistent with research showing a right-eye advantage for object categorisation in general (Vallortigara and Andrew 1991; Vallortigara et al*.,* 1999a).

Feeding is one of the activities routinely performed by an animal that draws on the specialisations of the left hemisphere (right eye use). Routine behaviour patterns are under control of the left hemisphere (MacNeilage, 2007). Foraging requires the animal to discriminate food items from non-food items. The animal needs to selectively focus on and categorise items it sees, which is also a function of the left hemisphere (Declerek et al., 2004). When tested monocularly, birds (chicks, pigeons, quails and zebra finches) using the right eye were more successful in distinguishing grain from pebbles than birds using the left eye (Rogers and Anson, 1979; Mench and Andrew, 1986; Dharmaretnam and Rogers, 2005; Güntürkün and Kesch, 1987; Alonso, 1998; Valenti et al*.,* 2003).

1.2.3 Lateralization of head-turning

Head-turning bias shown by Campbell’s monkeys in response to hearing an auditory stimulus depended on its emotional valence as well as on its social value (Basile et al., 2009b). Usually, the monkeys showed a left-hemisphere bias (right head turn) to conspecific calls, but when an intra-group threat call was heard they turned their head leftwards (Basile et al., 2009b). Research on dogs also showed head-turning responses to hearing a conspecific's bark may be influenced by emotional content of the vocalisation (Siniscalchi et al., 2008). Although dogs usually displayed a bias to turn their head rightwards (and thus used the left hemisphere) in response to conspecific vocalisations, stronger leftwards head-turning responses correlated with higher levels of reactivity.

The two sides of the brain differ, therefore, in the way that they process information. This thesis focuses on several functions, agonistic responses, attention to novelty/vigilance and reactivity, shown to be under control of the right hemisphere. It was expected that horses, like other vertebrate species, would display characteristic left-eye biases, and thus right-hemisphere processing, for these functions.

1.3 Function of lateralization

1.3.1 At the individual level

Lateralization of the brain may have evolved to overcome the problem of conflicting demands, that is, when pertinent stimuli are seen on both sides, the animal needs to make a choice as to which one it will respond (Bisazza et al*.,* 1998; Vallortigara et al*.,* 1999a). Another explanation for cerebral lateralization is that it minimises neural duplication, thus ensuring cognitive efficiency. This in turn enables parallel processing of information, allowing the animal to engage in two tasks simultaneously (Vallortigara and Rogers, 2005). A number of studies have provided support for this concept. Studies with chicks have shown lateralized chicks performed better than non-lateralized chicks when engaged in two tasks, predator vigilance and food discrimination, concurrently (Rogers et al., 2004; Dharmaretnam and Rogers, 2005). Dadda and Bisazza (2006) observed lateralized female fish could feed and simultaneously maintain vigilance for males in order to avoid copulation attempts better than non-lateralized females. The authors also found lateralized fish caught more prey than non-lateralized fish while simultaneously feeding and being vigilant for predators. Lateralization at the level of the individual is an advantage as it allows an animal to engage in two tasks at the same time.

Other research has shown lateralized animals perform better than non-lateralized animals even when apparently not engaged in simultaneous tasks. McGrew and Marchant (1999) observed chimpanzees showing hand preferences for termite fishing, regardless of the direction of preference, extracted more termites than chimpanzees that used both hands equally. Considering visual tasks, Güntürkün et al. (2000) found visual acuity was greater in lateralized compared to non-lateralized pigeons, that is, a positive correlation exists between degree of asymmetry and ability to discriminate grain from grit. Likewise, lateralized fish were better at spatial reorientation (Sovrano et al., 2005), at escaping (Dadda et al., 2010) and at schooling performance than non-lateralized fish; they showed greater cohesion and were more likely to occupy the centre of the school compared to non-lateralized fish that more frequently lost contact with the school (Bisazza and Dadda, 2005). The researchers of the latter study suggested fish use one eye to monitor conspecifics while the other eye scans the surroundings.

1.3.2 At the population level

As stated by various authors (e.g. Ghirlanda and Vallortigara, 2004; Vallortigara and Rogers, 2005), the benefits of individual lateralization for various functions can be gained without the need for the majority of animals to be lateralized in the same direction. Selection pressures independent of those for individual lateralization must underlie the existence of population biases. One proposal is that population-level lateralization is beneficial when an individual needs to interact with other lateralized individuals and thus, essentially, it is predicted that social species will exhibit population biases, whereas non-social species will not, although individuals may be lateralized (Vallortigara and Rogers, 2005). This is supported by the work of Bisazza et al. (2000) on 16 species of fish; those species categorised as shoaling species exhibited population level lateralization for predator viewing, whereas non-shoaling species were less likely to display a population bias. Similarly, a bias was found for left-eye viewing of their reflection in five species of social fish, whereas a non-social species displayed no bias (Sovrano et al., 1999).

Intuitively, population biases would appear to be disadvantageous, as prey is rendered predictable to predators. Also, decreased ability to detect threats on one side would be detrimental given that threats are likely to occur randomly on either side (Vallortigara and Rogers, 2005). Nonetheless, since population lateralization exists, one assumes the advantages must outweigh the costs.

CHAPTER 2 LATERALIZATION IN DOMESTICATED ANIMALS AND ANIMAL WELFARE

Domestic animals have been shown to possess lateralization (review, Morgante and Vallortigara, 2007) and this lateralization appears to follow the pattern found in other vertebrate species (review, Andrew and Rogers, 2002). Considering the close association between farm animals and humans over thousands of years, and the possibility that side biases may affect and be affected by animal-human interactions and management practices, it is important to study lateralization in farm animals.

2.1 Lateralized behaviour of large farm animals other than horses

Different species of farm animals undergo different management practices and such practices vary with breed and different production purposes (e.g. milk, meat or wool). Given that experience is known to influence lateralization (review, Schaafsma et al., 2009), different species and breeds of domestic animals may show differences in lateralization because they undergo different experiences (i.e. management practices). Unlike horses, production animals such as sheep and cows are not intensively trained.

Two studies have shown that lateralization in sheep is related to stress and therefore has relevance to welfare. Morgante et al. (2007) showed the strength of lateralization for a number of lateralized measures – free turning bias, turning around an obstacle and forelimb preference – is related to measures of stress in ewes. A subsequent study found strongly lateralized ewes showed metabolic differences that may indicate greater activation of the hypothalamic-pituitary-adrenal-axis compared to weakly lateralized ewes (Morgante et al., 2010).

A number of studies in sheep have focussed on eye-bias in recognising faces and shown that sheep are lateralized at the population level. Sheep showed a left-visual bias in recognising the faces of other sheep that are familiar, but they did not show a bias when looking at faces of unfamiliar conspecifics (Peirce et al., 2000). Sheep use different types of information to distinguish sheep (configurational coding) and human faces (non-configurational coding) (Peirce et al., 2001). Increased activation was found in regions of the right hemisphere while sheep processed the faces of conspecifics (da Costa et al., 2004). Versace et al. (2007) found that sheep showed a bias to detour rightwards around an obstacle in order to rejoin the flock, possibly because they used the left-visual field for face recognition. In contrast, sheep do not exhibit motor preferences for a number of measures, forelimb used to step onto a board or to initiate forward movement, or tail movements when suckling or lying down (Versace et al., 2007; Lane and Phillips 2004).

Studies on lateralization in cows have focused on the side on which they prefer to be milked, as measured by the side of the milking parlour they enter. Consistent individual preferences have been found in one-third to one-half of the animals assessed (Gadbury, 1975; Tanner et al., 1994; Hopster et al., 1998; Grasso et al., 2007). Hopster et al. (1998) found that cows, when milked on their non-preferred side, showed some signs of stress, such as increased pausing from feeding, increased heart rate and increased heart rate variability. Production of milk, however, was not affected. Paranhos da Costa and Broom (2001) also investigated side preference for milking and whether cows showed stress when milked on the non-preferred side. More than half of the cows showed consistent choices (>72% of choices) in entering a milking parlour on the left or right side but when milked on the non-preferred side these cows did not show signs of increased stress. A slight leftwards population bias was present that could be explained by an asymmetry in the milking parlour. As discussed by Grasso et al. (2007) and Paranhos da Costa and Broom (2001), choice of side of the milking parlour may be influenced by a variety of factors and, hence, it may not necessarily show a behavioural laterality. Nevertheless, Prelle et al. (2004) found cows that showed a stronger preference for a specific side on entering the milking parlour stood motionless for longer, were more aggressive, and were dominant in competition for food compared to cows showing a weaker preference.

Cows are also lateralized at the individual level in other ways. Kilgour et al. (2006) found roughly equal proportions of cows preferred to pass on the right side and left side of an obstacle in three out of three times, and one-fifth did not have a preference (i.e. two out of three times). They found that cows with a stronger rightwards laterality score had a higher heart rate in response to the close proximity of a human. Notably only three trials were used to determine laterality, which makes it likely that chance effects could influence the results. Robins and Phillips (2009) found cows turned so as to monitor approaching novel objects, carried by a human, in their left-visual field. This is a population-level laterality. When the stimulus had become familiar, no bias was shown.

Population-level lateralization has also been observed in cows on other measures. Arave and Albright (1981) observed a left-side preference in cows when they were lying down. However, Phillips et al. (2003) found no population bias for use of the hind leg to lie down in a herd of intensively managed dairy cows. Importantly, it was found that a herd of extensively managed (as opposed to intensively managed) cows displayed weaker lateralization of the same measures.

One further study on cows deserves mention because it shows how an understanding of side bias can be integrated into a simple management practice to improve not only production but also the welfare of domestic animals. Rizhova and Kokorina (2005) found feeding from the left compared to right side improves reproduction and affects lactation in cows. The authors attributed this effect to the direction of turning towards the food and the activation of the contralateral hemisphere in conjunction with a positive emotional response to the food and suggest that left turning might activate the right hemisphere and that this hemisphere may be involved in regulation of reproductive functions. Feeding of cows from the left increased lactation under good feeding conditions, whereas feeding from the right increased lactation under poor feeding conditions.

2.2 Lateralized behaviour of domestic horses (*Equus caballus*)

Initial studies on horses emphasised the practical aspects of side biases and as a result focused on limb preference and the resulting bias for a horse to turn or canter in one direction rather than the other. A horse with a strong limb preference might be more susceptible to injury than one that shows little preference. Van Heel et al. (2006) found behavioural limb preference exhibited by horses while grazing was associated with uneven hoof loading. Such faults in conformation are known to affect performance (Dalin et al., 1985; van Heel et al., 2010).

Grzimek (1968) made observations of 50 horses to determine lateralization and found individual preferences of forelimb preference in pawing, initiating walking and initiating galloping. Although only 20% of horses showed a limb preference during galloping, 70% of these showed a right-leading limb preference. Population level laterality of limb preference in horses has been reported and, although it appears to vary according to breed, it is possible that it is related to practice effects and hence training methods. For example, McGreevy and Rogers (2005) found a leftwards population bias in racing thoroughbreds for the measure of foreleg advanced in front of the other during grazing (McGreevy and Rogers, 2005). Right-leading limb preferences were displayed by racehorses of various breeds during galloping (Williams and Norris, 2007). Furthermore, standardbred horses, which are handled on both sides and not ridden, show a weaker leftwards forelimb preference while grazing than do thoroughbreds, and quarterhorses trained for cutting work, which requires agility, display no population bias (McGreevy and Thompson, 2005). The directional bias found in the thoroughbreds increases with age, due to either training or maturation (McGreevy and Rogers, 2005). Practice is known to have long-lasting effects on paw preferences in mice (Bulman-Flemming et al., 1996) and may do so in horses. Trotter horses that had undergone training displayed a bias when trotting for the stride length of the left diagonal (i.e. the left forelimb and right hindlimb touch the ground at the same time) to be shorter than the right, but this bias was not shown by untrained horses of the same age (18 months) or by younger horses (Drevemo et al., 1987). Wells and Blache (2008) found older horses trained for dressage exhibited a right-forelimb preference while grazing, whereas untrained younger horses showed no preference. It is unclear which variable, age or training, may be contributing to limb preference.

It has been inferred that the foreleg placed in front of the other while grazing may correspond to the preferred lead during cantering/galloping (McGreevy and Rogers, 2005). A study of thoroughbred racehorses (McGreevy and Rogers, 2005) was consistent with Deuel's and Lawerence's (1987) findings that racing quarterhorse fillies preferred the left lead twice as much as the right. A tendency to spend more time grazing with one particular foreleg in front of the other may place the horse in a position to automatically lead with the preferred leg (Figure 2.1) and, if the majority of horses display the same motor preference, it is more likely that they will turn in the same direction and stay together. In fact, it is the opposite hindleg that is used to 'push off' into these asymmetrical gaits and, as found by Williams and Norris (2007) using a large sample size (N = 9362), the majority of racehorses, irrespective of track direction, preferred to lead with the right forelimb. This result corresponds to initiation of the gallop with the left hind leg and, hence, control by the right hemisphere.

Wells and Blache (2008) failed to find a link between forelimb preference and canter lead. Their findings appear to conflict with the above hypothesis but they did not actually measure leading limb preference *per se*. Instead, they scored the time the horse spent cantering, whether it initiated cantering with the inside foreleg leading or not, how often the canter became disunited while the horse was cantering in one direction and then in the other. The authors found horses showed no side bias for cantering. This measure of canter lead may not correspond to a preference shown by a horse when unrestricted in an open area. In contrast, van Heel et al. (2010) showed a strong relationship between limb preference while grazing and side bias in the leading limb when horses make a transition from trotting to cantering.

It has also been suggested by McGreevy and Rogers (2005) that the forelimb preference while grazing may be related to temperament. Thoroughbred horses are known to be 'flighty' (Houpt and Kusunose, 2000) and they show a left-forelimb preference which might indicate activation of the right hemisphere. However, it is unclear which forelimb, the one placed in front or the one behind, is important in indicating the dominant hemisphere.

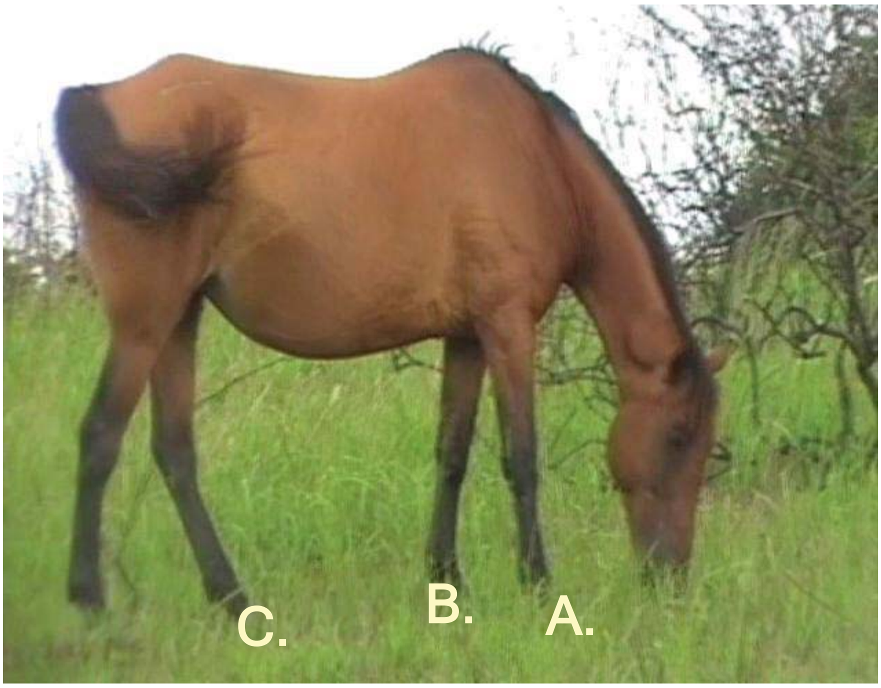

Figure 2.1 Limb placements during grazing. A, the limb preference scored in this thesis, the forelimb placed in front of the other. B, the supporting forelimb in a position to give purchase in a flight response. C, the hindlimb in a position to initiate galloping.

Another study (Murphy et al., 2005) on 'minimally' handled horses found population biases, but in opposite directions for males (leftwards) and females (rightwards). Murphy et al. (2005) assessed four measures, two of which might be regarded as visual rather than motor preferences since they involved passing an obstacle on one side or the other. The two other scores were side of rolling and foreleg used to initiate forward movement. The latter may be influenced by level of arousal since the cue used to initiate movement, waving a stick behind the horse, may increase arousal/fear. Nevertheless, there were significant correlations between measures.

A number of studies have approached lateralization of horses using an ethological methodology by investigating lateralization in a broader theoretical context to understand its causation, function, development and evolution. In the most part, side bias in horses follows the same trend observed in other vertebrate species. For example, Larose et al. (2006) revealed an association between eye use and emotionality; the more reactive the horse the more often it used the left eye to look at a novel object. Austin and Rogers (2007) found no overall population bias for escape turning from a potentially threatening novel object presented directly in front of the horse, but they did find the more flighty animals showed a preference to turn away from the object so that they could view it with their left eye. Moreover, Austin and Rogers (2007) demonstrated a left-eye bias in reactivity in that, horses showed a longer flight distance when a person holding a looming novel stimulus approached on the left side compared to the right side. Larose et al. (2006) also found a left-eye preference for horses to view the novel stimulus but only in trotter horses that are handled on both sides and not in French saddlebreds which are handled on the left side.

In contrast, de Boyer des Roches et al. (2008) observed a right-eye preference in horses for assessing a novel object considered to have neutral emotional value, which is contrary to research in other species showing right-hemisphere processing of novelty. In the same study the horses showed a right-nostril bias in sniffing the novel object. Since olfactory neural pathways are ipsilateral, this right-nostril bias corresponds to processing of novelty by the right hemisphere and accords with results from other vertebrates. The study also investigated side biases in attending to objects with negative and positive emotional value and found a right-nostril preference and tendency for left-eye exploration of a negative object.

Laterality of social behaviour has also been found in domestic horses. Olfaction is a major component of social behaviour in horses, whether used for conspecific identification or during mating (Waring, 1983). Young thoroughbred horses displayed a preference to smell stallion faeces with their right nostril (McGreevy and Rogers, 2005). It should be noted that this bias was lost in subsequent sniffing sessions, suggesting that novelty may play a role. By contrast, a left-hemisphere bias in auditory processing of conspecific vocalisations has been demonstrated in domestic horses but it depended on social familiarity (Basile et al., 2009a); horses were more likely to orientate their head rightwards on hearing the whinny of a non-group conspecific, but no bias was shown in response to the whinnies of group members. In addition, a different pattern of bias in ear orientation was shown to the vocalisation of a neighbour (right) compared to stranger whinny (no bias), which, the authors argued, may depend on level of arousal.

Further research appears to indicate lateralization in horses may be influenced by handling by humans under some conditions but not others. Irrespective of whether horses had been trained on both sides or trained conventionally on the left side, they displayed a left-eye preference for scanning their surroundings, and this left-eye bias was stronger in the presence of a familiar or strange human standing passively nearby (Farmer et al., 2009). However, the left-eye preference to view a human stranger was weaker in horses that had been trained on both sides than in horses trained on the left side. When a familiar human interacted with the horse, it displayed a stronger left-eye preference and this preference was not affected by prior bilateral or asymmetric training of the horse. The effect of type of handling on the strength of the left-eye bias to view a human seems to depend on the familiarity of the human. Farmer et al. (2009) suggested the left-eye preference to view humans might reflect right-hemisphere specialisation for social interactions and rapid responses. This could be similar to findings in other species (Chapter 1, *Section 1.2,* p. 2) but perhaps domestication has led to horses evolving a left-side bias for interacting socially with humans because this is the side, known as the near side, on which humans traditionally handle them.

2.3 Domestication

As yet, it is impossible to say whether asymmetries found in domestic horses and other farm animals are generated by human influence, whether by selective breeding or by handling and management practices.

2.3.1 Process of domestication

Domestication usually involves the selection of animals based on tameness, that is, animals that are more submissive towards humans are more likely to be kept as breeding stock. Selecting for one such trait can impact on the expression of other traits including morphology and behaviour (horses, Heird and Deesing, 1998). An experiment was conducted over 40 generations by D. Belyaev to test the effects of selective breeding for tameness on silver foxes (Trut, 2000). After six generations of selecting for tameness the foxes began to show behavioural traits similar to those shown by domestic dogs, such as tail wagging, whining and responding to their name. There are a number of adaptations in animals subjected to domestication, producing a phenotype different from the wild ancestor; these include: colour, body dimensions (e.g. leg length), reduced brain size, less fearfulness, increased susceptibility to predators and greater sociability (Jensen, 2006). Lateralization could also be affected in a similar manner. Moreover, due to human intervention, selective pressures, such as resource availability and predation, have been removed and, therefore, there may be less selection of animals that exhibit strong individual lateralization of these functions simply because they have not needed to engage simultaneously in vigilance while feeding.

Selective breeding for non-asymmetrical traits may also affect lateralization. It has been shown that selection for two morphological traits in modern eventing horses, longer legs and a smaller head, may influence motor preference. A study by van Heel et al. (2006) found foals with the above characteristics were more likely to display a limb preference, which the authors concluded is detrimental to conformation because it leads to uneven hoof loading. In other words, behavioural laterality resulted in morphological asymmetry. Similarly Dalin et al. (1985) found an association between size and asymmetry; bigger horses had greater hindquarter asymmetry in the heights of the tuber sacrale bones. It is possible that this relationship may also occur for perceptual side biases. For instance, selection of tameness may have inadvertently selected against lateralization of aggression.

Denenberg (2005) believes non-lateralization serves as a failsafe mechanism when there is little complexity in the environment and, therefore, there is no need for cognitive efficiency. Since complexity is likely to be lower in the domestic environment, domestic horses may have weaker lateralization than horses living in the natural environment and under natural social conditions. One of the effects of domestication is reduced brain size (see above) and this may indicate lower cognitive demands with reduced need for the cognitive efficiency that lateralization offers.

2.3.2 Domestication of horses

Recent evidence suggests horses were first domesticated 3500 BC (Outram et al., 2009). There is some disagreement on the nomenclature of the horse and how many subspecies of wild horse existed before domestication. Wilson and Reeder (2005) described three subspecies of the horse that have survived into modern times: *Equus ferus przewalski* (Przewalski), *Equus ferus ferus* (tarpan, now extinct 1909) and *Equus ferus caballus* (domestic horse). [Note that in most of the literature, domestic horses are usually referred to as *Equus caballus*.] According to Groves and Ryder (2000), three subspecies of horses existed throughout Eurasia prior to domestication; they were the steppe tarpan (*Equus ferus ferus*), forest tarpan (*Equus ferus sylvestris)* and the Przewalski horse (*Equus ferus przewalski*). Bennett and Hoffman (1999), on the other hand, described the geographic locations and morphologies of seven subspecies of horses belonging to *Equus caballus*, four of which existed in Europe, and these are believed to have founded the modern domestic horse.

Since the tarpan became extinct last century, the Przewalski horse is the closest undomesticated surviving relative of the domestic horse (Groves and Ryder, 2000). Przewalski horses are of similar appearance to the tarpan; they are short and buckskin coloured with a dorsal stripe. They differ from the modern domestic horses in karyotype (Przewalski horses have 66 chromosomes and domesticated horses have 64, Benirschke et al., 1965) and they exhibit more primitive physical characteristics, such as an erect mane and no forelock (Groves and Ryder, 2000). However, genetic studies have shown the Przewalski horse is unlikely to be the immediate ancestor of the domestic horse (Bowling et al., 2003; Lau et al., 2009).

It has been proposed that domestication occurred a number of times in separate geographical regions, involving different subspecies and giving rise to the founders of the different breeds of horses that we have today (Bennett and Hoffman, 1999). Jansen et al. (2002) and Vilà et al. (2001) concluded that, in order to account for the level of variation in mitochondrial DNA of the horse, a number of domestication events must have taken place at different geographical regions with multiple founders. High genetic variation is present in the matrilines (Vilà, 2001; Jansen et al., 2002; Lau et al., 2009), whereas low variation exists in patrilines (Lindgren et al., 2004; Lau et al., 2009), indicating the inclusion of numerous mares from different regions but few stallions. The same pattern of variation in maternal and paternal DNA has not been found in Przewalski horses (Lau et al., 2009).

Przewalski horses display more aggression towards conspecifics than do domestic horses (Feh, 1988) and, although both Przewalski and domesticated stallions generally exhibit similar types of social behaviour, the former spend more time closer to conspecifics, engage in more social interactions and mutual grooming, and differ in investigative behaviour of conspecifics (Christensen et al., 2002). In the latter study, however, the authors could not rule out the influence of other factors; the domestic stallions tested were all two years of age, had been weaned at four months, and were in a four-hectare enclosure, whereas the Przewalski stallions were of various ages and were free-roaming on a large reserve.

So far, attempts to tame Przewalski horses have been unsuccessful (Bowling and Ruvinsky, 2000). These characteristics of Przewalski's horse perhaps indicate that they may exhibit behaviour that is similar to that of the tarpan prior to domestication.

2.4 Relevance of lateralization to the handling and management of farm animals

2.4.1 Lateralization and safety

Knowledge of lateralization in large farm animals could be incorporated into handling practices in order to improve safety. One in five non-fatal farm accidents in the United States of America involves either a cow or a horse (Hendricks and Adekoya, 1998). Accidents involving horses, in particular, can be severe and sometimes fatal. According to hospital admissions data in Australia (summarised by Cripps, 2000), the most prevalent injury from horses is head injury resulting from being thrown off the horse. In the United States, there were on average 102 904 non-fatal equestrian injuries per year between 2001 and 2003 of which 11 502 resulted in brain injury (Thomas et al., 2006). Kriss and Kriss (1997) reported 40% of injuries involving horses result from being kicked. These findings are important because it is possible that some of these injuries could have been prevented by understanding side biases of aggression and reactivity in domestic horses if these are present.

2.4.2 Lateralization and welfare

Handling practices that take into account human safety are also beneficial to the welfare of the animal because they decrease stress. It has been shown that horses develop long-term negative or positive memories of humans based on repeated horse-human interactions, and these memories influence future interactions (Fureix et al., 2009; Sankey et al., 2010).

Stress in livestock, such as sheep, pigs and cows, is known to affect productivity and the wellbeing of an animal (e.g. Broom and Johnson, 1993; Voisinet et al., 1997; review, Grandin, 1998). Such stress may be minimised by accounting for side biases of the animals. The right hemisphere has been shown to control activation of the hypothalamic-pituitary-adrenal-axis in rats (Sullivan and Gratton, 2002) and is associated with the stress responses. For instance, elevated activity of the right hemisphere in marmosets, measured by tympanic membrane temperature, is associated with the stress of being captured (Tomaz et al., 2003). If farm animals show right-hemispheric functions similar to other animals (e.g. fear and aggression), stress might be reduced by handling and leading the animal on its right side so that it might use its left hemisphere to process information about an object or handler. Alternatively, use of the left hemisphere may inhibit fear responses thus enabling approach and learning.

As discussed previously (Chapter 1, *Section 1.2.1*, p. 6) limb preference may be associated with an animal's temperament and cognitive bias provided that, as discussed by Rogers (2010), the animal is in a relaxed state when limb preference is measured and the task used to measure limb preference does not demand the use of functions of a particular hemisphere. Limb preference can be assessed non-invasively and could be used to predict how an animal is likely to react in a given situation, i.e. whether the animal will be fearful of novel environments and objects. Furthermore, limb preference may be a predictor of an animal's cognitive bias and, as proposed by Rogers (2010), cognitive bias may be a reflection of which hemisphere of the animal is most activated.

Research investigating the effects of handling procedures has shown that, when released from restraint, sheep have a positive cognitive bias compared to unrestrained, control sheep (Doyle et al., 2010). Cognitive bias gives an insight into the coping style of an individual. As suggested by Rogers (2010), primates with a left-limb preference, measured when they pick up food, may show a negative cognitive bias and reactive coping style, whereas right-limb-preferring ones may show a positive cognitive bias and a proactive coping style. There is evidence this is the case in the common marmoset (Gordon and Rogers, 2010b). Animals with different coping styles may be suitable to different types of housing and handling procedures and limb preference might be a means by which animals can be selected for different purposes and housing conditions.

It is also possible that the animal's affective state may be deduced from the eye used to view objects; as discussed in *Section 2.2* (p. 16). Left-eye use in domestic horses is associated with greater reactivity (Austin and Rogers, 2007; Larose et al., 2006).

2.4.3 Lateralization and early experience

Management and handling practices, sometimes very intensive and stressful, may have an impact on lateralization of farm animals. Although they did not test it directly, Phillips et al. (2003) argued the stronger laterality shown by an intensively managed herd of cows, in comparison to an extensively managed herd, is a consequence of greater stress experienced by calves following early (1-day-old) removal from their mothers, after which the calves were housed indoors in separate pens. In contrast to intensively managed cattle, extensively managed cattle were living in large fields as a herd, mating was natural and calves remained with their mothers until six months of age.

It is known from empirical studies that early experience can influence lateralization (review, Schaafsma et al., 2009; Vallortigara and Rogers, 2005; Groothuis et al., 2010). It has been demonstrated that early experience of light stimulation on the embryos of chicks and pigeons resulted in development of asymmetry in certain visual pathways (Rogers and Bolden, 1991; Rogers and Deng, 1999; Manns and Güntürkün, 1997). Lateralization in these visual pathways produces lateralized behaviour of a number of functions (e.g. discrimination of food from non-food items, vigilance for and fear of predators, aggression), as demonstrated by comparing chicks and pigeons incubated in the light with those incubated in the dark (chicks, Rogers, 1982;

Zappia and Rogers, 1983; Rogers, 2000; Dharmaretnam and Rogers, 2005; pigeons, Güntürkün *et al.,* 2000; Skiba *et al.,* 2002). Such changes in side bias in aggression and fear in chicks have been shown to alter social dominance hierarchies measured in tests of food competition (Rogers and Workman, 1989) and the ability to detect predators (Dharmaretnam and Rogers, 2005). A number of studies on rats (as summarised by Denenberg, 1981, 2000; Cowell et al., 1997) have shown neonatal handling alters the development of lateralization of a number of functions, including taste aversion to lithium chloride and saline, muricide, turning in an open field, level of activity in an open field test, spatial learning in a Morris maze and paw preference. Neonatal handling involves removing rat pups from their mother for three minutes per day. It does not involve any known asymmetrical stimulation and yet it affects development of asymmetry.

Changes in lateralization resulting from early life experience might also affect lateralized behaviour of farm animals (Rogers, 2010). If so, understanding lateralization and how it is influenced by experience would be particularly applicable to livestock production. It would also be important for the management and handling of horses, especially since, as discussed in this Chapter 2, *Section 2.2* (p. 16), limb preference may be detrimental to performance.

2.5 Research conducted for this thesis

An observational study was conducted on three groups of horses - feral, domestic and Przewalski - with the aim of determining whether horses are lateralized. Given that horses are a social, prey species, side biases displayed during agonistic interactions and in response to potential threats were investigated. These types of behaviour are of particular relevance to horse-human interaction because horses are a large potentially dangerous animal prone to flight response. Limb preference was also considered because previous studies were inconclusive as to whether or not it is produced by human handling. Since the side biases found to be present in domestic horses might be entrained by humans, it was considered to be important to study feral and Przewalski horses in an attempt to establish whether or not lateralization is a species-typical characteristic of the horse. It was predicted that feral and Przewalski horses would show left-side biases in agonistic behaviour and responses to potentially threatening stimuli, based on the known lateralization of other vertebrate species (Chapter 1, *Section 1.2*, p. 2).

It was also hypothesised that the degree of lateralization might differ between Przewalski, feral and domestic horses since these groups have undergone different selective pressures, via the processes of domestication and feralisation, and live under different environmental conditions in which they undergo different experiences that might influence lateralization.

2.5.1 Feral horses (Equus caballus)

Lateralization of feral horses was the main focus of this thesis. No prior studies of lateralization have been conducted on feral horses. Indeed, very little research has investigated lateralization in feral animals to see whether they might differ from their captive, domestic counterparts.

Feral horses live under natural social conditions and this may be essential to the development of lateralization. Horses in their natural environment usually form two types of groups: harem and bachelor bands (Waring, 1983). The size of harem groups in Miskali free roaming horses (*Equus caballus*) range from one to nine mares according to the stallion's age (Kaseda and Khalil, 1996), whereas bachelor groups consist of two to six males (Khalil and Kaseda, 1998). In addition, bands that occupy the same home range may join together to form larger herds (Miller, 1983). Ponies roaming in New Forest, United Kingdom, usually form small bands of mares and their offspring, as stallion numbers are low owing to human management (Tyler, 1972). These social groupings were taken into account in collecting data.

Since horses are a social prey species, according to the theory proposed by Vallortigara and Rogers (2005), it was hypothesised they would exhibit population biases during social interactions and predator escape. Since it was thought that the strength of population bias in agonistic interactions might vary according to the type of interaction, data were collected separately for stallion-stallion fights and encounters between members of the same harem band.

2.5.2 Przewalski's horse (Equus ferus przewalski)

Domestication itself may have produced changes in lateralization, as discussed above in *Section 2.3.1* (p. 20), that might still persist in the feral horse populations studied. Therefore, research on the lateralized behaviour of the closest living 'wild' relative of the horse, the Przewalski horse, was undertaken. The other interest in studying lateralization in Przewalski horses was that they live under natural social conditions on a large reserve (in Le Villaret, France). Studies on Przewalski horses in Mongolia report harem bands usually consisting of one stallion, a number of mares ranging from four to eight and several offspring (King, 2002), and there are also bachelor groups (Boyd and Bandi, 2002). Any differences in laterality between feral and Przewalski horses would, therefore, not be attributable to social structure because both groups live under the same natural social conditions. It was hypothesised Przewalski horses would show stronger side bias in agonistic behaviour and reactivity than feral horses because during domestication selection of animals is likely to have been based on low aggression and reactivity so that animals would be more manageable and less prone to unpredictable flight. Since aggression and reactivity are associated with greater activation of the right hemisphere, lateralization of each might also have decreased in domestic and feral horses.

2.5.3 Further research on domestic horses

Further research was conducted on lateralization in domestic riding horses using the same observational methodology that had been developed to measure lateralization in feral and Przewalski horses, primarily to allow direct comparison between the three groups. It was hypothesised that domestic horses would be more weakly lateralized for agonistic behaviour and reactivity than feral horses for two reasons a) domestic horses do not need to be vigilant for predators while simultaneously grazing and b) domestic horses do not live in social groups which may be an important pressure in maintaining lateralization at the population level so that individuals coordinate with each other. Use of observational recording enabled ecologically valid measures of lateralization obtained from domestic horses to be compared to previous reports of lateralization in domestic horses measured using experimental approaches.

As discussed above in *Section 2.2* (p. 16), breeds show differences in lateralization but whether these differences are due to handling or characteristics of the breed is unclear. It is possible that selective breeding for particular traits may have inadvertently influenced lateralization along different lines according to breed. A group of Arab horses was investigated to see if this breed differs in laterality from other breeds investigated previously. This breed was chosen because Arab horses have been selectively bred for speed and endurance (Bowling and Ruvinsky, 2000) and they are considered to be the most flighty of the horse breeds (Hausberger et al., 2004). Given such high flightiness and the possibly of a link with limb preference, as has been suggested by McGreevy and Rogers (2005), it was hypothesised that Arab horses might show stronger limb preference than other domestic breeds.

The following chapter (Chapter 3) outlines the methods used throughout this research. Chapters 4 and 5 investigate lateralization in feral horses to determine whether it is species-typical and expressed in the natural environment. Some comparisons are made with previous research on laterality on domestic horses to see if human handling plays a role in its expression. Chapter 6 then examines lateralization in Przewalski’s horse to determine whether selection during the process of domestication may have influenced lateralization. In Chapter 7 lateralization in domestic horses is considered and compared to feral and Przewalski horses. In particular, the relationship between emotionality and limb preference is examined. The final chapter summarizes the findings in general and discusses them.

CHAPTER 3 GENERAL METHODS

3.1 Introduction

The project consisted of observational fieldwork. The procedure developed for scoring behaviour in feral horses was used for all groups and, importantly, also applied to domestic horses in order to make direct comparisons between these groups.

In all species with a long history of domestication, the question arises whether the traits they show in the present are a consequence of contact with and/or training and management by humans or whether observed behavioural traits have been retained and unchanged despite domestication. Especially possible is a loss of some traits over time or a strengthening of others. In horses, at least, it is still possible to find subjects that have been feral for generations and have had little to no handling. Thus, this species offers an opportunity to study the effect of handling and domestication on lateralization. Feral horses were studied to gain insight into whether lateralization is a characteristic of the horse as a species or the result of domestication. Since there are no truly wild horses remaining in their natural habitat, Przewalski horses were chosen to observe because, although they have been in captivity for the last 100 years, they are the closest, extant relative of *Equus caballus*.

Groups to study were selected using several criteria:

Feral: *Equus caballus* known to have had no direct handling and training, and living in the natural habitat and in a natural social structure (i.e. harem and bachelor bands).

Przewalski: *Equus ferus przewalski* free-roaming living under natural social conditions with harem and bachelor bands and genetically representative of the captive population.

Domestic: *Equus caballus* living in domestic conditions and known to have had direct handling and/or training. Domestic riding horses were chosen because they are predominantly handled on the left side. In addition, a subgroup of ‘unbroken’ (i.e. never been ridden) riding horses and a subgroup of purebred Arab horses were also studied.

Two groups of feral horses were chosen because they differed in the generations for which they had been removed from domestication and in their amount of contact with humans. Research on the domestication of foxes has shown that it takes six generations of selective breeding for tameness for them to show behavioural traits similar to dogs (Trut, 2000). By extension, it is possible that it might take a similar number of generations of feral living for horses to show changes in behavioural traits. The selection of groups of feral horses was determined by the following criteria:

1) Known to have been feral for less than six generations

2) Known to have been feral for more than six generations

Furthermore, the first group was known to have had regular and non-negative contacts with humans, whereas the second group had had minimal contact with humans and, when it had occurred it had been negative and traumatic.

It is necessary to define some terms used in this thesis. Group denotes different populations or types of horses: feral horses were subdivided into Group 1 located at Mona Mona “Aboriginal Mission” and Group 2 at Oxley Wild Rivers National Park. Band refers to a collection of horses that stays together as an independent social unit (i.e. harem and bachelor bands). Band is also applied here to domestic horses to denote field mates – a number of individuals housed together in the same field. Herd describes a collection of bands, which often congregate in the same area and are free roaming over the same home range.

3.2 Subjects and locations

3.2.1 Feral horses

A total of 76 feral horses (37 males and 39 females) were observed at two locations (Figure 3.1).

Individual feral horses were identified by sex, size, colour and distinguishing markings. Colours varied from black, grey, chestnut, buckskin to bay, and size ranged from 12 hands (pony) to 16 hands. Each band of horses was filmed and group size, sex, approximate age and distinguishing markings were recorded.

The ages of the feral horses were unknown and thus described as immature or adult. However, for immature horses it could sometimes be estimated when a horse had been born since the foal had not been present on previous field trips. Horses were then immature if they were still in their natal band and no more than two years old.

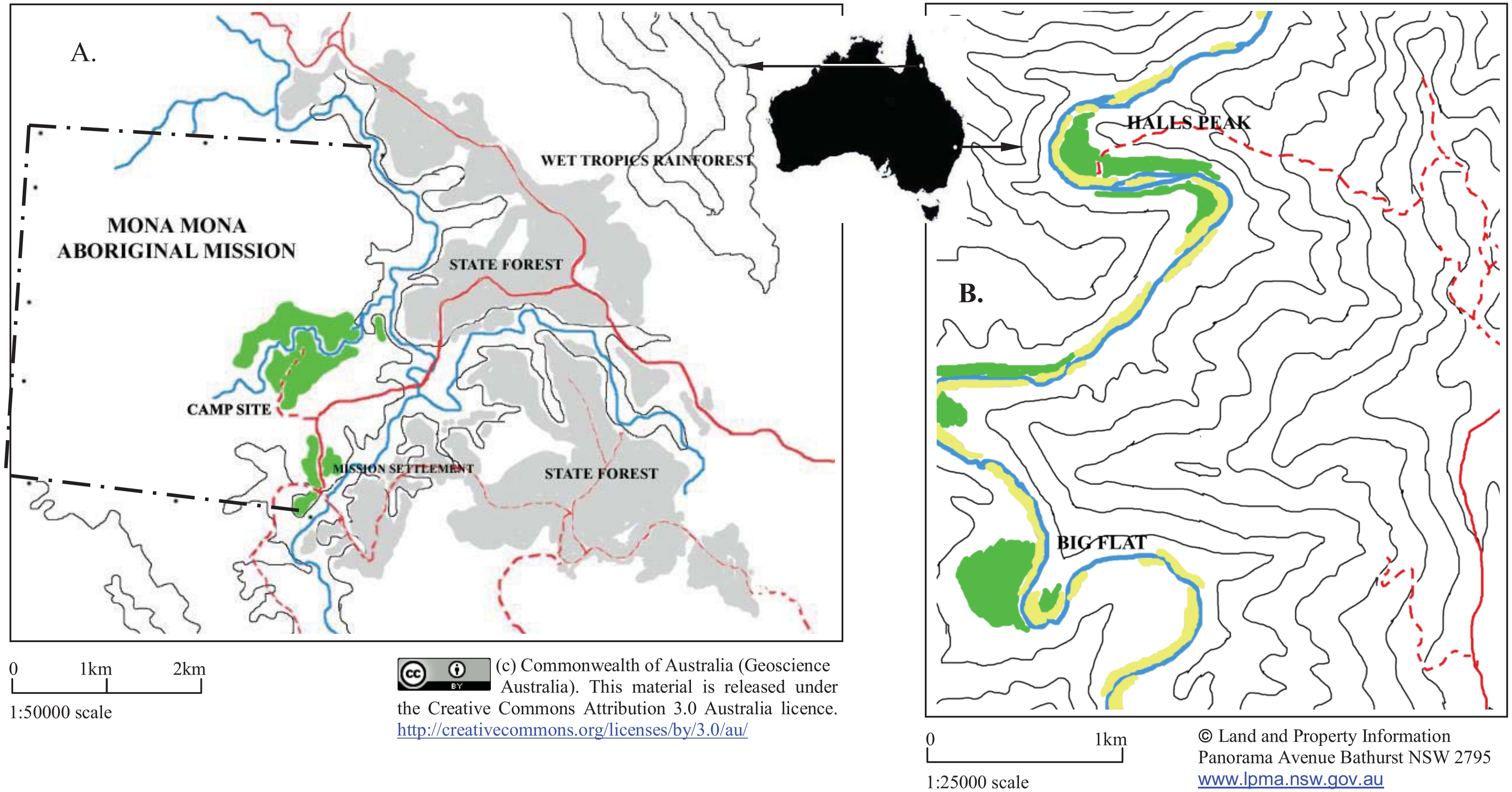

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Figure 3.1 Locations of feral horse fieldwork sites in Australia. A, Mona Mona "Aboriginal Mission". The majority of observations of the horses were made in the green areas and some in the grey areas. (Adapted from topographic maps supplied by Geoscience Australia). B, Oxley Wild Rivers National Park. The two main observations areas were Big Flat and Halls Peak. (Adapted from topographic maps supplied by Land and Property Information, Panorama Avenue Bathurst NSW 2795). Grey – pine forest, green – open grassland, yellow – rocky river bed, blue – waterway, red – unsealed road, red dashed – four-wheel-drive track, black – contour lines at 100m, black dashed lines – Mona Mona boundaries. Maps drawn by the author using data from the topographic maps.

3.2.1.1 Group 1

The first group of feral horses (Group 1) was observed at Mona Mona “Aboriginal Mission” (coordinates, S: 16°43′ 30″, E: 145°31′ 00″; Mona Mona was a mission from 1913 until 1962), situated on the Atherton Tablelands approximately 50km north-east of Cairns in tropical North Queensland, Australia. Data were obtained from 20 unhandled horses (10 males and 10 females), of which 14 were adults and 6 immature. Further details of Group 1 horses are provided in Appendix I. An additional three horses frequented the bachelor band and interacted with other stallions but behaviour was not scored for these horses because they had been handled previously.

The Group 1 horses consisted of four harem bands and one loosely affiliated bachelor band. The age of the immature horses at the first sighting ranged from one day to two years. It has been estimated that this group is two to five generations feral, although the exact number of generations removed from domestication is not known precisely. The horses are known to be descendents of horses released at Mona Mona in 1962, with an additional 12 horses released there in 1986–88 and another three in the mid-1990s. Some of the breeds released included Australian stock horses, Shetland ponies, Arab horses, quarterhorses, and cart-horses of unknown breeds and station horses of mixed unknown breeds.

The Group 1 horses roam freely over an area of 20 km^2, both within Mona Mona, which covers 1595 hectares, and in the surrounding State Forest (Figures 3.1A and 3.2). There is a small mission settlement and the horses are often seen grazing in its vicinity but no interactions take place between the horses and people. The study area was in a valley between two mountain ranges running NW/SE. Mona Mona contains several fields of open grassland and native forest (including tropical rainforest, she-oak and eucalyptus woodland). The surrounding area consists of a number of blocks of pine forest (State Forest) with trails leading through them, native forest and freehold farms on the southern edge. During the wet season the area becomes very boggy, slippery and unstable, making grazing areas very uneven and difficult to walk across.

The climate of North Queensland is tropical with well defined wet and dry seasons. The wet season involves monsoonal rain for four to six months and usually begins in December and ends in April. Data were collected in dry weather between August and December since the area is inaccessible at the height of the wet season and these are the months in which the horses are most likely to congregate in the more open areas.

Table 3.1 Fieldtrip locations and dates

Group	Location	Fieldtrip dates	Number of days observed
Feral Group 1	Mona Mona “Aboriginal Mission” in North Queensland (NQ)	**1.** October–December 2007	44
		2. July–September 2008	23
		3. November–December 2009	9
530 hours of observations		**Total days**	**76**
Feral Group 2	Oxley Wild Rivers National Park NSW	19–21 July 2008	*3*
		1–9 October 2008 & 13–28 October 2008	25
		23 May–4 June 2009	11
		3–20 October 2009	18
540 hours of observations		**Total days**	**57**
Przewalski	Takh Association, Le Villaret, France	**4.** 18 July–17 August 2009	31
340 hours of observations		**Total days**	**31**
Domestic (pilot study)	Kuranda district, NQ	**1.** January 2008	13
Domestic recreational and purebred Arab	Private properties, Kuranda district, NQ	**3.** November 2008–February 2009	23
Domestic (purebred Arab)	Copeland Arabian Stud, NQ	**3.** December 2008–January 2009	9
Domestic (working horse)	Blazing Saddles Trail Riding, NQ	**3.** February 2009	13
Domestic (purebred and crossbred Arab)	Centre for Eco-Ethos Research and Education, France	**4.** 14–16 July 2009 26 August-3 September 2009	12
440 hours of observations		**Total days**	**70**

1. Field trip to NQ from 21 September 2007–4 February 2008; 2. Field trip to NQ, 11 November 2008–4 March 2009; 3. Field trip to NQ from 30 July–3 September 2008; 4. Field trip to France, 14 July – 3 September 2009. Italics – no data collected.

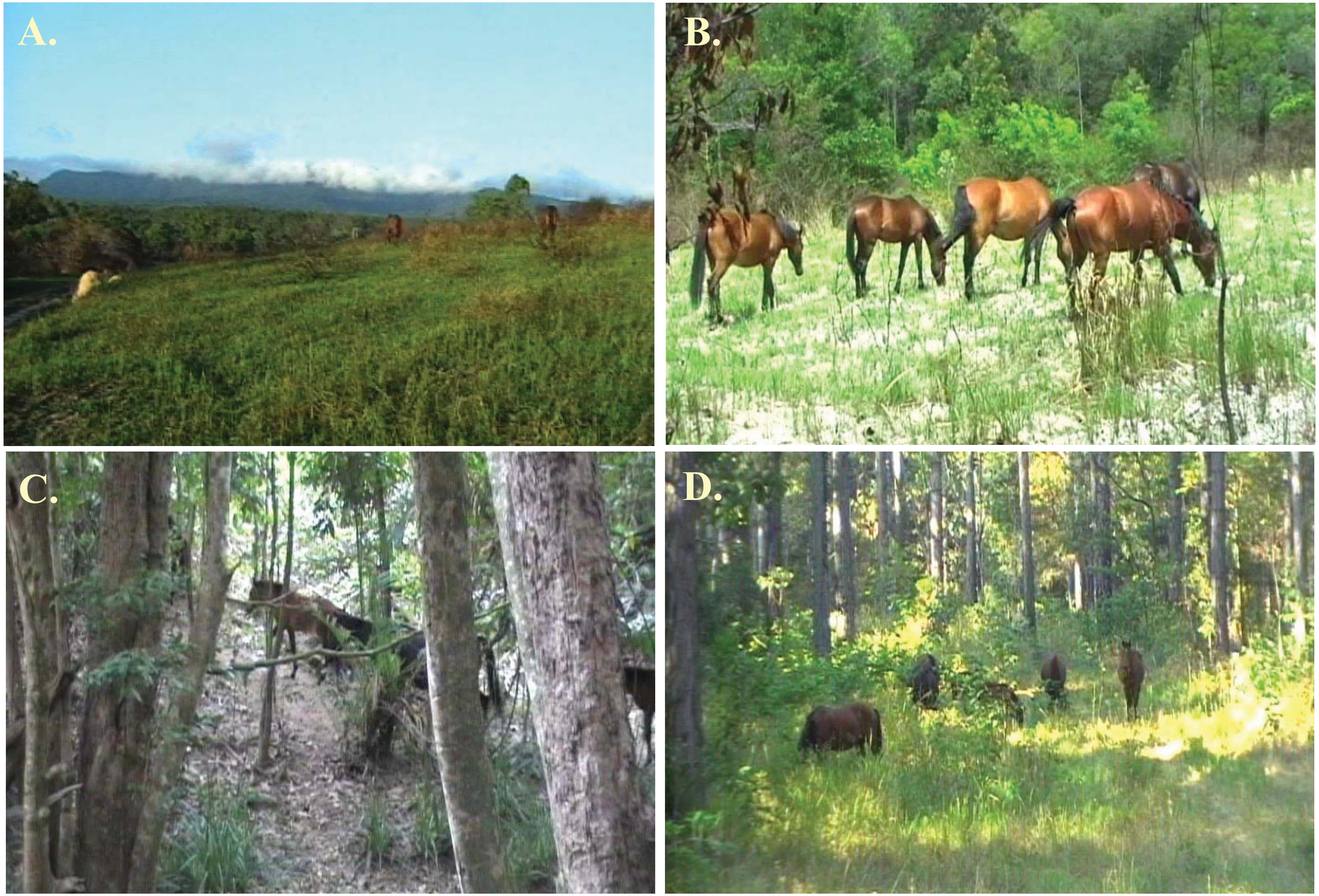


Figure 3.2 Mona Mona “Aboriginal Mission” showing terrain and horses. A, B and C: Mission. D: Pine Forest. Permission to use photographs given by Rhonda Brim, Djabugay elder.

A total of 76 days were spent in the field observing Group 1 feral horses in 2007 and 2008. The dates of fieldtrips, number of days spent in the field and total hours observed are summarised in Table 3.1. Behavioural data were collected between 07.00h and 18.00h depending on the movements of the horses, daylight, weather conditions and observer fatigue. Since on many occasions considerable time was spent finding these horses, actual observation hours each day ranged from six to eight hours. In total, Group 1 feral horses were observed for at least 500 hours. Finding the horses initially entailed checking open grassy areas that the horses frequented (knowledge was gained through talking to the Djabugay people) and driving along accessible roads to evaluate where recent activity had occurred; this was deduced from fresh faeces and hoof tracks. The main mode of transport used to locate horses was a mountain bicycle because it was quiet and, hence, allowed detection of sounds, such as snorting or sounds made by movement, indicating horses were nearby; the horses were often difficult to see in the forest. Once they were located, the bicycle was hidden behind vegetation and the horses were approached and followed on foot.

The horses for which data were collected had not been handled, although they were habituated to the presence of humans to the extent that, after raising their head to look at an approaching person, they soon returned to grazing.

3.2.1.2 Group 2

The second group of feral horses (Group 2) was located in a mountainous temperate climate zone in Oxley Wild Rivers National Park (coordinates S: 30°45′54″, E: 152° 00′27″), situated 50kms south-east of Armidale, New South Wales (Figures 3.1B and 3.3). Fifty-six horses (nine harem bands and one loosely affiliated bachelor band) were observed along the Chandler River. This sample comprised 27 males and 29 females, of which 19 (9 females, 10 males) were immature at the time of data collection on limb preferences (Appendix I). Age was estimated to range from 3 months to 20 years. The horses were free-roaming and unhandled by humans. Records obtained from National Parks and Wildlife Service show that these horses are descended from those released into the park in the 1910s and 1940s; these horses have been feral for 10–20 generations. The horses that were released were of mixed breeds.

The study area was in the heart of the Oxley Wild Rivers National Park and accessible only by four-wheel drive and only at certain times of the year. The Group 2 horses were observed in a deep gorge along a 7km stretch of the Chandler River. The habitat consists of native eucalyptus forest with grassy verges on which the horses graze. The horses were sighted on the steep sides

Figure 3.3 Oxley Wild Rivers National Park showing terrain and horses. A and C: Halls Peak. B and D: Big Flat. Photographs used with permission of Kenneth Pines, NSW National Parks and Wildlife Service.

of the gorge at times and moved up and down the river. The river bed was extremely rocky and unstable to walk over.

The majority of observations were conducted at two places. Both places had several vantage points from which the horses were clearly visible. The first place was at Halls Peak where a large group of 10 horses was observed. This consisted of a small open area and openly wooded flats along both sides of the river.

The second place was further down-river and involved driving or walking along the rocky river bed and crossing the river in a number of places. This second place was often inaccessible due to flooding and it was necessary on several occasions to hike the last 7km carrying camping and research equipment. This place was remote and not frequented by people. It was a large open grassy area. Bands congregated in this area only from October onwards into the summer months (December – February). Research was unable to be conducted after October because of threat of bushfires from the months of November to March.

Oxley has a temperate climate and some of the observations were made during the winter months when there were sub-zero night temperatures. Summer daytime temperatures often rose to above 30°C. Data were collected in the months of June/July and October.

Behavioural data for Group 2 feral horses were collected between 06.00h and 19.00h depending on the movements of the horses, daylight hours, weather conditions and observer fatigue. Fieldwork was conducted over 57 days in 2008 and 2009 (summarised in Table 3.1, p. 33), the horses being observed for 10 to 12 hours a day. These horses were unable to be observed for the originally planned amount of time because of restricted access (above); a total of at least 540 hours of observations were made. A number of planned fieldtrips were cancelled due to an unusual amount of flooding.

Unlike the horses at Mona Mona, the horses within this park have been culled and mustered on occasion (NSW National Parks and Wildlife Service, 2006) and are fearful of humans. Since mustering had last occurred in 1998 in a different area of the park, it was unlikely that any of the horses in the study had undergone this experience, but this cannot be ruled out. In fact, since they had had very little experience with even sighting humans, they were initially highly vigilant and reactive to the presence of the observer, who could watch from a distance of no closer than 100m while remaining stationary. Later, some of the horses approached the observer and grazed at distances between 20 to 100m.

3.2.2 Przewalski Horses

An opportunity arose, made possible by Dr. Marthe Kiley-Worthington from the Centre for Eco-Ethos Research and Education situated in the Rhone Alps France, to conduct a short term study of one month on laterality in Przewalski horses in France. This is a captive herd being bred by the Takh association, founded by Dr. Claudia Feh, as part of a breeding and research program to re-introduce the Przewalski horse back into its natural habitat on the Mongolian Steppes. Importantly, as stated by Association pour le cheval de Przewalski: TAKH (2008), the 11 founder horses at Le Villaret are genetically representative of the entire captive population.

The Przewalski horses were observed July/August 2009 (Table 3.1, p. 33) on a 200 hectare reserve at Le Villaret (N: 44°15'06.56", E: 03°26'37.09") situated on the Causse Mejan, Lozere. This is a limestone plateau chosen for its similarity to the habitat from which the Przewalski horses originate. The reserve is open, with very few trees, and is hilly and extremely rocky (Figure 3.4). The reserve was established by Dr. Feh in 1992.

At the time of this study the herd consisted of 33 horses (20 males and 13 females; Appendix II). The respective ages of the Przewalski horses were obtained from the Takh Association, which keeps precise records of these horses. The horses roam freely under natural social conditions as family and bachelor bands. There were four family bands and four bachelor bands. These horses have been studied extensively and are consequently used to being followed and observed at close distances.

Behavioural data for the Przewalski horses and domestic horses were collected between 06.00h and 21.00h with the same constraints as described above for observing feral horses. Przewalski horses were observed usually for 12 hours a day.

The Przewalski horses were difficult to identify since they were similarly coloured, all being light brown/yellow to light reddish brown with black manes and tails and, unlike the domestic and feral horses, they had very few distinguishing markings. The horses were identified using the same practice as used by the researchers at Le Villaret. Most of the markings used to identify the Przewalski horses are transient; new scars are routinely recorded on a weekly basis. Copies of drawings of the outline of the horses from different angles were provided by the Association and any distinguishable markings and scars were drawn onto these. During the period of observation, bands remained stable and this aided identification of individual horses. The researcher was able to identify all horses and this was checked on two occasions by the caretaker of the horses.

Figure 3.4 Takh Reserve at Le Villaret, France, showing terrain and Przewalski horses. Photographs used with permission of Dr. Claudia Feh.

3.2.3 Domestic horses

A total of 84 domestic riding horses (41 males and 43 females) were observed from November to February 2008 (Table 3.1, p. 33). These included 23 purebred Arab horses (9 males and 14 females), 32 recreational riding horses of various breeds excluding purebred Arab (14 males, 18 females) and 29 working trail-riding horses (18 males and 11 females). An additional six horses (five males and one female) were observed in the first field trip (January 2008) but these were unavailable for observation in the following year due to having been sold, the owner being ill, or the owner moving away. Data from these latter horses were used only in the pilot study on limb preference. One purebred Arab and one crossbred Arab horse were excluded from analysis of limb preference because of chronic lameness.

Seventy-five of these domestic horses were observed on the Atherton Tablelands to the east of Cairns in North Queensland, Australia (within 20 km of coordinates S: 16° 49' 0, E: 145° 39' 0). They were housed on 10 privately owned properties, the Copeland Arabian breeding stud, and at Blazing Saddles trail riding. The properties on which the recreational horses were housed varied in size from 2 to 100 hectares (Figure 3.5). Fields were usually undulating, and often hilly, and vegetation varied from open grassland to denser forest (which the horses do not enter) on two of the properties. The Copeland Arabian stud is 10 hectares with both relatively flat open fields and forested undulating fields on uneven ground. These fields vary in size and the horses were rotated from one field to the other. The horses from Blazing Saddles were observed in a five hectare relatively open field in which they were housed during the day; at night they were released into a larger 400 hectare field of undulating open woodlands and grasslands.

Observations of the recreational riding domestic horses belonging to private owners were carried out between 09.00–17.00h depending on property access, feeding times, and weather conditions and those of the trail riding horses between 10.00 – 16.00h. On average the privately owned and trail riding horses were observed for six hours a day. The horses at Copeland Arabian Stud were observed for nine hours a day between 08.00 – 17.00h.

A second smaller group of purebred and crossbred Arab domestic horses ($N = 9$) was observed in July and September 2009 at the Center for Eco-Ethos Research and Education in Saillans (N: 44°41′51″, E: 5°11′53″), France, owned by Dr. Kiley-Worthington. The property is 172 hectares and is extremely steep (Figure 3.6). This is a temperate climate with warm dry summers and extreme cold temperatures with snow during the winter. Data were collected between 06.00h and 21.00h.

Figure 3.5 Some of the domestic horses from North Queensland and the fields in which they were housed. A and B: Private properties. C: Blazing Saddles. D: Copeland Arabian Stud. Photographs used with permission of the owners of the horses.

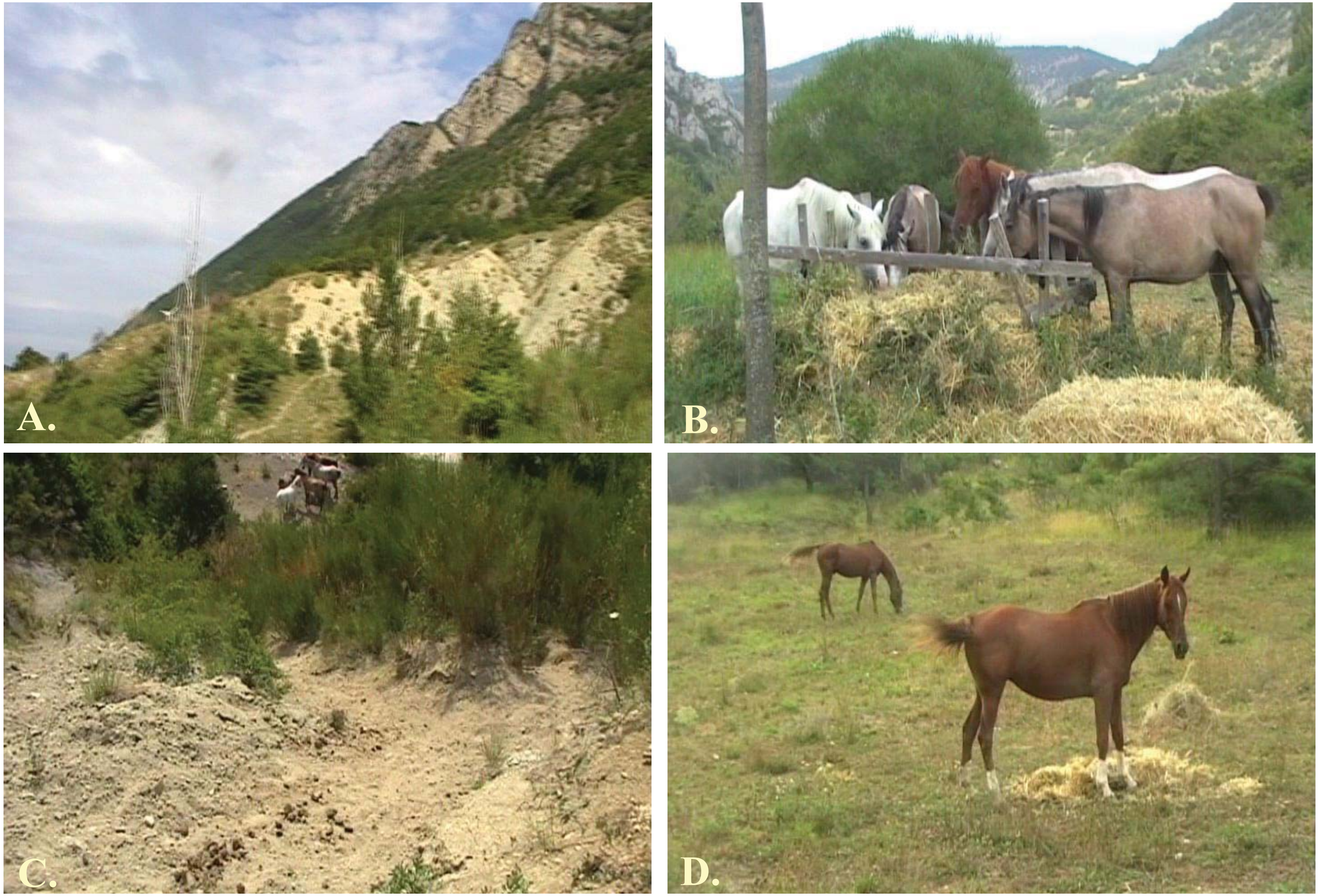


Figure 3.6 Arab horses and the terrain at the Center for Eco-Ethos Research and Education in France. Photographs used with permission of Dr Kiley-Worthington.

The group of purebred Arab horses belonged to five private recreational horse owners, the Copeland Arabian Stud, and the Centre for Eco-Ethos Research and Education. There was also a subgroup of the riding horses that had never been 'broken in' to saddle and, therefore, had never been ridden ($N = 11$). Further details are provided in Appendix III.

The majority of the domestic horses were known and easily identifiable. The owners provided information such as age, breed and some history of use. Distinguishing markings, such as leg markings (e.g. white socks or stockings) and the shapes of white markings on the head (e.g. blaze, stripe, star), were noted, as well as colour and approximate height.

3.2.4 Observer effects

The main concern with observing the feral horses in particular, was not to startle them. In order to avoid stress, horses were gradually habituated to the presence of an observer. At no time was food provisioned. The direction from which the horses were observed depended on visibility but was considered to be random since they often changed their orientation with respect to the observer.

On approaching Group 1 bands (feral horses in Queensland) the observer was attentive to any behavioural indicators of stress such as cessation of grazing and looking at the observer. If any such responses occurred the horses were approached no closer. Horses were watched from this distance and allowed to approach the observer themselves. Group 1 feral horses were able to be followed on foot and watched at distances of no less than 10 metres but most observations were made usually from 20 to 50m away. Where possible, these horses were watched by the observer in a seated position, but more often vegetation obscured viewing so they had to be watched from a standing position. Care was taken to make no sudden movements. If, at any time, horses did show nervousness at being watched, recording was ceased.

The particularly fearful Group 2 horses (feral horses at Oxley) were not approached. Instead the observer watched them from vantage points while adopting a seated position and remaining stationary. Over time these horses did approach to within a distance at which data could be collected. Some bands took less than an hour to do so, whereas others took one week. Observations were made from 20 to 200m away; observations at the greater distances were made using binoculars and some stallion fights were filmed at these distances.

The Przewalski horses were habituated to being watched by humans. The area in which they were observed was large, open grassland with very little vegetation. They could be watched from many vantage points while the observer was seated or they could be followed and watched while standing. They were usually watched from a distance of 20 to 50 metres. Observation of the domestic horses was conducted similarly to that of Przewalski horses.

3.2.5 Materials

3.2.5.1 Equipment

Research equipment included a JVC mini DV digital video camera with 30x optical zoom, a pair of Pentax binoculars with 10x magnification and a Hewitt and Packard palm computer with global satellite positioning capability. The palm computer and a portable keyboard were also useful for working on the data in the field. A monopod rather than a tripod was used to steady the camera since the monopod was easier and lighter to carry when the horses were moving constantly; it was also easier to set up on uneven ground and did not startle the horses. A mountain bicycle was used as transport at Mona Mona.

To observe the feral horses at both sites it was necessary to camp for one to three weeks at a time, using minimal equipment. A small solar panel and a 12 volt battery were used to charge the palm computer, camera batteries, and other batteries. First Aid equipment and an Emergency Position Indicating Radio Beacon (i.e. for helicopter rescue) were necessary for safety when camping at Oxley Wild Rivers National Park because the area is very remote and potentially dangerous, and no other people were nearby. Also a satellite phone was used to make daily contact calls since no other form of communication was possible.

3.3 Methods

Most behavioural observations were recorded in the field with pencil and paper using a shorthand code to notate the different types of behaviour. More complex interactions, such as stallion fights, were video-taped and scored later. Session times were recorded with stopwatches, as were the interval times used in sampling of limb preference. The time of day, date, climatic conditions, area and environmental events that could possibly affect behaviour were noted. Some video-tape of stallion fights for Group 1 horses filmed in the year prior to commencing this PhD was analysed and included in the present dataset.

3.3.1 Types of behaviour recorded

Three categories of behaviour were recorded: side bias in vigilance, percent reactivity and high alert; eye use during agonistic encounters with conspecifics; and limb preference. The following behavioural categories were recorded for the three groups of horses (feral, domestic and Przewalski). Sampling sessions for all behavioural types commenced when a focal animal/band was grazing and clearly visible. Head-turning bias in vigilance, high alert and percentage reactivity, and eye bias in agonistic interactions were sampled continuously using focal animal/band sampling. All occurrences of the behaviour being sampled were recorded. For limb preference, interval sampling was used. A more detailed description of session times for each group is provided in the respective chapters.

3.3.1.1 Head-turning bias in vigilance, high alert and percentage reactivity

Head-turning behaviour shown by horses in response to the detection of stimuli in their surroundings was recorded. Head-turning bias in vigilance was measured as the number of times a horse lifted its head from grazing in order to look to its left or right, irrespective of the height to which the horse lifted its head. In addition, percentage reactivity and side bias in high alert measures took into account how high the horse lifted its head as well as the direction to which it turned its head. These responses are unpredictable and happen quickly so they were recorded exclusively in the field whenever they occurred using a pen and paper record. Recording was carried out while the horses were grazing. Each time a horse raised its head from the position adopted during grazing in order to look at a stimulus the following were recorded:

- the direction in which it turned its head,
- the height to which it raised its head,
- ear position,
- where and what the horses could have been looking at, and
- whether the horse held its head up for more than 3 seconds.

The height to which the horse raised its head was recorded according to the position of the poll (top of the head) on a scale of 1 to 5 (Figure 3.7). When the poll was above the level of the withers a score of 4 or 5 was given, at wither level a score of 3 and below the level of the withers a score of 1or 2. When the poll was at the level of the withers the top line of the neck was horizontal.

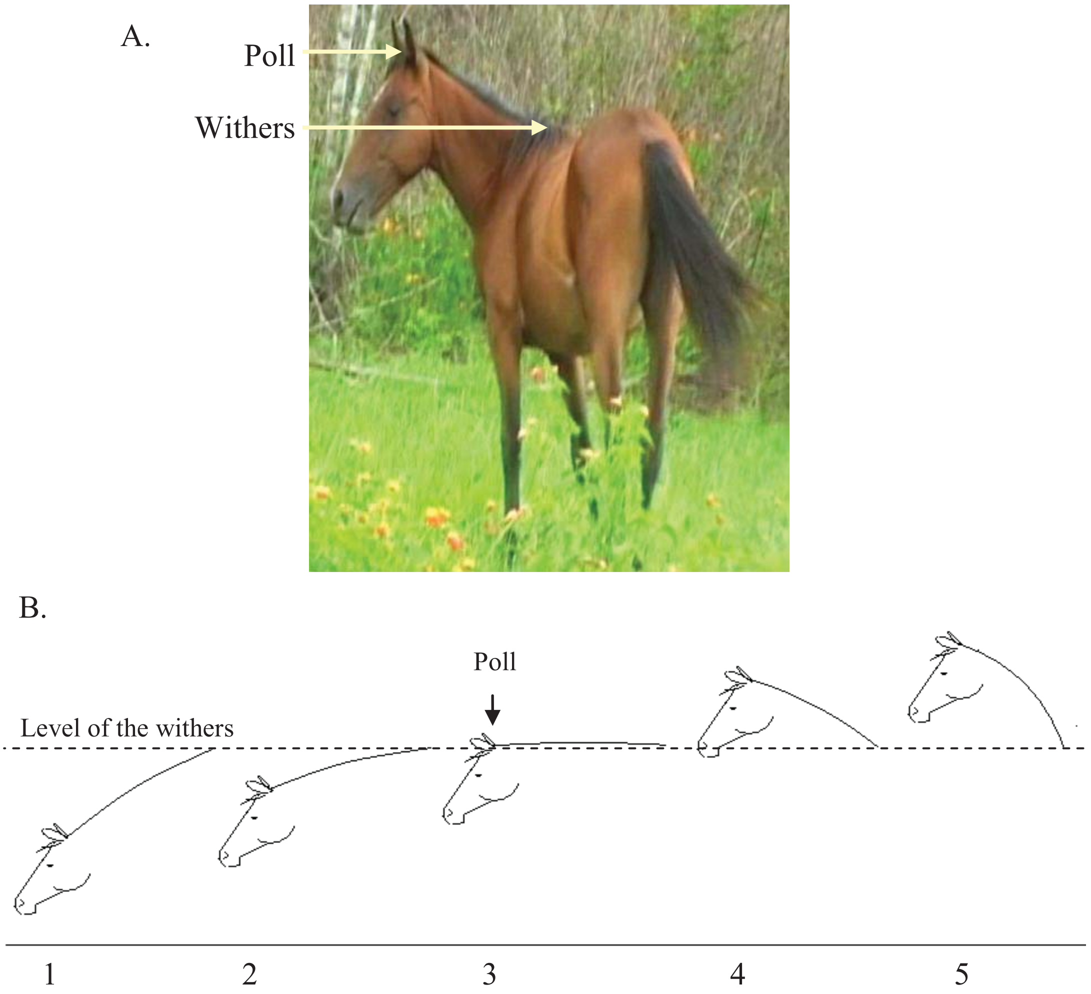


Figure 3.7 A, photo of a horse showing the positions of the withers and poll. B, The heights to which horses raised their head to look at a stimulus. Head height was scored, from left to right: 4 or 5 – poll above the level of the withers; 3 – at wither level; 1or 2 – below the level of the withers. Dashed line indicates the level of the withers.

Head lifts above the level of the withers were considered high and head lifts at or below the level of the withers were considered low. A high postural tonus while looking at a stimulus, known as the alert posture (and referred to in this thesis as high alert), indicates higher arousal and preparedness for locomotion (Kiley-Worthington, 1976; Waring, 2003). Research has shown head height is positively associated with heart rate (Visser, 2002; Rietmann et al., 2004). In addition, Rietmann et al. (2004) showed that a higher head was associated with lower heart rate variability and, importantly, greater activation of the sympathetic branch of the autonomic nervous system.

To calculate laterality, only those scores that involved the horse turning its head by more than 30 degrees from the midline were used. The laterality index of vigilance was calculated for each horse as (right - left looks / left + right looks). A percentage reactivity score was calculated as the proportion of looks that involved the head above wither level (high head / high + low head) for looks to the left and for looks to the right from each horse. A laterality index of high alert was calculated for each horse from looks that involved only head lifts that were above the level of the withers.

In addition to the scores for lateralization, bands of feral horses were also observed for a fixed accumulated sampling period of 10 hours, during which all head lifts from each feral horse were scored (including head lifts with no turning of the head) so that a score for level of reactivity could be calculated. Sampling times differ, however, for individual horses because they were not always in sight and three bands were rarely sighted.

3.3.1.2 Eye preference during agonistic interactions

Agonistic behaviour was recorded using pen and paper. The more complex interactions (i.e. stallion fights) were video-taped and some agonistic interactions between members of harem bands were also captured on video-tape while groups were moving to water or during copulation attempts. Video-taped interactions were scored later using slow-motion and frame by frame playback. Interactions within harem bands, which included mare-mare, stallion-mare, mare-immature and stallion-immature and immature-immature, were analysed separately from interactions between stallions.

All interactions between stallions were video-taped and scored later using slow motion and frame-by-frame playback. Agonistic interactions between stallions were classified as ‘stallion fights’, and such fights included interactions between stallions belonging to both bachelor and harem bands. These interactions could occur between bachelor stallions within the bachelor band or between bands (e.g. a harem stallion guarding a harem band from a bachelor or another harem stallion). Such interactions ranged from single threats to full attacks involving neck biting, rearing, kicking and pushing. They were divided into bouts according to eye use.

First, the eye used by the stallion to look at the opponent’s head region immediately before and during a sequence of agonistic responses was recorded as right monocular, left monocular or binocular (only monocular eye use was analysed). Each look was called a ‘looking bout’ and ended when the stallion looked at its opponent with the other eye, changed opponent or engaged in a new behaviour such as vigilance.

During stallion fights, changes in eye used to view an opponent while engaging in agonistic responses were recorded. Behaviour was considered to be agonistic if the horse pinned its ears back against its head and this response could range from the ears simply facing backwards to the ears being flat against the head (Figure 3.8). Whether used intentionally by the horse to communicate or as an indicator of emotion, pinning of the ears is known as a threat cue (Kiley-Worthington, 1987; Waring, 2003).

Figure 3.8 An example of a horse with its ears pinned (arrow) during an agonistic interaction.

Second, the types of agonistic responses the stallions engaged in within each looking bout were recorded. The responses recorded in the present study are based on the ethograms produced by McDonnell and Haviland (1995) and Heitor et al. (2006). Agonistic responses belonging to eight categories (Table 3.2) were scored. These categories were grouped according to whether they involved threat or attack responses. 'Attack' although referring to responses that involved actual contact or attempted contact (i.e. the recipient moved out of the way) also included the same motor actions (i.e. indistinguishable from those occurring during actual attacks) when the horses were up to a body length apart and there was no obvious gesture of recoil by either horse. For example, two stallions may have reared up and 'boxed' with their forelimbs while remaining in a stationary position at a horse-length apart from each other (illustrated in Figure 3.9). Although it appeared that injury would have occurred had the opponent been in striking distance, and this did occur at other times, intent to injure could not be assumed. Other examples included striking and kicking outwards in the direction of the opponent with one or two limbs, which were also scored as attack.

By contrast, 'threat' included only low level intensity threats such as head movements and lifting of a limb while pinning the ears and there was no contact between the two horses. Sometimes threats involved some contact such as rubbing, touching or sniffing of the opponent while the ears were pinned but there was no biting or pushing of the opponent. Hence, 'threats' were low-level agonistic interactions and 'attacks' were high-level agonistic interactions.

Figure 3.9 Two stallions boxing without contact being made.

Table 3.2 Agonistic responses recorded.

Behaviour categories		Responses
Threat	Head threat	Ears pinned, nose directed at horse Bite threat, head toss (nod or shake), head bow, head turned towards horse
	Kick threat	Tail swish, lifting of a limb, pawing, movement of hindquarters towards opponent, hop (both hind legs lifting off the ground but not projected outwards)
	Other threats	Resting head on the back of conspecific (precursor to mount) Simple rear with no contact or boxing, the forequarters are raised with both forelimbs off the ground
Attack	Bite	Bite Grasp, grasp and head shake
	Kick	Lifting of one or two limbs with outwards projection towards opponent, contact not necessary
	Push	Using the shoulders, hindquarters or head to push another horse away, involves contact or an obvious movement towards an opponent that results in it moving out of the way
	Rear	Mount or rear with contact Boxing (rearing and striking out with forelegs) Dancing (rearing with both horses high and locked together)
	Chase	Lunge (rapid movement towards opponent at distance of less than a body length) Charge (gallop towards horse) or pursuit at a gallop

Based on ethograms produced by McDonnell and Haviland (1995) and Heitor et al. (2006).

Each looking bout was recorded as a ‘threat’ or an ‘attack’ bout. A ‘threat’ bout contained only low level threats and an ‘attack’ bout contained at least one attack response. Therefore, a bout could be a left-eye threat, right-eye threat, left-eye attack or right-eye attack. Some examples of the method of scoring looking bouts according to visual field and type of response are presented in Figure 3.10.

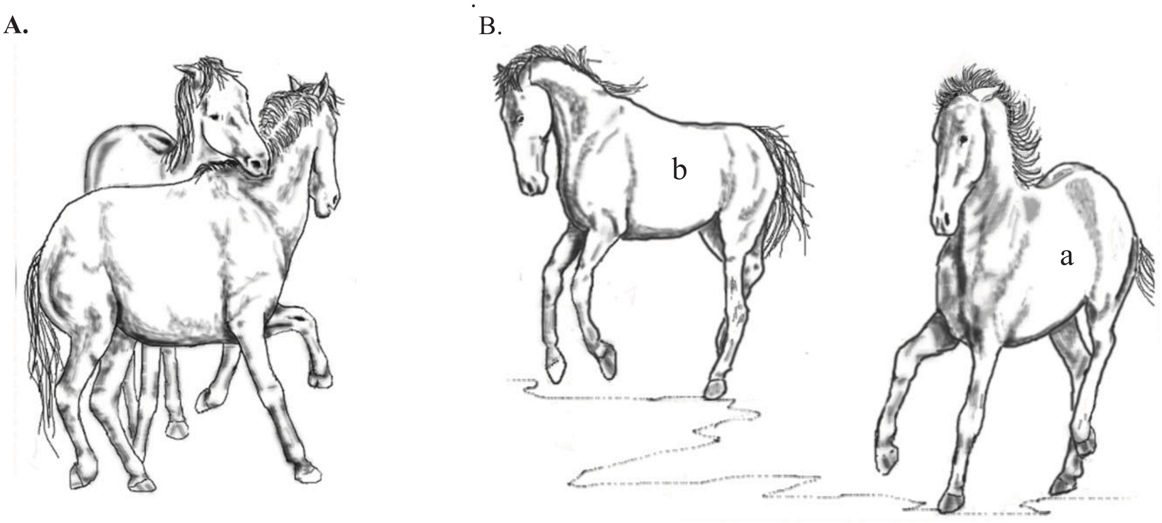


Figure 3.10 Examples of agonistic interactions to illustrate the scoring method. A, This frame was scored as left-eye attack for both of these stallions. The horse in the background is biting and pushing, the horse in the foreground is also pushing and giving a head threat (mouth open to bite). B, In this frame the horse in the foreground (a) is performing a right-eye threat (head threat) and the horse in the background (b) is performing a left-eye threat (simple rear).

The same procedure of scoring was applied to agonistic interactions within harems. These interactions, except for some interactions including attempted copulation, were generally less complex and much shorter in duration than stallion fights; they usually consisted of one to two looking bouts per horse. They were scored in the field using pen and paper. Agonistic behaviour, especially attack, was rare for certain individuals. Therefore, when such an occurrence happened, it was recorded. This sampling often took place when the individual was not part of the current focal group, engaged in the behaviour in clear view and recording did not interfere with the current sampling session. When an interaction occurred between stallions any other sampling ceased in order to video-tape the interaction.

To obtain a score for frequency of aggression for each feral horse, agonistic responses were also scored for a cumulative period of at least five hours per horse/band while they were grazing.

3.3.1.3 Limb preference

In order to learn to identify individual horses by becoming familiar with each horse, the first behaviour to be sampled was forelimb preference while grazing. This required focusing solely on one or two horses at a time.

A pilot study was conducted first in order to determine the most accurate interval for sampling. At set intervals, the forelimb that the horse had placed in front of the other was recorded as left or right (Figure 3.11). If no forelimb was placed in front of the other or the horse was not grazing (i.e. moving or had its head lifted), no score was recorded.

Figure 3.11 The stance adopted by the horse when grazing. The black arrow indicates measure of limb preference recorded in this study, the forelimb placed in front of the other. In this picture a right score would have been given since the right forelimb is in front of the left forelimb.

This preliminary study was based on 15 minutes of video-tape of each of 20 domestic horses while they were grazing. Forelimb position (one relative to the other) was recorded at 30- and 60-second intervals. The percentage of time spent with the left forelimb placed in front of the right was calculated from the instantaneous sampling scores for both the 30- and the 60-second interval as (the number of $\text{left}_{\text{scores}}$ / $\text{left}_{\text{scores}}$ + $\text{right}_{\text{scores}}$). The actual time (continuous sampling) during which each foreleg was placed in front of the other was also determined from the video-tape. Relative duration of the left forelimb placed in front of the right forelimb was calculated as $\text{Left}_{\text{time}}$ / $\text{Left}_{\text{time}}$+$\text{Right}_{\text{time}}$.

Results showed clearly that 30-second-interval sampling gave a more accurate estimate of the time spent with one forelimb advanced of the other (Appendix IV). Thirty-second-interval sampling was more strongly correlated with the measure of actual duration ($r(18) = 0.82$, $p < 0.001$) than 60-second-interval sampling ($r(18) = 0.65$, $p = 0.002$). The absolute differences in percentage scores between the measures of duration of each limb in front of the other and the 60-second-interval sampling data (mean difference = 0.11, $SE = 0.02$) were significantly greater than that of the scores sampled at 30-second intervals (mean difference = 0.07, $SE = 0.01$; $t(18) = 3.27$, $p = 0.004$). Hence, the 30-second interval was chosen for use in the field.

Forelimb preference during grazing was determined using a method similar to that of McGreevy and Rogers (2005) but a record was made of which forelimb was advanced in front of the other at 30-second intervals (every 30 seconds). No record was taken if neither foreleg was in front of the other.

Scores of leg position could be made only when the horse's forelegs were not obscured by vegetation or another horse, or were not obstructed by body orientation (e.g. facing away). Since it was not possible to observe each horse continuously for the entire period (i.e. the horse changed behaviour or moved out of sight) observational periods were accumulated. Each horse was observed for a total of 50 to 70 minutes. Limb-preference data for foals were not collected until they were at least one-month-old.

3.4 Inter-rater reliability

Due to remoteness, logistical problems and the need for special permits for research concerning both groups of feral horses, and the Przewalski horses, observations in the field were conducted exclusively this researcher (NA). However, an inter-rater reliability test was conducted on the agonistic responses captured on video-tape. The second observer was familiar with the behaviour of horses. Descriptions of the various behaviour types were given to the second observer and a sequence of video clips were made showing examples of each behaviour and eye use. This observer, who was naive to the hypothesised side bias, was trained to recognise eye use and the different types of agonistic responses until she felt confident to commence scoring; this training took approximately seven hours over three days. The second observer then scored a set of nine clips that had been chosen by an independent person. The correlations between the two observers for the four different types of looking bout were: left looking bouts with threats only (Spearman's $r_s(7) = 0.75$), left looking bouts with an attack (Pearson's $r(7) = 0.71$), right looking bouts with threats only (Pearson's $r(7) = 0.87$) and right looking bouts containing an attack (Pearson's $r(7) = 0.88$).

3.5 Statistical analysis

3.5.1 Laterality index

A laterality index (LI) was calculated from the data of each horse (LI = R–L /R+L, where L and R are the number of left and right scores, respectively) for vigilance, high alert, threat, attack and limb preferences. *Z*-scores $(L - (L+R/2) / \sqrt{(L+R)/4})$ were used to determine whether each horse displayed a significant individual preference. The strength of laterality index was calculated as the absolute value of the laterality index; it did not take into account the direction of preferences.

3.5.2 G-test

Since the number of scores of vigilance, high alert, threat and attack that were obtained varied greatly between individuals, the *G*-test was used to determine eye preference. The *G*-test is based on the log-likelihood test (Sokal and Rohlf, 1995). Calculations were from the goodness of fit test based on a hypothesis intrinsic to sampled data given by Sokal and Rohlf (1995). The *G*-value for each horse was computed according to the equation: G = observed*ln (observed/expected), where the observed frequency was the number of left scores and the expected frequency was the average value between left and right scores (i.e. no bias). These values were summed and adjusted using William's correction for Type 1 error (Sokal and Rohlf, 1995) and using N-2 degrees of freedom compared to the Chi-squared distribution with alpha set $p = 0.05$. This test takes into account each individual's contribution to the total number of scores.

3.5.3 Other analyses

Statistical tests, with the exception of the binomial *Z*-score test and the *G*-test were conducted using SPSS (PASW18). For all analyses alpha was set at 5% and tests were two-tailed. A Runs test was conducted on forelimb-preference scores to determine whether each score was independent of the previous scores. One sample t-tests were used to determine whether any population biases existed for limb preference because the number of scores obtained did not vary greatly between individual horses.

Normality was assessed using the one-sample Kolmogorov-Smirnov test and equal variance by using Levene's test. Providing these assumptions of normality and equal variance were not violated, ANOVAs and t-tests were conducted on the LI-scores and absolute LI-scores (strength of bias) when sample sizes were roughly equal. The majority of comparisons (age, group etc.) involved different sample sizes. Therefore non-parametric Mann-Whitney tests were conducted. Non-parametric tests were used when normality and equal variance could not be obtained by transformation of data.

The values of percentage reactivity, obtained by calculating the proportion of high head lifts for left and right looks, were arcsine transformed according to Zar (1999). A Wilcoxon signed ranks test (when normality or equal variance could not be obtained by transformation of the data) or t-test (when data were normally distributed) was used to determine significance.

3.6 Ethical note

The study had approval from the University of New England Animal Ethics Committee (AEC) and was in accordance with Australian Government; Natural Health and Medical Research Council (2004) *Australian code of practice for the care and use of animals for scientific purposes,* 7th ed. Retrieved from http://www.nhmrc.gov.au/files_nhmrc/file/publications/synopses/ ea16.pdf

AEC approval numbers are: 07/122, 07/164, 08/030, 08/119, 08/156, 09/056 and 09/097. Permission was granted by the Djabugay people to observe the horses at Mona Mona Aboriginal Mission and a NSW National Parks scientific license (no. 12581) was obtained to observe the horses at Oxley Wild Rivers National Park. No permits were required to observe the Przewalski and Arab horses in France, but permission was gained from Dr. Feh and Dr. Kiley-Worthington, respectively.

CHAPTER 4 FERAL HORSES PART 1

4.1 Introduction

This chapter reports on the investigation of lateralization in feral horses (*Equus caballus*). Since it could be argued any biases found in domestic horses are a product of the traditional practice of handling horses on their left side or of other management practices (Chapter 2, *Section 2.2,* p. 16 and *2.4.2,* p. 23), it was considered important to study horses that have never been handled. Furthermore, domestic horses are not kept under natural social conditions and this may affect not only laterality of agonistic behaviour but also coordination as a group when confronted by a potential threat. Hence, the feral horses were selected for investigation because they had not been handled and were living under natural social conditions.

A feral animal is defined as one belonging to a species which has been domesticated (i.e. has been tamed via selective breeding over a long period of time) but is now living in the wild (Martin and Hine, 2008). Throughout the world there are numerous populations of feral horses that have been studied extensively but not in terms of lateralization. These populations include New Forest ponies in England (Tyler, 1972), the Camargue horses in France (Feh, 1999), the Sorraia in Portugal (Heiter et al., 2006) and the Mustangs of America (Houpt and Keiper, 1982) but by far the largest population of feral horses, numbering more than 400 000, exists in Australia (Dawson, 2006).

Horses were introduced to Australia when the First Fleet of white settlers arrived in 1788 (Dobbie et al., 1993). Initially, Arab horses were imported. Other breeds, such as ponies and draft horses were introduced later, followed by thoroughbreds when racing became popular (Dobbie et al., 1993). Australian feral horses, known as brumbies, were first sighted in 1804 near Sydney (Dobbie et al., 1993). They have since gained the status of a pest species and, as a consequence, previous studies of the brumby have focused on its distribution, population dynamics and ecological impact with an emphasis on management or eradication (e.g. Dawson, 2005). No ethological research had been published on the Australian brumby prior to the research reported here.

The potential for learnt patterns of behaviour to be passed on to subsequent generations (as shown in rats by Denenberg, 2000) needed to be considered. It is possible handling prior to becoming feral may have influenced laterality of the feral horses by being passed on to subsequent generations. Therefore, two groups of feral horses that differed in the number of generations for which they had been feral were studied: Group 1 was estimated to be two to five generations feral, whereas Group 2 was 10 to 20 generations. Any differences that might be present between Group 1 and 2 would not likely to be due to breed because both populations are made up of mixed breeds. Also predators, dingoes and wild dogs, were present at both study locations and the substrate on which the horses walk and run is similar in both locations. The main difference, therefore, between Group 1 and 2 was the duration of feral living.

4.2 Methods

4.2.1 Subjects

Two groups of feral horses were studied. Group 1 was located at an "Aboriginal Mission" in North Queensland and Group 2 was in the Oxley Wild Rivers National Park in NSW. Group 2 feral horses had been feral for a longer period of time (10–20 generations) and were more fearful of humans than Group 1 horses (2–5 generations). A total of 20 horses were observed in Group 1 and 56 in Group 2. More details of these horses are given in Chapter 3 (*Section 3.2.1,* p. 30).

4.2.2 Data collection

During the first field trip (October–December, 2007) to observe Group 1 feral horses, all agonistic responses that were clearly visible were recorded whenever they occurred throughout the day. Total sampling time differed for each horse. On the second field trip (July–September, 2008) to observe Group 1, each band was observed for at least six hours of cumulative time to gather agonistic recordings.

The majority of bands (7 bands, 42 horses) in Group 2 (over the entire study period) were observed for at least 10 hours but some additional scores were collected for three bands (number of horses per band were 4, 5 and 3; one of the harem stallions was missing an eye and therefore agonistic data was not scored for this horse) sighted infrequently.

Agonistic data for interactions within harem bands were obtained for 67 horses (15 stallions, 27 mares, 12 fillies and 14 colts; 1 of the fillies was a mare in the second year of recording; further details are provided in Appendix I). Agonistic data were scored as described in Chapter 3, *Section 3.3.1.2* (p. 47). Briefly, a looking bout was the period of time in which the recipient was in the aggressors left or right monocular visual field; a change of eye used to view the conspecific corresponds to a change of looking bout. Left and right looking bouts were recorded as threats or attacks.

Approximately 5 and 14 hours of video-tape of stallion fights were collected from Groups 1 and 2, respectively. Only stallion fights that were captured on video-tape were scored and analysed. Agonistic data of stallion fights were gathered from 24 stallions, 7 from Group 1 and 17 from Group 2 (Appendix V). Of these, 10 were harem stallions and 11 were bachelors. Three stallions were not included in the comparison of bachelor and harem stallions: two stallions that changed from bachelor to harem status during the study period and one of unknown status due to rare sighting.

Head lifting and turning responses were also recorded mostly over a cumulative period of at least 10 hours per horse to determine laterality of vigilance and laterality of reactivity. As described in Chapter 3, *Section 3.3.1.1* (p. 45), record was made of the number of times each horse lifted its head from grazing and looked to the left or right, and what the horse was possibly looking at when it did so. The height to which the horse lifted its head was also scored as either above or below the height of the withers. A measure of laterality index of vigilance was calculated using all the head-turning scores, whereas percentage reactivity was calculated as the number of scores in which the horse raised its head above the level of the withers divided by the number of scores in which the horse lifted it head to any height for left and for right head turns. A measure of laterality index of high alert was calculated using only those head lifts that were above the level of the withers. Head lifting and turning scores of vigilance and high alert were obtained from 66 and 65 horses respectively (Appendix I).

4.2.3 Statistical analysis

Methods of statistical analysis are given in Chapter 3, *Section 3.5* (p. 53). For correlation analyses, unless otherwise stated, Pearson's correlations were used. Bonferroni adjustments were made if two tests were conducted at the same time (i.e. males and females, immature and adult horses) to compensate for Type 1 error. The adjusted p-value was 0.05/2 = 0.025.

The number of horses included in analyses for the different behavioural categories differed from the total number of horses due to insufficient data being attained from some subjects.

4.3 Results

4.3.1 Eye bias during agonistic interactions

4.3.1.1 Within harem bands

A total of 1886 agonistic interactions were recorded within harem bands for 18 feral horses in

Group 1 and 2557 agonistic interactions for 49 feral horses in Group 2 (one horse was excluded from analysis because he was missing an eye). These interactions were those between any members of the harem band including one stallion in a harem band. There was a mean of 1.5 left or right looking bouts per agonistic interaction. A detailed description of a looking bout is given in Chapter 3, *Section 3.3.1.2* (p. 47), but briefly, looking bouts were defined as the periods in which a horse was using either its left or right eye to view monocularly the head of a conspecific during an agonistic encounter. Of all agonistic looking bouts recorded, 89% (Group 1, 86% and Group 2, 90%) contained threats only and 11% (Group 1, 14% and Group 2, 10%) contained an attack. Descriptions of threats and attacks are given in Table 3.2 (p. 49)

First an analysis was conducted to check whether or not a funnel effect was present (i.e. whether the strength of laterality was dependent on the number of scores obtained for each horse; Palmer, 2002). A significant negative association was found between the absolute value of the LI-score and the total number of scores recorded for looking bouts containing only threats ($r(64) = -0.25$, $p = 0.045$; Figure 4.1); horses with higher absolute LI-scores tended to be those horses for which fewer scores had been obtained. No such association was found for looking bouts containing an attack (Spearman's rho, $r_S(64) = -0.03$, $p = 0.800$). This indicates that no funnel effect was present for 'attacks' but there was a funnel effect for 'threats'. In order to remove this funnel effect in the threat scores, it was necessary to exclude 7 horses, for which less than 15 scores had been obtained. The resulting dataset showed no significant association between the number of scores per horse and the absolute LI-score ($r(57) = -0.24$, $p = 0.069$) and thus no significant funnel effect, although it is recognised that a trend to significance was still present. Mean number of scores obtained for each horse was 90 and ranged from 15 to 309.

Group 1 and Group 2 did not differ significantly in the LI-scores of looking bouts involving agonistic responses of any type (i.e. including threats and attacks) (Mann-Whitney U, $Z = -1.76$, $p = 0.079$), although there was a trend for Group 2 horses to show a stronger leftwards bias than Group1 horses. Left and right looking bouts were then separated into 'threat', if during the bout the horse engaged in low-level threats only, and 'attack', if the bout contained at least one high level threat/attack. Group 1 and Group 2 did not differ significantly in the number of looking bouts involving threats only (Mann-Whitney U, $Z = -1.13$, $p = 0.260$, $N = 59$) or in those with attack (Mann-Whitney U, $Z = -1.62$, $p = 0.105$, $N = 62$). Moreover, males ($N = 27$) and females ($N = 39$) did not differ significantly on any of these measures (total agonistic responses Mann-Whitney U, $Z = -0.37$, $p = 0.712$; threats Mann-Whitney U, $Z = -0.20$, $p = 0.841$, attack, Mann-

Whitney *U*, *Z* = -1.32, *p* = 0.186). Hence, there was no effect of sex or the number of generations horses had been feral on laterality of agonistic responses. The groups and sexes were then combined to give a sample size of 59 and 62 feral horses for threat and attack looking bouts, respectively.

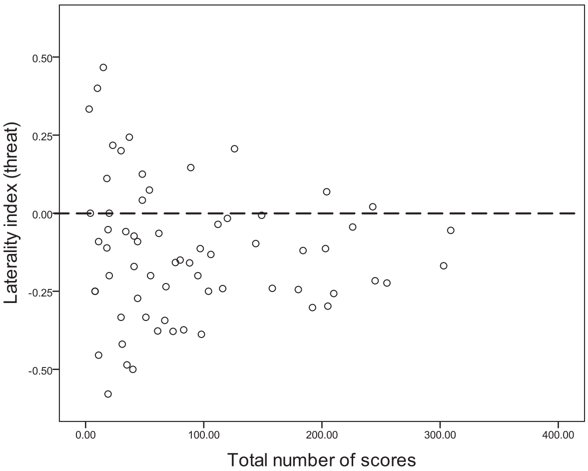


Figure 4.1 Scatterplot showing a funnel effect for 'threats'. There was a significant association between absolute LI-scores and the total number of scores (p = 0.045). LI-scores and the total number of scores obtained from each horse are represented on the Y and X-axis, respectively. The dashed line indicates no bias. $N = 66$

Analysis of the data for all 66 horses revealed a significant leftwards population bias of eye used in looking bouts that contained any type of agonistic response (G-test, $G(64) = 1130$, $p < 0.001$), and separately for looking bouts containing only threats (G-test, $G(57) = 960$, $p < 0.001$) and looking bouts containing attack (G-test, $G(60) = 186$, $p < 0.001$; Figure 4.2). The mean leftwards population bias of looking bouts with threats was 57% and of those with attack was 63%. The difference in the strength of left-side bias between threat and attack was not significant (paired t-test, $t(56) = 1.84$, $p = 0.071$). Of the 62 horses that showed an attack response, 4 showed a left-eye bias. Of the 59 horses that displayed threat responses, 21 showed a significant left-eye bias and 1 a right-eye bias in threats. More details on the scores gained for each horse are provided in Appendix III. Notably substantially more scores were obtained from each horse for threat responses than attack responses, which may account for the difference in numbers of significantly lateralized individuals.

In summary, significant left-eye biases were found for threat and attack responses during interactions between members of harem bands (including, offspring, mares and one stallion).

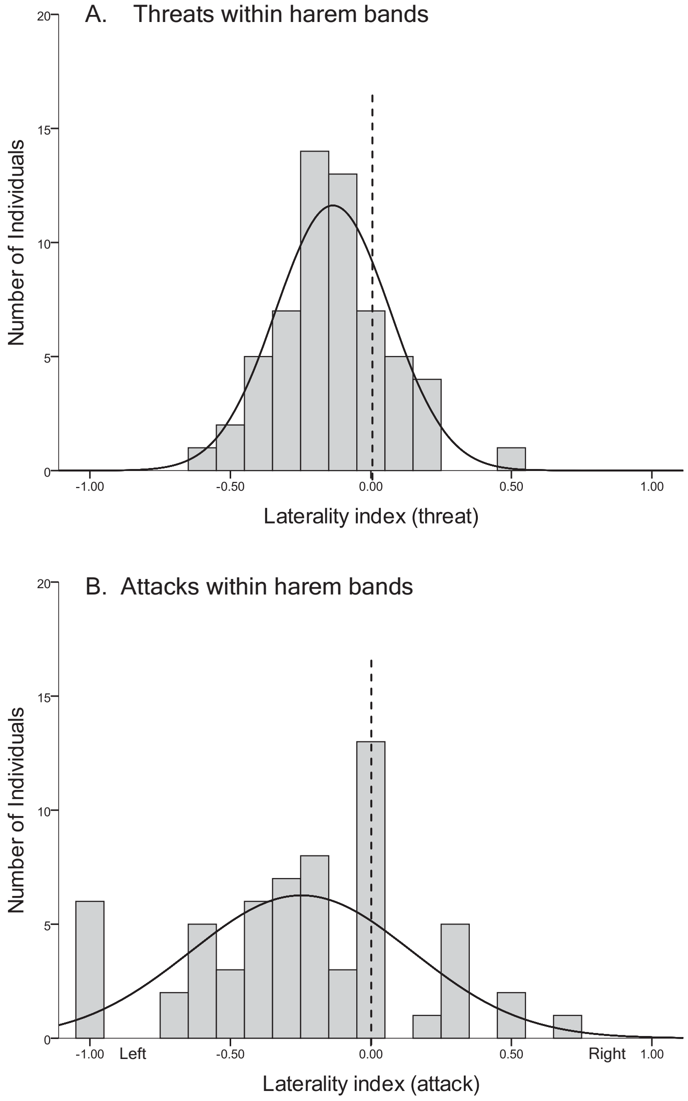


Figure 4.2 Frequency histograms (with normal curve plotted) of laterality indices for agonistic interactions within harems ($N = 66$). A, agonistic looking bouts contained threats only and B, looking bouts containing an attack. Note that the distribution for 'attack' is shifted more strongly leftwards than that of 'threats' and shows greater variance. The dotted line indicates the zero value (no bias). Values to the left of the dotted line indicate a leftwards laterality and values to the right indicate a rightwards laterality. Both plots are skewed significantly to the left.

4.3.1.1.1 Age effects on laterality in agonistic responses

Next, the strength of biases was considered irrespective of direction, using the absolute LI-scores. Immature ($N = 21$) feral horses were found to have significantly stronger absolute LI-scores than adults ($N = 38$) in looking bouts consisting of threats only (Mann-Whitney U, $Z = -3.10$, $p = 0.002$, $N = 56$), but not for looking bouts that contained an attack (Mann-Whitney U, $Z = -0.07$, $p = 0.924$, $N = 59$; Figure 4.3). When direction of bias was considered, bias direction (right or left) was independent of whether horses were immature or adult (threat, Chi-squared test, $\chi^2(1) = 0.1$, $p = 0.836$, $N = 63$; attack, Chi-squared test, $\chi^2(1) = 0.4$, $p = 0.544$, $N = 50$; zero scores were excluded). Hence, strength but not the absolute direction of bias varies with age. Immature horses showed more strength of laterality than adults for threat but not attack.

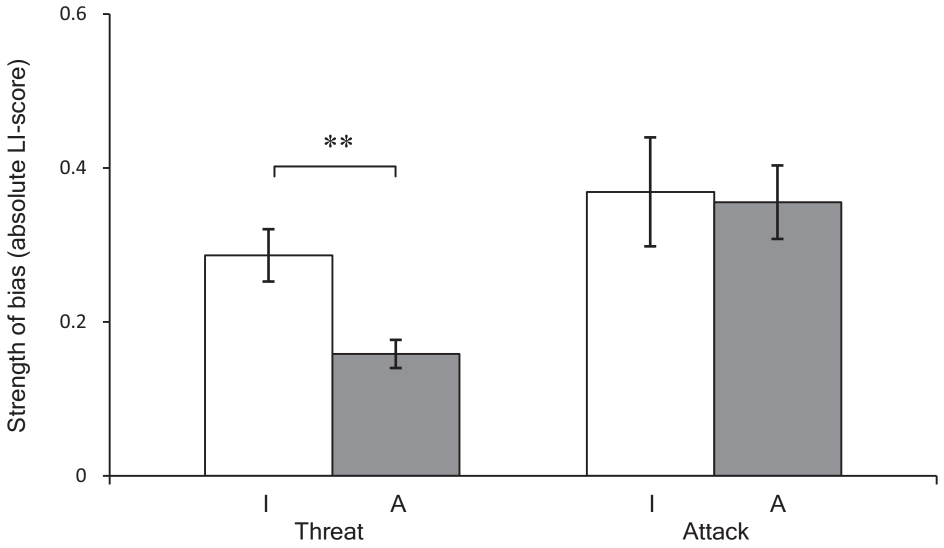


Figure 4.3 The effect of age on strength of eye bias in threats and attacks. The mean strength of laterality and standard error has been plotted. Immature horses ($N = 21$) showed significantly stronger strength of laterality than adults ($N = 38$) in threats. White bars represent immature horses (I) and grey bars represent adults (A). ** denotes significance at $p < 0.01$

4.3.1.2 Stallion fights

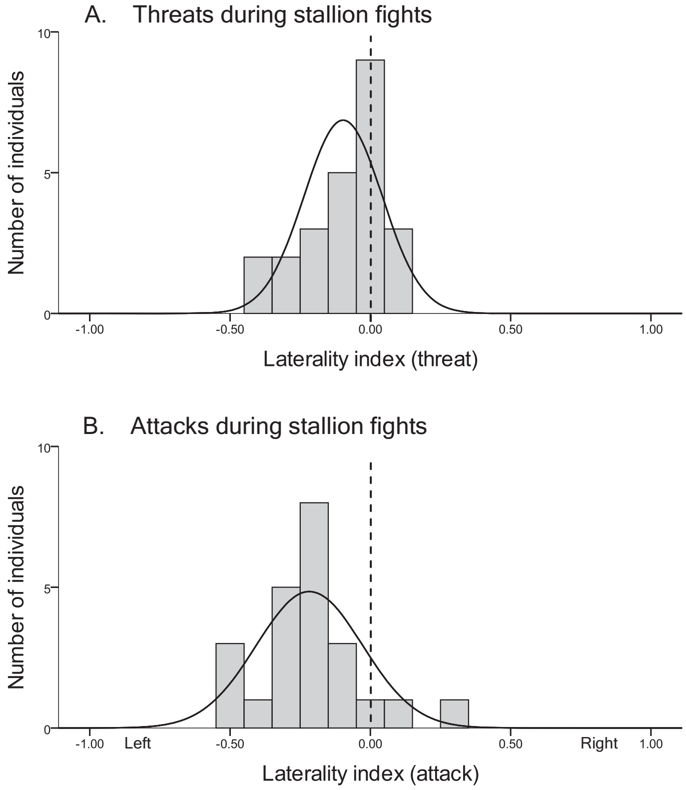
A total of 141 agonistic interactions between stallions were recorded in Group 1 horses and 452 in Group 2 horses and there was mean of 8 left or right looking bouts per interaction. The mean number of left plus right looking bouts scored per stallion was 149 ($SD = 53$) in Group 1 and 219 ($SD = 164$) in Group 2. Of all agonistic bouts recorded, 30% of those recorded for Group 1 and 69% of those recorded for Group 2 contained threats only, whereas 70% of those for Group 1, and 31% of those for Group 2 contained an attack.

An analysis was conducted to determine whether a funnel effect was present. No association was found between the strength of the LI-score and the total number of scores recorded for either looking bouts containing threats only ($r(22) = -0.18$, $p = 0.397$) or looking bouts containing an attack ($r(22) = -0.18$, $p = 0.397$). Therefore, the scores of strength of laterality in threats and attack were not influenced by the number of scores that had been obtained for each horse.

No significant differences in LI-scores were found between Group 1 ($N = 7$) and Group 2 ($N = 17$) in looking bouts performed by stallions that contained any type of agonistic response (Mann-Whitney U, $Z = -095$, $p = 0.340$). The same absence of group difference was found in looking bouts containing only threats (Mann-Whitney U, $Z = -1.08$, $p = 0.280$) or containing an attack

(Mann-Whitney *U*, $Z = -1.01$, $p = 0.315$; attack scores were not obtained for one horse). Hence, both groups were combined to give a sample size of 24 stallions.

Analysis of the data for Groups 1 and 2 combined revealed a significant left-eye population bias for looking bouts containing threats only (G-test, $G(22) = 339$, $p < 0.001$; mean left group bias of 55%) and for looking bouts containing an attack (G-test, $G(21) = 316$, $p < 0.001$; mean left group bias of 61%; Figure 4.4). The left-eye bias in attack was significantly stronger than in that in threats (paired t-test, $t(23) = 2.76$, $p = 0.011$, Cohen's $d = 0.85$). Of the 24 stallions, 7 showed a significant left-eye bias in looking bouts with threats only and 9 showed a significant left-eye bias in looking bouts with an attack. No horses showed a right-eye preference in either threats or attacks.


A. Threats during stallion fights

B. Attacks during stallion fights

Figure 4.4 Frequency histograms (with normal curve plotted) of laterality indices for agonistic interactions during stallion fights ($N = 24$). A. agonistic looking bouts containing threats only and B. looking bouts containing an attack. Both plots are skewed significantly to the left. Note that the distribution for attack is shifted more strongly leftwards and shows greater variance than that of threat. The dotted line indicates the zero value (no bias). Values to the left of the dotted line indicate a left laterality and values to the right indicate a right laterality.

The stallions were further divided into those with a harem ($N = 11$) and those that were bachelors ($N = 10$) because these two types of stallion form two different types of band, harem and bachelor bands, which differ in social structure. Stallions in harems had lower LI-scores of eye preference in looking bouts with threats only than did bachelor stallions ($t(11.8) = -2.75$, $p = 0.018$, Cohen's $d = 1.60$; unequal variance assumed) but not in looking bouts with attack ($t(18) = --0.71$, $p = 0.485$, Cohen's $d = 0.33$; Figure 4.5). Hence, bachelor stallions displayed a stronger left-eye preference when engaging in threats than did stallions in harems but left-eye preference when engaging in attacks was not significantly different between these two types of stallion, although there was a trend in the same direction.

Figure 4.5 Comparison between harem ($N = 10$) and bachelor ($N = 11$) stallions of eye bias for looking bouts containing threats only (T) and looking bouts containing attack (A) during stallion fights. Stallions in harems showed a weaker left-eye bias for looking bouts containing only threats than did bachelor stallions but there was no such significant difference in eye bias in looking bouts containing an attack. Laterality index is plotted on the Y-axis: negative value represents a left bias and a positive value represents a right bias. * denotes significance at $p < 0.05$

Similar to interactions between harem members, interactions of stallions with other stallions showed a significant left-eye bias for both threats and attack. There was a significant difference between bachelor and harem stallions in the strength of bias but for threats only and not for attack.

4.3.2 Side bias in vigilance and reactivity

4.3.2.1 Laterality index in vigilance

A total of 511 ($M = 30$, $SD = 17$) and 2116 ($M = 42$, $SD = 33$) left and right scores of vigilance behaviour were obtained for Group 1 and Group 2, respectively. An analysis was conducted to check whether the strength of laterality might be dependent on the number of scores obtained for each horse. No significant association was found between the strength of the LI-score and the total number of scores recorded for vigilance ($r(64) = -0.12$, $p = 0.324$), indicating that no funnel effects had occurred. Hence, the strength of laterality was not influenced by how many scores had been obtained for each horse.

There was no significant difference in LI-vigilance scores between Group 1 ($N = 17$) and Group 2 ($N = 49$) (Mann-Whitney U, $Z = -0.38$, $p = 0.708$) and no significant sex difference ($t(64) = -1.04$, $p = 0.302$, Cohen's $d = 0.26$; males $M = -0.10$, $SE = 0.05$, $N = 32$; females $M = 0.03$, $SE = 0.05$, $N = 34$). Hence group and sex were combined giving a sample size of 66 horses and this analysis showed that the horses were more likely to stop grazing and lift their head to look at a stimulus detected on their left side than on their right side (G-test, $G(64) = 177$, $p < 0.001$). They showed a leftwards population bias of 54% (Figure 4.6) and 8 of the 66 horses showed significant individual biases: 5 left and 3 right.

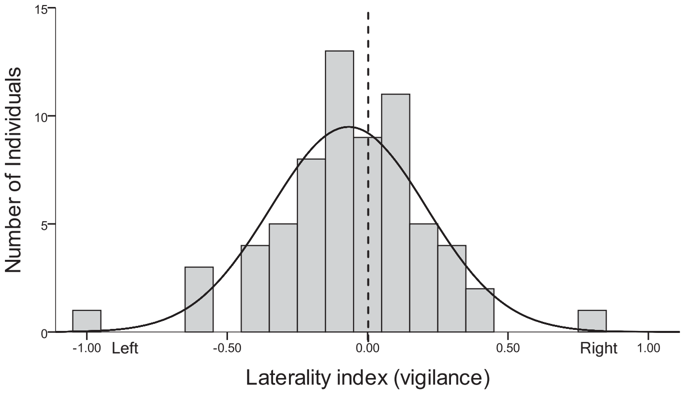


Figure 4.6 Frequency histogram of laterality indices for vigilance ($N = 66$). A weak but significant population bias to the left was detected. The plot is presented as in Figs 4.2 and 4.4.

4.3.2.1.1 Effect of age on laterality in vigilance

In addition, an analysis was conducted on the absolute values of the LI-vigilance scores. Immature ($N = 21$) horses displayed stronger absolute LI-scores than adults ($N = 41$; Mann-Whitney, $Z = -2.08$, $p = 0.038$, 4 horses that changed age status during the observational period were excluded from analysis; Figure 4.7). However, the direction of bias was independent of whether horses were immature or adult (Chi-squared test, $\chi^2(1) = 1.5$, $p = 0.214$, $N = 59$). Hence, strength but not the direction of bias was influenced by age.

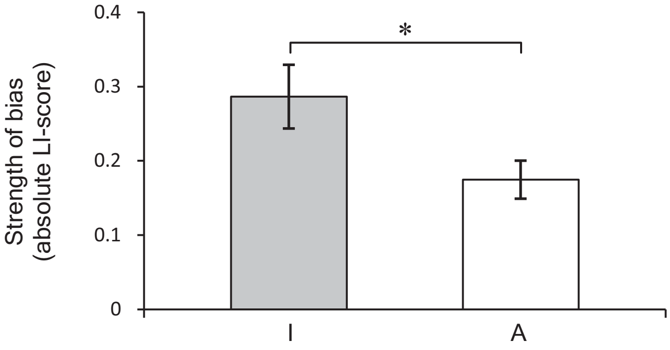


Figure 4.7 The effect of age on strength of side bias in vigilance. Immature horses (*N* = 21) showed significantly stronger strength of laterality than adults (*N* = 41) for threats. The mean strengths of laterality and standard errors have been plotted. White bar represents immature horses (I) and grey bar represents adults (A). * denotes significance at $p < 0.05$

4.3.2.2 Percentage reactivity

A total of 1229 (*M* = 72, *SD* = 39) and 2463 (*M* = 49, *SD* = 30) left plus right head lifts from grazing, to any height, were obtained for Group 1 and Group 2, respectively. This score was called percentage reactivity and differs from laterality in vigilance, in that, percentage reactivity takes into account the height to which the horse raised its head; head lifts above the level of the withers were considered to indicate greater reactivity than head lifts that were below the level of the withers. Percentage reactivity was not calculated as a laterality index but was instead calculated as head lifts above the withers divided by total head lifts, for left and for right head turns. The horses raised their head so that the poll was above wither level in a mean of 43% of the total number of left and right head turns. An ANOVA with side of look (as the within subjects factor) and sex (as the between subjects factor) was conducted, after arcsine transformation of scores of the percentage of left and right looks above wither level. A significant main effect for side ($F(1, 63) = 57.7$, $p < 0.001$, partial $\eta^2 = 0.48$) was revealed; looks to the left contained a significantly greater percentage of head lifts higher than wither level (54%) than did looks to the right (34%), indicating greater reactivity elicited by stimuli detected on the left side. There was no significant main effect for sex ($F(1, 63) = 1.02$, $p = 0.316$, partial $\eta^2 < 0.02$) and no significant interaction between sex and side of looking ($F(1, 63) = 0.61$, $p = 0.436$, partial $\eta^2 = 0.01$). Therefore, the scores of males and females were combined.

Group 1 horses (*N* = 17) showed significantly higher head lifts than Group 2 horses (*N* = 48) provided they were looking on their right side (Mann-Whitney *U*, $Z = -4.11$, $p < 0.001$) but not when they were looking to the left (Mann-Whitney *U*, $Z = -0.73$, $p < 0.468$). Both Groups 1 and 2 showed higher head lifts when the looks were to their left than to their right side (Group 1, paired t-test, *t*(16) = -2.35, *p* = 0.032, Cohen's *d* = 0.37; arcsine transformed; Group 2, Wilcoxon signed-ranks, *Z* = -5.28, *p* < 0.001; Figure 4.8) This shows that both groups were left lateralized but Group 2 was more strongly left lateralized than Group 1.

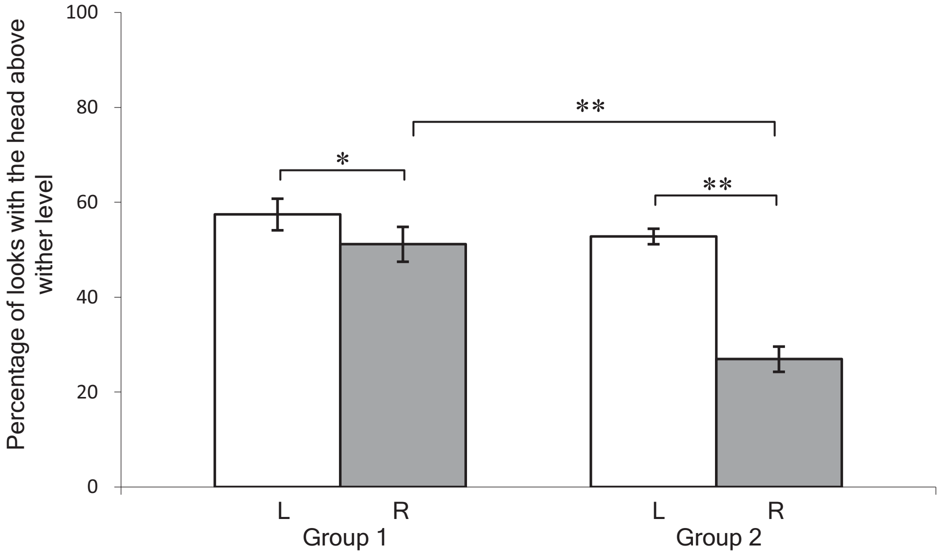


Figure 4.8 Percentage reactivity elicited by stimuli detected on the left side and on the right side. Horses from both groups were significantly more likely to raise their head above wither level when attending to a stimulus detected on their left than right side. Group 1 (*N* = 17) horses lifted their head significantly higher than Group 2 (*N* = 48) horses when looking to the right. Means of the percentage of head lifts that were above wither level and standard errors have been plotted. White bars represent looks to the left (L) and grey bars represent looks to the right (R). * denotes a significant difference at *p* < 0.05, ** denotes a significant difference at *p* < 0.01

4.3.2.2.1 Age effect on percentage reactivity

Next, Groups 1 and 2 were combined in order to determine age effects. Four horses were excluded because they had been sampled when they were immature and again when they were adults and therefore data would not have been independent. Although adult horses (*N* = 40) lifted their heads above wither level more often than did immature horses (*N* = 21) when performing both left (Mann-Whitney *U*, *Z* = -2.91, *p* = 0.004) and right looks (Mann-Whitney *U*, *Z* = -3.38, *p* = 0.001), significant left-side biases were found for both adults (Wilcoxon *U*, *Z* = -4.84, *p* < 0.001) and immature horses (*t*(19) = -4.13, *p* = 0.001, Cohen's *d* = 1.36; arcsine transformed, Figure 4.9). Hence, both adults and immature horses were more reactive on the left side than on the right side.

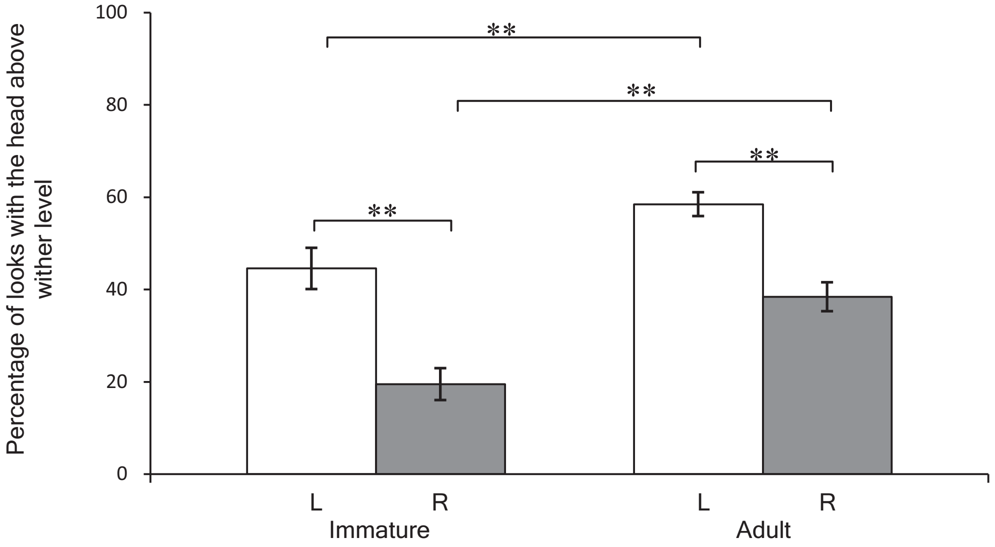


Figure 4.9 Both immature (*N* = 21) and adult horses (*N* = 40) exhibit significantly higher percentage reactivity towards stimuli detected on the left than right side. Also it can be seen that adult horses lifted their head higher than immature horses for both left and right looks. The mean and standard error has been plotted. White bars represent looks to the left (L) and grey bars represent looks to the right (R). ** denotes a significant difference at $p < 0.01$

4.3.2.2.2 Effect of the type of stimulus on percentage reactivity

Head lifts were subdivided according to type of stimulus that elicited each head lift; conspecific, observer (human), cow, another visual stimulus, sound, or unknown. Further analysis was conducted only on the data (arcsine transformed) of head lifts in response to a conspecific and the observer because sufficient scores had been obtained in these categories for the majority of horses. A 2x2 within subjects ANOVA found a main effect for side ($F(1,50) = 35.23$, $p < 0.001$, partial $\eta^2 = 0.41$) but no main effect of stimulus ($F(1,50) = 2.66$, $p = 0.109$, partial $\eta^2 = 0.05$) and no interaction between these two factors ($F(2,50) = 0.59$, $p = 0.447$, partial $\eta^2 = 0.01$). Hence, higher reactivity on the left side was found for responses to both conspecifics and the observer.

Further analysis considered a potential effect of group of horse since Group 2 was more fearful than Group 1 (Chapter 3, *Section 3.2.1*, p. 30). Due to sample size variability between Group 1 and 2, non-parametric tests were conducted. Sample sizes in the separate analyses of the different stimuli were; conspecific (Group 1, *N* = 16; Group 2, *N* = 43) observer (Group 1, *N* = 17; Group 2, *N* = 38). The analysis revealed that Group 1 horses were more reactive than Group 2 horses when looking at a conspecific on their right side (Mann-Whitney *U*, $Z = -4.33$, $p < 0.001$) but there was no group difference when looking at a conspecific on their left side (Mann-Whitney, $Z = -0.46$, $p = 0.644$). By contrast, horses in Group 2 were more reactive when looking at the observer on the left (Mann-Whitney *U*, $Z = -2.24$, $p = 0.025$) but not when looking at the observer on the right (Mann-Whitney *U*, $Z = -1.12$, $p = 0.264$). Hence, eye bias in percent reactivity to the two stimuli is dependent on group (Figure 4.10).

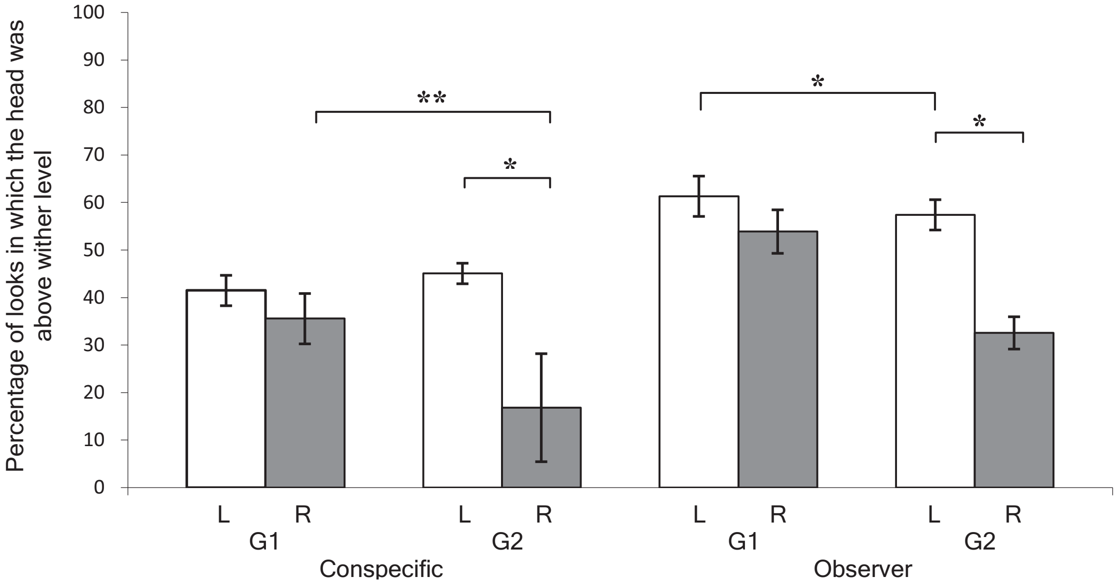


Figure 4.10 Percentage reactivity, as measured by the percentage of looks in which the horse lifted its head above the wither level, (Y-axis) shown by Group 1 and Group 2 horses when looking at either a conspecific or the observer detected on their left or right side. Means and standard errors have been plotted. Group 2 horses were significantly more reactive than Group 1 horses when the human observer was detected on their left side. Group 2 horses showed significantly lower reactivity than Group 1 horses to a conspecific seen on their right side. Group 2 horses showed significant left bias whereas Group 1 horses showed no significant bias. The symbols G1 and G2 represent Group 1 and Group 2 respectively. White bars represent looks to the left (L) and grey bars represent looks to the right (R). * denotes significance at $p < 0.05$, ** denotes a significant difference at $p < 0.01$

Since the above tests detected group differences, further analyses of the same measures involving separate 2x2 within subjects ANOVAs were conducted on each group (arcsine transformed data). Group 1 showed no significant main effects (stimulus, $F(1,15) = 1.40$, $p = 0.256$, partial $\eta^2 = 0.09$; side of look, $F(1,15) = 0.30$, $p = 0.594$, partial $\eta^2 = 0.02$) or interaction ($F(1,15) = 0.46$, $p = 0.510$, partial $\eta^2 = 0.03$). Results from Group 2 revealed a significant main effect for stimulus ($F(1,34) = 9.99$, $p = 0.003$, partial $\eta^2 = 0.23$) and a significant main effect for side of look ($F(1,34) = 54.29$, $p < 0.001$, partial $\eta^2 = 0.62$) but no significant interaction between these two factors ($F(1,34) = 0.30$, $p < 0.585$, partial $\eta^2 = 0.01$). Group 1 horses were not lateralized (at least when reactions to these two stimuli were considered) and did not display any difference in reactivity to either stimulus. By contrast, Group 2 horses were left lateralized and more reactive towards an observer than to a conspecific (Figure 4.10).

4.3.2.3 Side bias in high alert

This measure considered only the head lifts from grazing that were above the level of the withers. A significant negative association was found between the absolute value of the LI-score and the total number of scores recorded ($r(61) = -0.39$, $p = 0.002$; Figure 4.11); horses with stronger LI-scores tended to be those horses for which fewer scores had been obtained. Therefore, the strength of laterality in high alert may have been influenced by the number of scores.

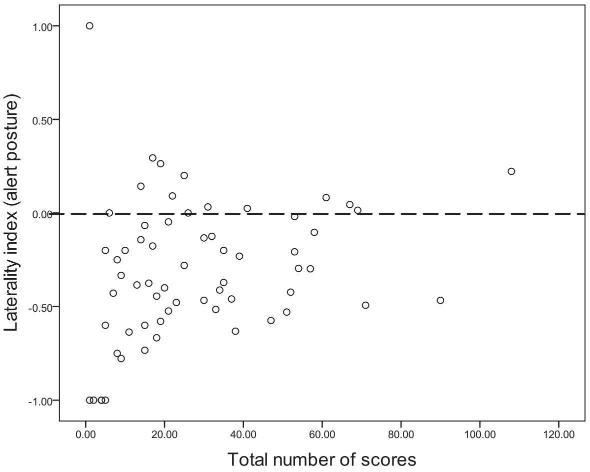


Figure 4.11 Scatterplot showing a funnel effect for high alert. There was a significant association between absolute LI-scores and the total number of scores ($p = 0.002$). LI-scores and the total number of scores obtained from each horse are represented on the Y and X-axis, respectively. The dashed line indicates no bias. $N = 63$.

In order to remove the funnel effect it was necessary to exclude from further analysis five horses, for which fewer than five scores had been obtained. The resulting dataset, once re-analysed, showed no significant association between the number of scores obtained from each of the horses and the absolute LI-score ($r(56) = -0.24$, $p = 0.070$) and thus no significant funnel effect. Mean number of scores obtained for each horse was 31 and ranged from 5 to 108.

A significant leftwards population bias of 64% was found for high alert (G-test, $G(56) = 514$, $p < 0.001$, $N = 58$; Figure 4.12). Twenty-three horses out of the total 58 showed a significant left-side bias and one showed a significant right-side bias.

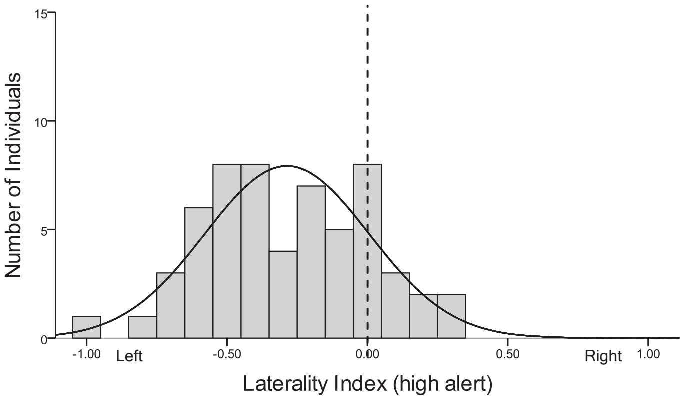


Figure 4.12 Frequency histogram of laterality indices for high alert (*N* = 58). A strong significant population bias to the left was revealed. The plot is presented as in Fig 4.6.

4.3.2.3.1 Effect of age on laterality index in high alert

In addition, the absolute values of LI-scores in high alert were used to determine whether there was an age effect on strength of bias. Immature horses ($N = 17$) and adults ($N = 38$) did not differ significantly in the strength of side bias in high alert (Mann-Whitney *U*, $Z = -1.88$, $p = 0.061$). The direction of bias, irrespective of strength, was independent of whether horses were immature or adult (Chi-squared test, $\chi^2(1) = 0.04$, $p = 0.840$, $N = 52$, zero values were excluded; Table 4.1). Neither strength nor direction of bias differed according to age.

Table 4.1 Number of horses showing left or right LI-scores

	Direction of LI-score	
	Left	Right
Adult	28	7
Immature	14	3

4.3.3 Correlations between different measures of laterality

Given that social behaviour in general and aggressive behaviour may be broadly regulated by the same neural circuits, as shown in humans and rats (Nelson and Trainor, 2007), and both are involved in threat and attack behaviour, it was thought that eye bias for threat and attack might be related. Nonetheless, correlation analysis showed that these two variables were not significantly associated in the feral horses ($r(51) = 0.09$, $p = 0.504$, $R^2 < 0.01$).

However, there was a moderate and significant association between LI-scores of high alert (head lifts above the level of the withers) and LI-scores in threats ($r(50) = 0.31$, $p = 0.024$, $R^2 = 0.10$).

This relationship was limited to female horses (*r*(21) = 0.44, *p* = 0.017, R^2 = 0.19; Figure 4.13B). Female feral horses that displayed a left-side bias for high alert were more likely to show a left-eye bias for engaging in threats directed at other members of their harem band.

There was no significant association between eye bias in attack and side bias in high alert (*r*(48) = 0.19, *p* = 0.192, R^2 = 0.04). Nevertheless, there was a strong, positive association in immature horses of side bias in high alert and side bias in attack, although this was not significant after adjusting for Type 1 error (*r*(14) = 0.52, *p* = 0.038, R^2 = 0.27; Figure 4.14A).

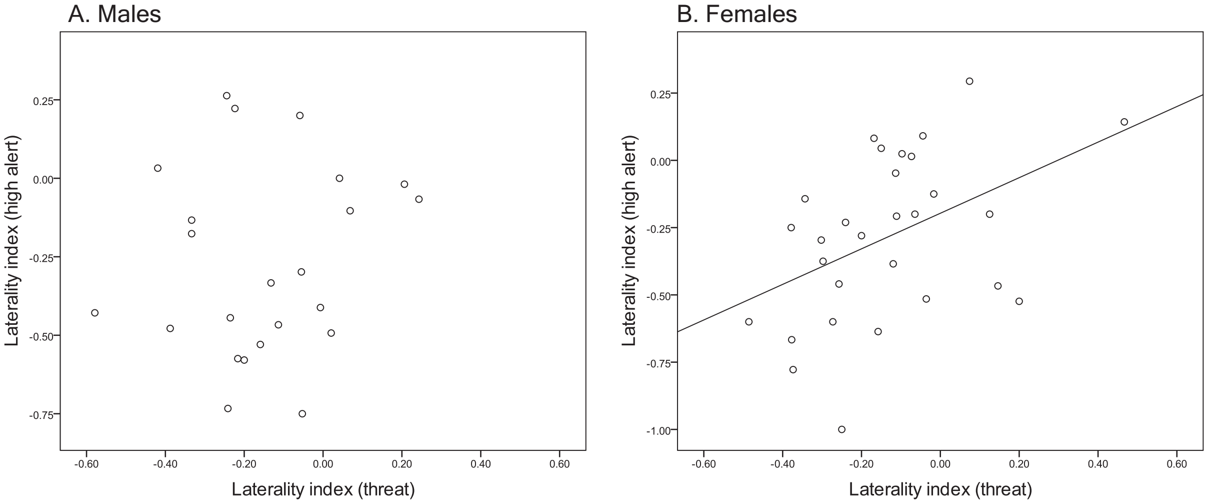


Figure 4.13 Scatterplots showing the relationships between side-bias in high alert (Y-axis) and eye-bias (X-axis) in threats. Males (A) and females (B) are plotted separately. Females showed a significant relationship. A line of best fit is plotted for females (R^2 = .19).

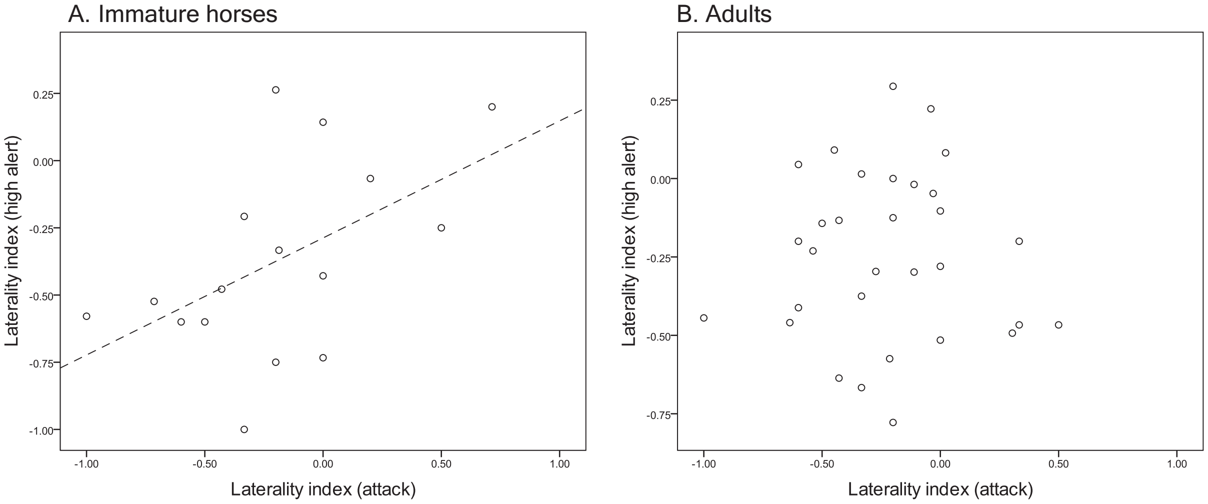


Figure 4.14 Scatterplots showing the relationships between side-bias in high alert (Y-axis) and eye-bias (X-axis) in attack. Immature horses (A) and adults (B) are plotted separately. The association in immature horses was not significant after a Bonferroni adjustment had been made (alpha = 0.025). A line of best fit (dashed) is plotted.

4.4 Discussion

The findings reported in this chapter are summarised in Table 4.2.

Table 4.2 Summary of the findings on side biases in feral horses

Type of agonistic interaction	Behavioural measure	*N*	Population bias	% Left bias
Within harem band	Threat	59	L *	57
	Attack	57	L *	63
Stallion fight	Threat	24	L *	55
	Attack	23	L *	61
	Vigilance	66	L *	54
	High alert	63	L *	67

L* denotes significant left bias. Note that all side biases were significant and leftwards.

Left-side biases were found in the scores of threat and attack. These side biases were almost certainly in response to visual, and not auditory, stimuli because, agonistic responses were scored according to which monocular visual field the opponent occupied and during the response the horse's ears were pinned against its head. With the ears in this position, it is unlikely that the horses were paying attention to auditory inputs during agonistic interactions. On the other hand, initiation of the head-lifting responses in high alert and vigilance could have been in response to hearing a sound. Given that the vision of Group 1 horses was frequently obstructed by high vegetation when the horses were grazing and in a substantial number of cases the stimulus could not be identified by the observer, it is probable that aural detection initiated some head lifts. Auditory laterality has been demonstrated in the domestic horse for the processing of conspecific calls (Basile et al., 2009a): the researchers found that ear orientation often preceded head-turning responses. Since ear orientation prior to a horse lifting its head was not recorded in the research reported in this thesis, it cannot be determined whether head-turning biases were in response to visual or auditory stimuli. Hence, the calculated biases of reactivity and vigilance, but not agonistic responses, are referred to as side biases, rather than eye preferences.

The left-side biases reported above correspond to right-hemisphere processing of agonistic behaviour, as well as vigilance and high alert. Hence, feral horses follow the same pattern of right hemisphere attention to unexpected or novel stimuli, as found in other species (discussed by Rogers, 2010; MacNeilage et al., 2009).

The leftwards biases of attack and reactivity were particularly strong (62% and 67%) and are consistent with the known right-hemisphere specialisation of aggression (Zappia and Rogers, 1983; rats, Denenberg, 1984) and reaction to fear-inducing stimuli (toads, Lippolis et al., 2002; dunnarts, Lippolis et al., 2005) found in other vertebrates. The strength of left-eye bias in attack in horses is comparable to the 65% left-eye bias for aggressive displays found in lizards (Deckel, 1995), and the 61% left-eye bias found in toads for attack (Robins et al., 1998). In Gelada baboons the left-visual-field bias in agonistic interactions is stronger (91%) than in feral horses (Casperd and Dunbar, 1996). It is unknown why horses would have a weaker left-eye bias for agonistic interactions than baboons. It is unlikely that differences in monocular and binocular visual fields alone account for difference in the strength of left-side bias between horses and baboons because toads have a large binocular visual field like the baboons and they show a left-eye bias of similar strength to that of horses. The difference could, of course, be due to methods of scoring in each case.

4.4.1 Eye preference in agonistic behaviour

Right-hemispheric control of agonistic responses was independent of whether encounters occurred between members of the harem band or between two stallions. It was also the same in males and females. Most studies of other species thus far have focused on laterality of aggression in male–male encounters but there is evidence from lizards of left-eye biases in both males and females. For instance, males of the genus *Urosaurus* turned so as to display aggressively to other males on their left side (Hews and Worthington, 2001) and females from a closely related genus *Sceloporus* did so during courtship rejection displays (Hews et al., 2004). The results suggest that right-hemisphere control of agonistic responses may be resilient to social experience and the influence of sex hormones in horses.

There was no change in the strength of bias in attack with age. However, the strength of bias in threats decreased with age indicating modulation by experience or maturation. This suggests that threat and attack may be lateralized independently and that laterality of attack rather than that of agonistic responses is unchanged by experience or maturation. Indeed, there was no correlation between eye bias in threat and that of attack.

The left-eye biases for attack during encounters within harems (63%) and between stallions (61%) were at least 5% stronger than those for threats in harem interactions (57%) and during stallion fights (55%); this difference was significant for stallions and approached significance for interactions within harems. Lizards (*Anolis*), as well as toads, also show stronger left-eye bias with increasing aggression. Deckel (1995) found *Anolis* preferentially used their left eye in the most aggressive displays (as evidenced by light body colour) but showed no eye bias during less aggressive threat posturing. Likewise, as mentioned in the first chapter, in their work on toads (*Bufo marinus*), Robins et al. (1998) found more aggressive tongue strikes (those aimed at the opponent's eyes) were directed at conspecifics occupying the left hemifield, whereas less aggressive tongue strikes (those not aimed at the opponent's eyes) were not lateralized. Hence, left-eye bias in agonistic responses increases with aggression.

The feral bachelor stallions in this study displayed stronger left-eye preferences for threats than did harem stallions. Since it is known that harem stallions have elevated levels of testosterone compared to bachelor stallions (MacDonnell and Murray, 1995), hormone levels may possibly explain this difference in strength of lateralization. In fact, testosterone can modulate laterality, as shown in other species (e.g. chicks, Zappia and Rogers, 1987; meta-analysis on birds and mammals, Pfannkuche et al., 2009). However, feral horses showed no sex differences in the eye-preference in agonistic behaviour. An alternative explanation could be that ritualised behaviour influences the strength of lateralization. Harem stallions may use learned ritualised displays during fighting. If such learned behaviour patterns are a function of the left-hemisphere specialisation for controlling routine behaviour (as discussed in Chapter 1, *Section 1.2,* p. 2), the weaker left-eye bias displayed by harem stallions in comparison to bachelor stallions could be due to some threats by harem stallions being ritualised signals rather than aggressive responses *per se* performed to inflict harm.

4.4.2 Side bias in vigilance and reactivity

Feral horses showed biases to turn their head to the left in measured vigilance and high alert. Laterality of vigilance included all head lifts involving the horse turning its head to the left or the right, whereas laterality of high alert included only head lifts that were above the level of the withers. These results are consistent with known right-hemisphere functions, including superior ability to process global cues and spatial relations and attention to novelty (reviews, MacNeilage, 2009; Rogers and Andrew, 2002) since these must play a role in detecting and attending to potential threats. The left-side bias in high alert at the group level was particularly strong (67%): feral horses lifted their head higher when responding to a stimulus detected on the left side than to a stimulus on their right side. Moreover, the strength of bias was not influenced by age. As pointed out by Kiley-Worthington (1976) and Waring (2003), such high postural tonus, known as the alert posture, indicates readiness for locomotion of flight or fight. Indeed, head height correlates positively with heart rate (Visser, 2002; Rietmann, et al., 2004). In addition, Rietmann et al. (2004) have shown that the higher the head is lifted the lower the variability of heart rate and the greater the activation of the sympathetic nervous system. If the right hemisphere has greater control of activation of the hypothalamic-pituitary-adrenal axis than does the left hemisphere, as known in rats (Sullivan and Gratton, 2002) and humans (Wittling, 1997), the higher postural tonus displayed by horses when attending to a stimulus detected on their left side is consistent with greater readiness for the flight/fight response to stimuli detected on the left than on the right side. In an entirely different species, the Australian magpie, an alert posture has been reported to occur in conjunction with left-eye use to view a model predator (Koboroff et al., 2008), indicating right-hemisphere control of alert behaviour in this species also.

Not only is left-eye bias in high alert in feral horses congruous with left-eye reactivity to model predators shown in other species (toads, Lippolis et al., 2002; dunnart, Lippolis et al., 2005), but also it is consistent with findings from previous studies of domestic horses showing they react with a greater flight distance when confronted by a looming, threatening stimulus presented on the left compared to right side (Austin and Rogers, 2007) and in a finding showing an association between higher reactivity and preferred left-eye use to a view a novel stimulus (Larose et al., 2006).

A weaker left-side bias of 54% was obtained for vigilance than for high alert. Given that vigilance was calculated using all left and right head lifts, including those of high alert, this weaker bias may simply reflect that a left-side bias was present mainly for high head lifts (high alert).

4.4.3 Is laterality influenced by how long horses have been feral?

Although the biases found here in feral horses cannot be attributed to direct experience of being handled by humans, they could, nonetheless, be due to handling in previous generations before the horses became feral. There was no evidence that this might have been the case in so far as both groups showed a significant leftwards laterality in all the measures reported in Table 4.2 (p. 72). If any effect of handling on previous generations had been passed on to subsequent generations, Group 1 (2–5 generations feral) and Group 2 horses (10–20 generations feral) would have shown a difference in laterality.

There was, however, a difference between Group 1 and 2 horses in the left-side bias in percentage reactivity, measured as the percentage head lifts above the withers for left head turns and right head turns. Group 2 horses were more strongly lateralized than Group 1 horses. Since Group 2 horses had been feral for much longer than Group 1 horses, it is unlikely that they have retained lateralization that may have been present in the domestic ancestors. Any lateralization in Group 2 horses is unlikely to be due to handling influences passed on from the domestic ancestors. Group 1 horses may show some carry over effect of handling that occurred before they became feral.

The percentage reactivity revealed a group difference when only two of the stimuli recorded to have elicited the response, the human observer or a conspecific, were considered in the analysis. Group 1 horses did not show a side bias for the percentage of high head lifts to attend to either of these stimuli, whereas Group 2 horses lifted their head higher when they looked to the left than to the right for both stimuli. It is possible this group difference in laterality was influenced by the horses' reactivity to the presence of a human observer since Group 2 horses were more fearful of the observer than Group 1 horses (discussed in *Section 3.2.1,* p. 30). Indeed they may have been generally more reactive in the presence of an observer (i.e. increased activation of the right hemisphere) and expressed it by being more reactive to any type of stimulus seen or heard on their left than right side. It seems likely the difference in strength of left-side bias in percentage reactivity to observer and conspecific stimuli between Groups 1 and 2 may be due to differences in fear of humans rather than to a strengthening of laterality of reactivity over generations of feral living. It should be noted that when all stimuli were considered both Groups 1 and 2 showed greater left than right-side percent reactivity.

4.4.4 Conclusion

Feral horses display significant left-side biases of agonistic responses, high alert, and vigilance. Since feral horses are unhandled and Group 1 and Group two horses showed the same direction of lateralization, findings suggest that lateralization of the horse is a species characteristic that has not been entrained by humans. However, there were some group differences in whether a bias was present in response to observer and conspecific stimuli that may have been influenced by past experiences with humans.

CHAPTER 5 FERAL HORSES: PART 2 LIMB PREFERENCE

5.1 Introduction

Given that a number of studies of lateralization in the horse have focussed on limb preference it was thought that this potential measure of lateralization deserved special consideration in a separate chapter. Limb preference in domestic horses was discussed in Chapter 2, *Section 2.2* (p. 16).

In addition, it was considered important to investigate whether level of aggression and/or level of reactivity might correlate with forelimb preference. As discussed in Chapter 1, *Section 1.2.1* (p. 6), limb use may be associated with predominant activation of the contralateral hemisphere (e.g. rats, Vyazovskiy and Tobler, 2008; humans, Harmon-Jones, 2006) and so consistent use of a preferred limb can be associated with expression of the behaviour controlled by the contralateral hemisphere. A left-handed animal may be more reactive than a right-handed animal, and possibly show a negative cognitive bias (Rogers, 2010). Hence, it was predicted that high levels of reactivity might be associated with left-limb preference, as has been shown in other species (Chapter 1, *Section 1.2.1*, p. 6). In fact, one of the explanations given, amongst others, by McGreevy and Rogers (2005) for the left population bias in limb preference shown by thoroughbred horses was the high reactivity of this breed. However, this explanation is problematic not only because of the confounding effects of human entrainment on limb preference, but also because it relies on drawing a link between the described, but not measured, reactivity of the thoroughbred breed (Houpt and Kusunose, 2000) and a group bias in limb preference. Hence, the relationship between limb presence and reactivity was investigated in feral horses in order to explore this idea.

5.2 Method

5.2.1 Subjects and locations

The subjects were the same as those in Chapter 4.

5.2.2 Data collection

Limb preference scores were obtained for 71 feral horses in Groups 1 and 2. Details of the method of sampling are given in Chapter 3, *Section 3.3.1.3* (p.51). The forelimb which was placed in front of the other during grazing was scored every 30 seconds (i.e. at 30 seconds and 60 seconds). Each horse was observed for an accumulated total 50–70 minutes to record limb preference. Scores per horse ranged from 75 to 133. Six horses from Group 2 were scored in October 2009 until 100 scores had been reached.

Repeat scoring of limb preference was conducted on a subsample of horses in order to determine its stability over time. Four horses from Group 2 were sampled again one week after the initial scores had been recorded to determine whether limb preference changed over this short period of time. In addition, seven immature horses (one from Group 1 and six from Group 2) were sampled during their first year (< 1 year of age) and again in their second year (1–2 years of age). Scores of 11 adult horses from Group 1 were compared to scores obtained in the previous year (2006) for the same horses.

Level of aggression was measured as the number of agonistic interactions per hour from the timed observational periods described in the previous chapter (*Section 4.2.2,* p. 56). Level of reactivity was scored as the number of times a horse lifted its head from grazing in order to attend to something detected in its vicinity in an accumulated period of 10 hours for each band. This measure of level of reactivity included right and left turns of the head plus head lifts in which the horse did not turn its head.

5.2.3 Statistical analysis

Statistical analysis was the same as in the previous chapter and more detail is given in Chapter 3, *Section 3.5* (p.53). A laterality index of limb preference was calculated as $\text{Right}_{\text{scores}} - \text{Left}_{\text{scores}} / \text{Total}_{\text{scores}}$. A one-sample t-test was used to determine whether the population bias was significant.

5.3 Results

All raw data for individual horses are provided in Appendix VI.

5.3.1 Forelimb preference

The scores obtained from 30 second interval sampling were checked using the Runs test. No significant runs were found for any of the sequences of left and right scores of limb preference per individual (Z-scores ranged from 0.02 to 1.81, p-values ranged from 0.07 to 0.99). Hence, each left and right score could be regarded as independent.

There was a significant negative correlation between the total number of scores obtained and the strength of individual limb preference (Spearman’s rho, $r_s(69) = -0.38$, $p = 0.001$; Figure 5.1), indicating a funnel effect. Stronger LI-scores were more likely to be found when fewer scores had been collected. Further analysis used a fixed number of scores per individual. The first 80 scores per horse (from all 71 horses, 2 horses that had total scores of 75 and 77) were used to calculate a LI-score.

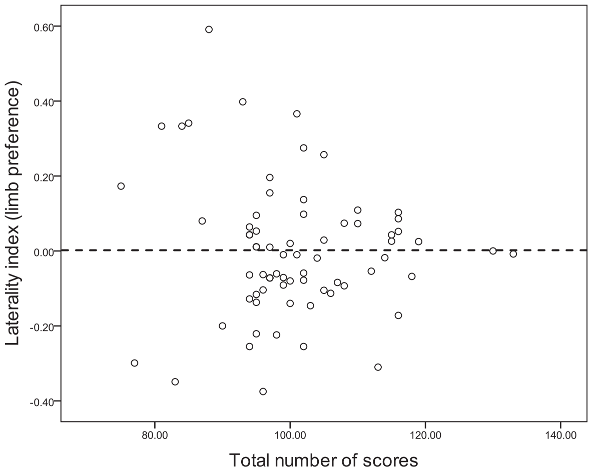


Figure 5.1 Scatterplot showing a funnel effect for limb preference. There was a significant association between absolute LI-scores and the total number of scores (p = 0.001). LI-scores and the total number of scores obtained from each horse are represented on the Y and X-axis, respectively. The dotted line indicates the zero value (no bias).

There was no significant difference in forelimb LI-scores between Group 1 (N = 20) and Group 2 (N = 51; Mann-Whitney U, Z = -1.00, p = 0.318) or between males (N = 34) and females (N = 37; $t(69)$ = -0.47, p = 0.638, Cohen's d = 0.11). Hence the LI-scores of both groups and sexes were combined. No significant population bias was found (one-sample t-test, $t(70)$ = -0.20, p = 0.844; M = -0.004, SE = 0.02; Figure 5.2). There were also no significant population biases when groups were considered separately: Group 1 ($t(19)$ = 0.99, p = 0.334; M = 0.03, SE = 0.03), Group 2 ($t(50)$ = -0.15, p = 0.878; M < 0.01, SE = 0.03).

Immature horses showed higher LI-scores (more rightwards) than adults (Mann-Whitney U, Z = -2.13, p = 0.033). Neither adults ($t(44)$ = -1.91, p = 0.063, M = -0.03, SE = 0.02) nor immature horses ($t(25)$ = 1.34, p = 0.194, M = 0.07, SE = 0.05; Figure 5.2) showed significant population biases. On inspection of the frequency histograms, the distribution of immature horses appeared to follow a bimodal distribution. Twelve of 25 immature horses showed significant limb preferences, whereas only 4 of 46 adults showed significant forelimb preferences.

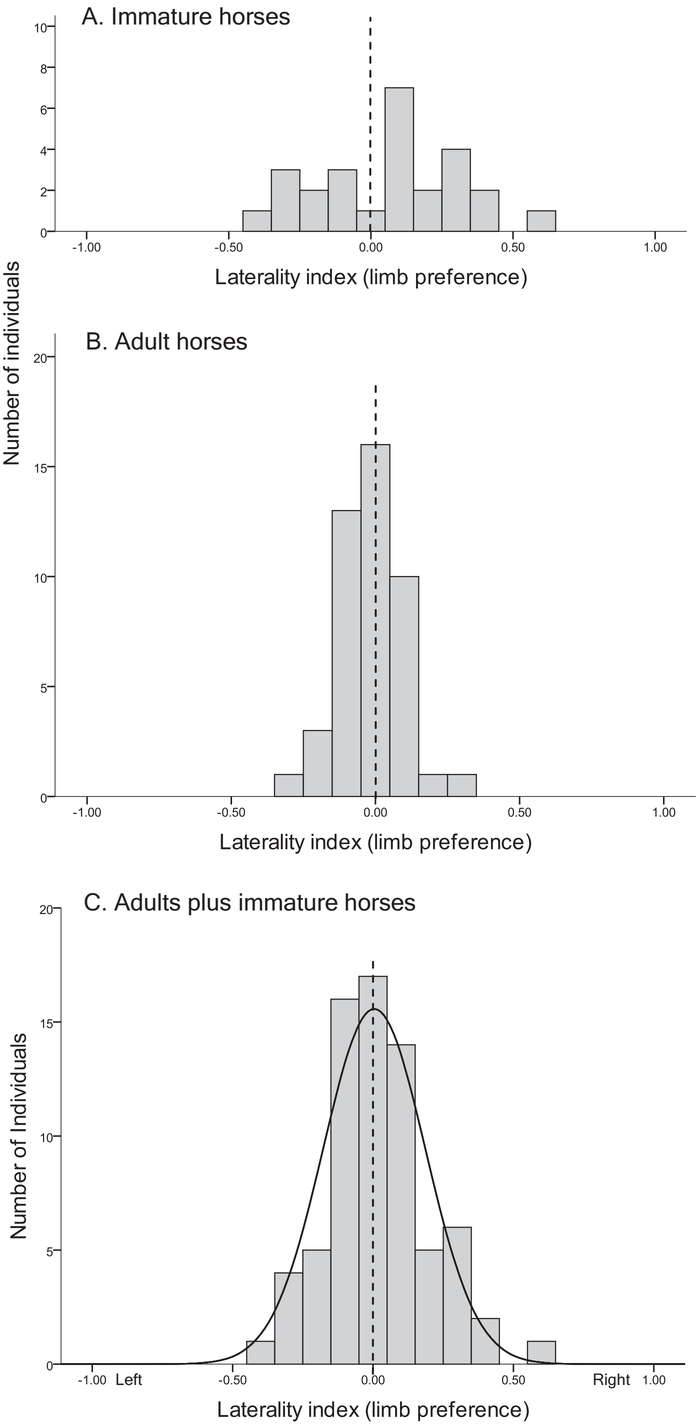


Figure 5.2 Frequency histograms (with normal curve plotted) of laterality indices for forelimb preference during grazing. A, immature horses B, adults and C, adults plus immature horses (N = 71). No significant bias was detected. The dotted line indicates the zero value (no bias). Values to the left of the dotted line indicate a left laterality and values to the right indicate a right laterality.

Since previous studies of limb preference in domestic horses had used scores collected employing 60 second interval sampling, the analysis was repeated using scores obtained at 60 second intervals to calculate the LI, and a similar absence of a significant population bias was found ($t(70) = 1.30$, $p = 0.202$; $M = 0.03$, $SE = 0.02$). Also, there was no significant group difference (Mann-Whitney *U*, $Z = -1.09$, $p = 0.277$) or sex difference ($t(70) = 0.34$, $p = 0.736$).

5.3.1.1 Effect of age on strength of laterality in limb preference

The strength of bias, regardless of direction, was also investigated using the data of forelimb preference based on 80 scores only and using the 30-second-interval sampling data. There was a significant effect of age on the absolute LI-scores: immature horses ($N = 26$) had stronger limb preferences than did adult horses ($N = 45$; Mann-Whitney *U*, $Z = -4.26$, $p < 0.001$; Figure 5.3). Direction of bias, irrespective of strength, was dependent on whether horses were immature or adult (Chi-squared test, $\chi^2(1) = 5.44$, $p = 0.020$, $N = 65$; Table 5.1). Hence, strength and the direction of bias are affected by age.

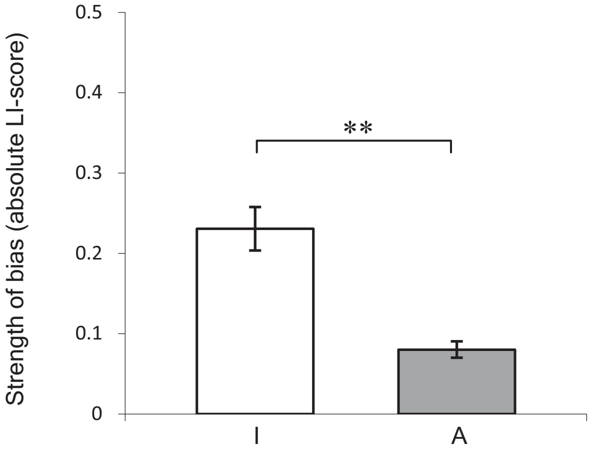


Figure 5.3 The effect of age on strength of limb preference. Immature horses ($N = 26$) showed significantly stronger limb preferences than adults ($N = 45$). The mean strength of laterality and standard error has been plotted. White bars represent immature horses (I) and grey bars represent adults (A). ** denotes significance at $p < 0.01$

Table 5.1 Number of horses showing left or right LI-scores

	Direction of LI-score	
	Left	Right
Adult	25	14
Immature	9	17

5.3.1.2 Stability in forelimb preference over time

Some checks were made in order to determine the stability over time of this particular measure of forelimb preference. There was a strong positive and significant correlation between the initial measure of limb preference and a second measure collected from recordings made one week later ($r(2) = 0.99$, $p = 0.007$), showing that forelimb preference is stable over a one-week period. The correlation was also very strong when the strength of the LI-scores were analysed but this was not significant ($r(2) = 0.88$, $p = 0.122$). However, scores taken one year apart for the immature horses (< 1year old and at 1-2 years of age) were not significantly correlated ($r(5) = 0.38$, $p = 0.400$), although they did not differ significantly in strength of LI-score ($t(6) = 0.16$, $p = 0.876$). This indicates that, while the strength remains the same the direction of a preference may change over time. Scores for 11 of the adult horses from Group 1 taken in 2007 were correlated with scores taken one year previously (in 2006) and no significant association was found ($r(9) = -0.09$, $p = 0.800$), but as in the case of the immature horses, preferences did not differ significantly in strength of LI-score ($t(9) = -0 .74$, $p = 0.516$) over that period.

5.3.2 Correlation of limb preference with level of reactivity

A significant, moderate, positive association was found between limb preference and level of reactivity. Feral horses that displayed a stronger right-forelimb preference tended to have a higher level of reactivity ($r(57) = 0.33$, $p = 0.011$, $R^2 = 0.11$; Figure 5.4).

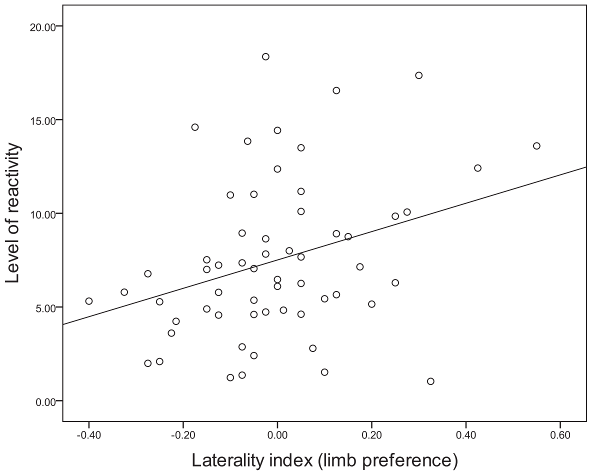


Figure 5.4 Scatterplot showing the relationship between level of reactivity (Y-axis) and forelimb preference (X-axis) expressed as Laterality Indices. A mild but significant association ($p = 0.011$) was found using the scores of 59 horses. A line is best fit is plotted; $R^2 = 0.11$. Level of reactivity was measured as the number of times a horse lifted its head per hour.

Level of reactivity in only the horses that showed a significant left or right-limb preference was examined. Right-forelimb preferring horses (*N* = 7) showed significantly higher scores of reactivity than left-forelimb preferring horses (*N* = 7; *t*(12) = -2.68, *p* = 0.020, Cohen's *d* = 1.55; Figure 5.5).

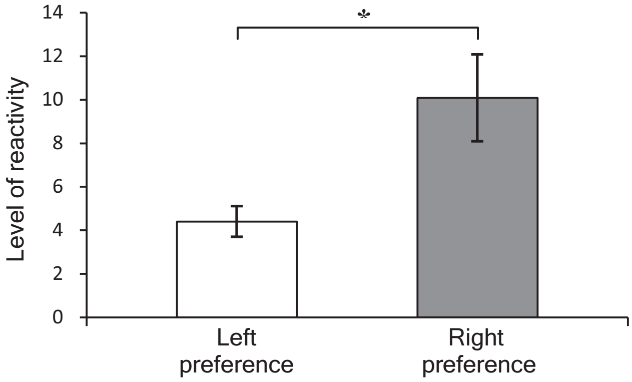


Figure 5.5 Level of reactivity, measured as the number of times a horses lifted its head from grazing per hour, exhibited by horses that were significantly left-forelimb (*N* = 7) and right-forelimb preferring (*N* = 7). Right-limb preferring horses showed significantly higher reactivity than left-limb preferring horses. Means and standard errors are plotted. * $p < 0.05$

Since there was an age difference in the strength of limb preference, this association was examined further by analysing immature and adult horses separately. The relationship for more reactive horses to display increasing right-forelimb preference was present in immature horses (*r*(18) = 0.52, *p* = 0.018, R^2 = 0.27; *N* = 20; Figure 5.6A) but no significant association was found in the adult horses alone (*r*(37) = 0.12, *p* = 0.484; *N* = 39; Figure 5.6B).

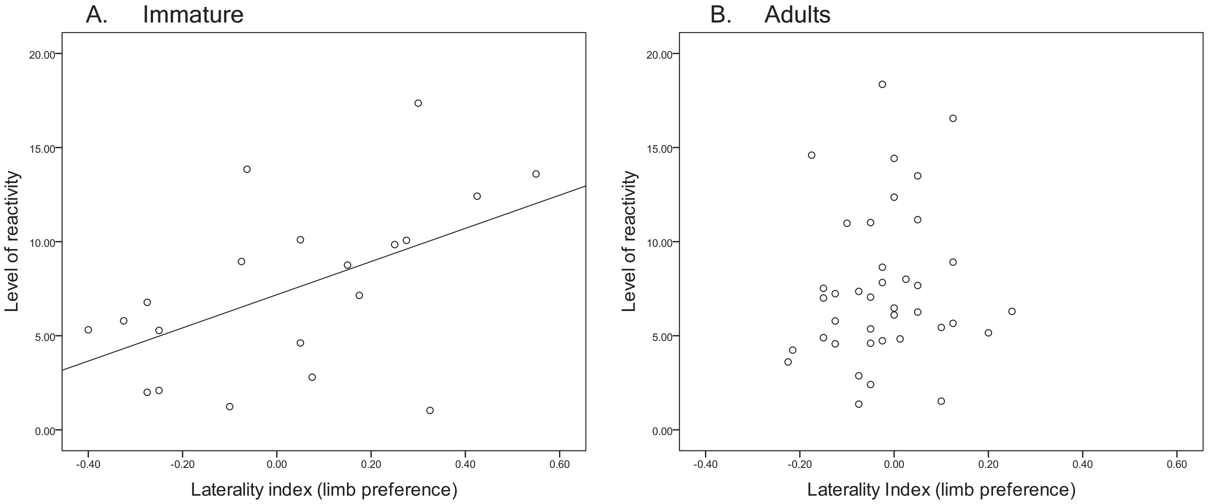


Figure 5.6 Scatterplots showing the relationship between level of reactivity (Y-axis) and forelimb preference (X-axis) expressed as Laterality Indices. A, immature feral horses B, adult feral horses. A significant association (p = 0.012) was found in the immature horses. A line is best fit is plotted; R^2 = 0.27. Level of reactivity was measured as the number of times a horse lifted its head per hour.

5.3.3 Correlation of limb preference with level of aggression

The results obtained for correlations between level of aggression and forelimb preference while grazing are given in Table 5.2. No significant associations were found.

Table 5.2 Correlations between aggression and limb preference.

Level of aggression correlated with		*N*	*Correlation coefficient (r)*	*p-value*
Forelimb preference	All horses	53	-0.13	0.347
	Males	25	-0.12	0.536
	Females	26	-0.15	0.459
	Immature	14	0.05	0.862
	Adult	29	-0.19	0.298

After Bonferroni adjustments for Type 1 error, p = 0.025 for correlations of males and females and of immature horses and adults, Pearson's r values are given, N = sample size. No significant relationships were found.

5.4 Discussion

5.4.1 Limb preference

No evidence of limb preference at the population level was found using the measure of forelimb placed in front of the other while grazing. Only 16 out of 71 (23%) horses showed significant individual limb preferences and, amongst these, there were equal numbers of left- and right-limbed horses. Absence of limb preference in feral horses, compared to previous reports of population biases in some breeds of domestic horses (McGreevy and Rogers, 2005; McGreevy and Thomson, 2005), suggests limb preference in domestic horses may be entrained by human handling. It is possible that conventional handling of horses on their left side may accentuate a pre-existing preference or even change its direction producing a left-limb preference as has been shown in thoroughbreds and standardbreds via a practice effect. Notably, the left-limb preference was weaker in the standardbred horses (McGreevy and Thomson, 2005), which are trained less predominantly on their left side compared to the training of thoroughbreds.

Nearly half of the immature horses (12 out of 25) compared to very few adults (4 of 46) showed significant individual limb preferences. Immature feral horses exhibited stronger limb preferences, irrespective of the direction of the bias, than adult feral horses. By contrast, findings from some groups of domestic horses show that limb biases strengthened with age (McGreevy and Rogers, 2005; McGreevy and Thompson, 2005; Wells and Blache, 2008). A study of domestic horses by van Heel et al. (2006) reported no change in limb preference with age but the horses were still immature at the time of the second recording. Similarly, in the present study there was no change in the strength of scores taken one year apart when horses were immature at the time of both recordings. Feral and domestic horses, therefore, show a different pattern of age effect on limb preference, giving support to the idea that limb preference is entrained in domestic horses.

The stronger limb preference in immature than adult feral horses shows that limb preference weakens with ageing. This too might be an effect of experience in the natural habitat. The substrate at both study sites was unstable and uneven. Under such conditions it may be a disadvantage to exhibit limb asymmetry, especially if it could lead to a morphological asymmetry of the hooves or limbs. Stronger forelimb preference is associated with uneven hoof loading (van Heel et al., 2006) and is linked to poor performance in horses (van Heel et al., 2010). Such a physical weakness on one side could be a serious disadvantage in the wild, especially when agility on uneven ground is essential (e.g. during flight from predators). Mandel et al. (2008) argue that some forms of otherwise advantageous lateralities may be suppressed in certain contexts because they may have negative consequences. As an example, Mandel et al. (2008) found no side bias (either at the individual or population level) in swallows avoiding obstacles during flight; they attribute this absence of a side bias to the fact that asymmetry in wing asymmetry would be detrimental to flight. Birds do, however, express brain lateralization strongly as side preferences in the processing of visual, auditory and olfactory stimuli (summarised by Rogers, 2008) and may even possess strong foot preferences associated with feeding (parrots, Harris, 1989; chicks, Rogers and Workman, 1993). In other words, a species may or may not display a limb preference but still be lateralized in other ways. The horse appears to be another example of this. Furthermore, limb preference may be expressed on some tasks and not others (Rogers, 2007).

An alternative explanation for stronger limb preference at the individual, but not population, level in immature horse is that the limbs of immature horses are longer relative to body size than those in adult horses and, therefore, younger horses need to spread their forelimbs further apart in order to reach the ground to graze. Van Heel et al. (2006) found a positive association between longer legs (and smaller heads) and stronger forelimb preferences in domestic horses. Hence, it is possible that longer legs relative to body size may contribute to the stronger preferences in the immature feral horses than in adult feral horses.

Absence of limb preference at the level of the population in feral horses is very informative given that studies of lateralization in domestic horses have focused on limb preference (e.g. Deuel and Lawrence, 1987; Murphy et al., 2005; Wells and Bache, 2008). It may be concluded that limb preference is an unreliable indicator of lateralization in horses and it may be better to determine lateralization in horses using eye preference, as shown in Chapter 4 (p. 55).

5.4.2 Reactivity and limb preference

An association was found between level of reactivity and limb preference. In immature, but not in adult horses, an increasing right-forelimb preference was associated with increasing reactivity. It may be possible that the immature horses, in particular, show a relationship between these two measures because they display a wider range of LI-scores of limb preferences (as can be seen in Figure 5.2, p. 80) than do the adults. When only horses that showed a significant individual forelimb preference were considered (N = 14, four of which were adults), right-limb preferring horses were found to be more reactive than left-limb preferring horses.

The finding in feral horses that right-forelimb preference is associated with a higher level of reactivity may, at first, be seen as contrasting to previous findings in primates showing that left-hand preference is associated with greater reactivity (e.g. Hopkins and Bennett, 1994; Cameron and Rogers, 1999). However, limb preference in horses is a different measure than is handedness in primates because horses and primates differ in the way they use their limbs and in the constraints of posture. As proposed by MacNeilage et al. (1987), postural constraints may play a role in the expression of hand preferences. Horses are quadrupeds and, unlike primates, do not use their limbs for manipulation. In horses it may be the leg giving postural support that is contralateral to the active hemisphere, and so, the behaviour expressed will be that controlled by the hemisphere opposite the limb placed behind and not the one in front. This is consistent with research on chicks demonstrating that the leg used for postural support when a chick scratches the ground in search of food is contralateral to the activated hemisphere (Tommasi and Vallortigara, 1999; Dharmaretnam et al., 2002). As noted by McGreevy and Rogers (2005), it is the forelimb that is placed behind that is in a position to give purchase if a flight response is necessary; this is the forelimb that is closer to being beneath the horses center of balance (i.e. the withers). Hence, a horse that is said to have a preference to place the right-forelimb in front of the left forelimb during grazing may actually have a left-supporting forelimb preference and be more reactive because the right hemisphere is dominant (Figure 2.1, p. 18).

No previous study has explored a specific relationship between level of reactivity and limb preference in horses but evidence of such a relationship exists in other vertebrate species (Rogers, 2009, discussed in Chapter 1, *Section 1.2.1*, p. 6). For example, common marmosets and chimpanzees with a left-hand preference display a more fearful temperament compared to right-handed individuals (described in Chapter 1, *Section 1.2.1,* p. 6). The few investigations of a potential association between limb preference and reactivity in non-primate, four-legged animals have not found the same relationship. One study in dogs showed no association between reactivity and paw preference (Siniscalchi et al., 2008), whereas another showed an association between reactivity to the sound of thunderstorm and strength, but not direction, of paw preference to hold food (Branson and Rogers, 2006). In mice, left-pawed females were more anxious than left-pawed males and ambidextrous females (Mrabet et al., 2000). These findings and those reported here from the feral horses highlight the importance of investigating the association between reactivity and paw preference in four-legged animals because weight bearing functions of the limbs may mask or modify it.

The suggestion by McGreevy and Rogers (2005) that the population bias for thoroughbred horses to place the left forelimb in front of the right, and thus use the right forelimb preferentially as support, might reflect the known reactive temperament of the thoroughbred breed is not supported by the findings reported here. It is perhaps more likely that the preference to place the left forelimb in front of the right forelimb in thoroughbred horses is due to asymmetrical handling by humans.

5.4.3 Conclusion

The absence of limb preference in feral horses during grazing is in contrast to the presence of side biases of agonistic responses and high alert reported in the previous chapter. Indeed, limb preference may not be a reliable indicator of lateralization in *Equus caballus* since it may be modified with age and change over time. Instead, limb preference may indicate a horse's level of reactivity as has been shown in other species. The exact nature of this relationship appears to be influenced by postural constraints with increasing left-supporting forelimb preference being associated with greater reactivity, and presumably right-hemispheric dominance.

CHAPTER 6 PRZEWALSKI HORSES

6.1 Introduction

The previous chapter presented results showing that laterality is a characteristic of feral *Equus caballus*. However, since feral horses are domestic horses that have returned to the wild, they cannot be considered to be entirely devoid of human influence. In addition, it is possible selective breeding over almost 6000 years of domestication (Bowling and Ruvinsky, 2000; Outram et al., 2009) may have modified the original laterality of the horse and this modification may persist in extant feral populations. Selection of horses by humans for riding and working was most likely based on traits such as tameness, speed or strength (Bowling and Ruvinsky, 2000). Selection of these characteristics may have resulted in a change in brain lateralization. Another genetic line was therefore studied: the closest living wild relative of *Equus caballus*, the Przewalski horse (*Equus ferus przewalski*; Boyd and Houpt, 1994; Chapter 2, *Section 2.3.2,* p. 21). They are not, however, the direct ancestor of the domestic horse (Chapter 2, *Section 2.3.2*, p. 21).

The herd of Przewalski horses studied has formed a social structure comparable to that of feral horses (Association pour le cheval de Przewalski: TAKH, 2008). This makes them a suitable group to compare to the feral horses because any effect of social structure on lateralization is unlikely. Differences between both groups in lateralized behaviour would more than likely be due to genetic selection.

However, there are some limitations associated with using Przewalski horses to gain indication of laterality of the wild horse. They are an endangered species, now extinct in the wild, and have undergone two bottlenecks of captive breeding, limited breeding success in the original horses taken from the wild and loss of captive breeding populations during World War II, resulting in loss of genetic diversity (Ryder, 1994). As a result, the genetic pool is small; current populations can be traced back to only 13 founders (Bouman and Bouman, 1994). Furthermore, one of those founder horses was a Mongolian domestic horse (*Equus caballus*; Bouman and Bouman, 1994; Ryder, 1994). It is not known how this may have affected lateralization. Despite these limitations, Przewalski horses may give the best approximation of laterality in horses before domestication.

Przewalski horses have been held in captivity in zoos and on reserves and have, consequently, undergone some selective breeding, although not domestication; for example the most stressed individuals from the original wild-caught herds did not breed (Bouman and Bouman, 1994). The genealogy of Przewalski horses held in captivity has been traced back to 1889 and a studbook has been kept since 1959 (Volf, 1994). Unlike domestic horses, they have been bred in captivity for only 2–12 generations (Houpt and Boyd, 1994) and recent breeding has served to retain genetic diversity rather than being for the purpose of domestication (Geyer et al., 1989). More recently, emphasis has been placed on natural breeding by releasing animals into semi-wild reserves and allowing them to form natural social bands.

6.2 Method

6.2.1 Subjects

A herd of 33 Przewalski horses was observed on a reserve at Le Villaret, France, the reserve is managed by the Association pour le cheval de Przewalski, TAKH. There were 13 bachelor stallions forming four bands and 20 horses belonging to four harem bands (5 stallions, 10 mares, 3 fillies and 2 colts). At the time of the study (July to August 2009) there was one three-year-old stallion that still remained with his natal band.

In addition to the details supplied in Chapter 3, *Section 3.2.2* (p. 38), further details of band membership, age and sex for the Przewalski horses are presented in Appendix II.

6.2.2 Data collection

Each band was observed for an accumulated time of at least 10 hours to gather recordings of agonistic interactions within harem bands. Bands were also observed at other times while they were resting and moving and, in both cases, any agonistic interactions clearly observed during those times were recorded and added to the data set.

Eight hours of video-tape of stallion fights were obtained and these were analysed. Data on agonistic interactions between stallions (stallion fights) were gathered from 18 stallions (5 harem stallions and 13 bachelors). Agonistic data were scored as described in Chapter 3, *Section 3.3.1.2* (p. 47).

In addition, each band was observed for 13 hours to record laterality scores for vigilance, percentage reactivity and high alert. Scoring was as described in Chapter 3, *Section 3.3.1.1* (p. 45). Vigilance, high alert and percentage reactivity data were obtained for all 33 horses.

Limb preference data were collected until 100 total left and right scores had been attained for each horse. Limb preference data were acquired for 31 horses; the remaining two horses were lame and, therefore, not recorded.

6.2.3 Statistical analysis

Methods of statistical analysis are given in Chapter 3, *Section 3.5* (p. 53).

6.3 Results

All data for individual horses are provided in Appendix II.

6.3.1 Eye preference during agonistic interactions

6.3.1.1 Within harem bands

A total of 2393 agonistic interactions, each containing a mean of 1.3 left or right looking bouts, were recorded within harem bands for 20 Przewalski horses. Briefly, looking bouts were periods within an agonistic interaction in which a horse was looking at a conspecific using only its left eye or right eye (i.e. when the conspecific occupied either the left or right monocular visual field). The mean number of left and right looking bouts scored per horse was 155.9 (SE = 17.7). Of all agonistic bouts recorded, 76% consisted of threats only and 24% contained an attack.

Males showed significantly lower LI-scores and, therefore, a stronger leftwards bias than females for agonistic looking bouts that contained any type of agonistic response (Mann-Whitney U, $Z = -2.26$, $p = 0.024$). Hence, males had a stronger bias to look with the left eye, or engage in an agonistic response when the conspecific occupied the left monocular visual field, than did females but both sexes showed a significant left-eye bias in agonistic responses (G-tests, females, $G(11) = 432$, $p < 0.001$, $N = 13$, and males $G(5) = 322$, $p < 0.001$, $N = 7$; Figure 6.1). As seen in Figure 6.1, the magnitude of the difference between males and females was small.

When the two categories of agonistic response, threat and attack were considered separately, no significant sex difference was found for either threats (Mann-Whitney U, $Z = -1.11$, $p = 0.267$) or attacks, Mann-Whitney U, $Z = -1.71$, $p = 0.088$). Hence, males and females were combined to give a sample size of 20 horses. Such analysis revealed a significant leftwards population bias of eye used in looking bouts containing only threats (G-test, $G(20) = 433$, $p < 0.001$) and looking bouts containing an attack (G-test, $G(20) = 335$, $p < 0.001$; Figure 6.2). The leftwards population bias of looking bouts with threats only (Mean 58% left laterality index) was significantly weaker than that of bouts containing an attack (Mean 68% left laterality index; paired t-test, $t(19) = 2.10$,

$p = 0.050$, Cohen's $d = 1.69$). Furthermore, of the 20 horses, 5 showed a significant left-eye bias for looking bouts that contained only threats and 16 showed a significant left-eye bias for looking bouts containing an attack. Taken together, eye bias for threats was weaker than that for attack at both the population and individual levels.

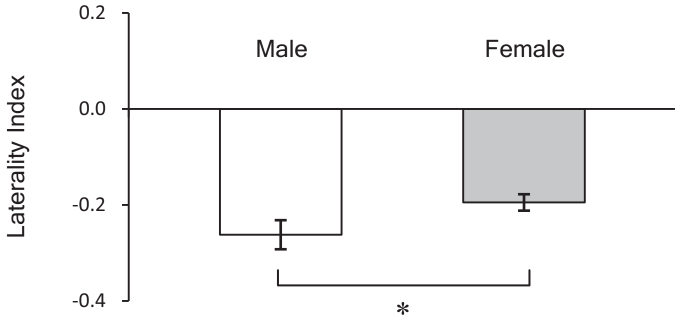


Figure 6.1 The laterality index of all agonistic responses (threat plus attack) is plotted for males (white bar) and females (grey bar), as means and standard errors. Males displayed a stronger left-eye bias than females for total agonistic responses (threat and attack). ** denotes a significant difference at $p < 0.05$

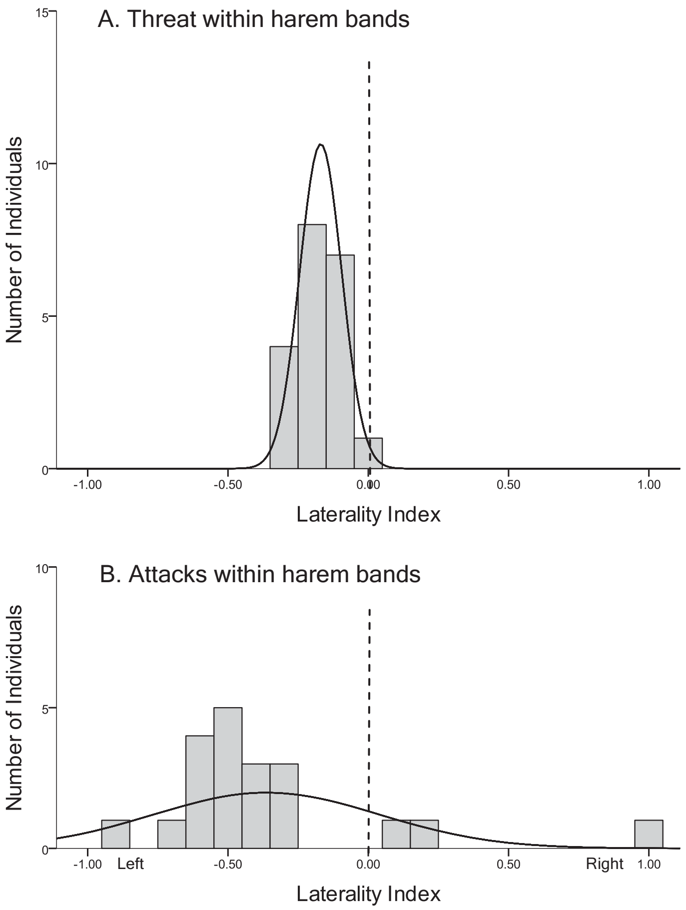


Figure 6.2 Frequency histograms of laterality indices for agonistic interactions within harems ($N = 20$). A, agonistic looking bouts consisting of threats only and B, looking bouts containing an attack. Note that the distribution for attack is shifted more strongly leftwards and shows greater variance. The dashed line indicates the zero value (no bias). Values to the left of the dotted line indicate a left laterality and values to the right indicate a right laterality. Both plots are skewed significantly to the left.

Next the strength of lateralization (absolute LI-scores) was analysed. Immature ($N = 5$) horses showed stronger LI-scores than adults ($N = 15$) for looking bouts containing an attack (Mann-Whitney U, $Z = -2.14$, $p = 0.032$), but they did not differ significantly from adults for looking bouts consisting of only threats (Mann-Whitney U, $Z = -0.48$, $p = 0.631$). Hence, irrespective of the direction of biases, immature horses were more strongly lateralized than adults for attacks but not threat (Figure 6.3).

Finally, an analysis was conducted to check whether the strength of laterality was dependent on the number of scores obtained for each horse. No significant association was found between the strength of the LI-score and the respective total number of scores recorded for either looking bouts containing only threats ($r(18) = -0.21$, $p = 0.385$) or looking bouts containing an attack ($r(18) = -0.19$, $p = 0.427$). Therefore, the strength of laterality was not influenced by how many scores had been obtained for each horse.

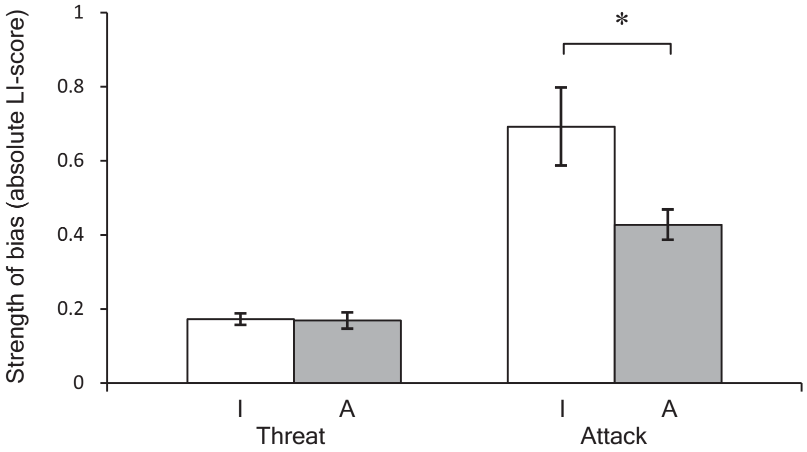


Figure 6.3 The effect of age on strength of side bias in threats only and attack. The mean strength of laterality and standard error has been plotted. Immature horses ($N = 5$) showed significantly stronger strength of laterality in attack than adults ($N = 15$). White bars represent immature (I) and grey adults (A) * denotes significance at $p = 0.05$

In summary, significant left-eye biases were found for threat and attack responses during interactions within harem bands, those for attack being stronger than those for threat, and immature horses showing stronger laterality than adults for attack but not threat.

6.3.1.2 Stallion fights

A total of 400 interactions were recorded for 18 stallions with a mean of 12.7 left or right looking bouts per interaction. The mean number of left and right looking bouts scored per stallion was 283.2 ($SD = 192$).

There was a left-eye population bias for looking bouts containing any type of agonistic response (G-test, $G(16) = 459$, $p < 0.001$), for looking bouts containing of threats only (G-test, $G(16) = 209$, $p < 0.001$; Mean 52% left bias) and for looking bouts containing an attack (G-test, $G(16) = 267$, $p < 0.001$; Mean 63% left bias; Figure 6.4). Left-eye bias in attack was significantly stronger than that of threat (paired t-test, $t(17) = 4.90$, $p < 0.001$, Cohen's $d = 0.66$). Of the 18 stallions, one showed a significant left-eye bias for looking bouts with threats only and six showed a significant left-eye bias for looking bouts with an attack.

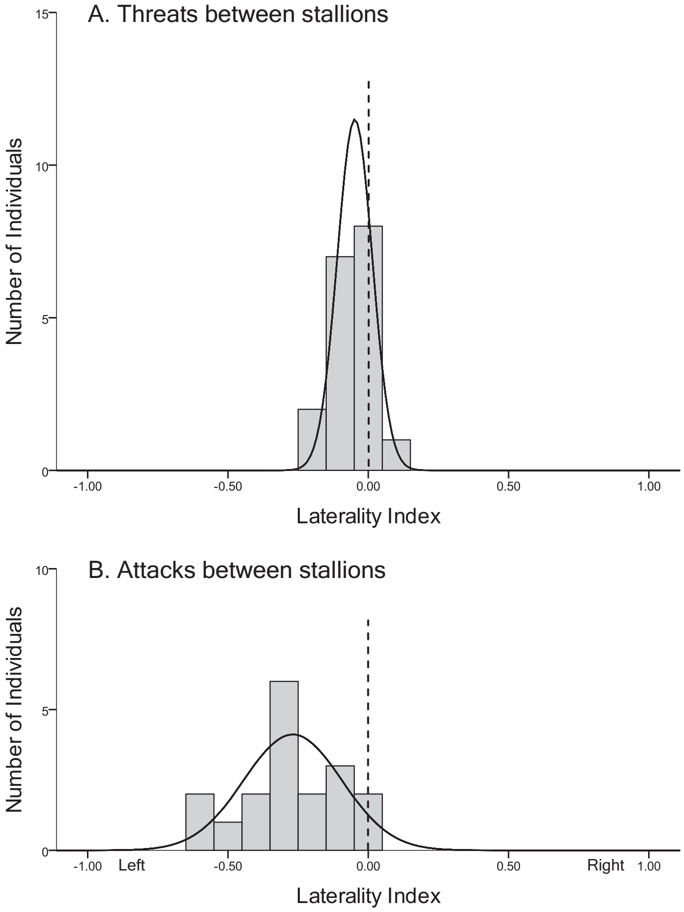


Figure 6.4 Frequency histograms of laterality indices for agonistic interactions during stallion fights ($N = 18$). A, agonistic looking bouts containing of threats only and B, looking bouts containing an attack. Note that the distribution for attack is shifted more strongly leftwards and shows greater variance than that of threat. The dashed line indicates the zero value (no bias). Values to the left indicate a left laterality and values to the right indicate a right laterality.

Stallions were then subdivided into those belonging to a harem and those that were bachelors because they differ in experience of fighting (Khalil and Kaseda, 1998) and it has been found that harem stallions have higher levels of testosterone than bachelor stallions (MacDonnell and Murray, 1995). Stallions in harems ($N = 5$) did not differ significantly from bachelor stallions in their LI-scores of eye use in looking bouts with threats only (*N* = 13; Mann-Whitney *U*, *Z* = -0.25, *p* = 0.805) or in looking bouts with an attack (Mann-Whitney *U*, *Z* = -0.74, *p* = 0.459).

No significant association was found between the strength of the LI-score and the total number of scores per individual for either looking bouts containing only threats (*r*(16) = -0.18, *p* = 0.475) or looking bouts containing an attack (*r*(16) = -0.40 *p* = 0.105). Hence, laterality was independent of the total number of scores obtained for each horse.

Stallions interacting with other stallions showed a significant left-eye bias in threats and attacks during stallion fights. There was no significant difference between bachelors and harem stallions in the strength of bias in either type of response.

6.3.2 Comparison of left-eye bias between interactions within harem bands and those during stallion fights

There were five stallions for which both agonistic data were obtained in stallion fights and in interactions within harem bands. Analysis revealed a significantly weaker left-eye bias for attack in stallion fights than in interactions in harem bands (paired t-test, *t*(4) = -3.16, *p* = 0.034, Cohen's *d* = 2.16) and no significant difference in threat (paired t-test, *t*(4) = -2.06, *p* = 0.108, Cohen's *d* = 1.58). It should be noted that the failure to find significance in the latter test is most likely due to sample size given a large effect size was shown (Cohen, 1988).

6.3.3 Side bias in vigilance

A total of 2710 scores of lifting and turning the head to the left or right side (i.e., vigilance) was obtained, with a mean of 82 scores (*SD* = 38) per horse. These scores, referred to as vigilance, included all head lifts regardless of the height to which the horse raised its head. There was no significant sex difference in laterality index of vigilance (Mann-Whitney *U*, *Z* = 0, *p* = 1.00; males *M* = -0.06, *SE* = 0.04, *N* = 20; females *M* = -0.06, *SE* = 0.10, *N* = 13). Hence, the sexes were combined, giving a sample size of 33 horses, and analysis showed a leftwards population bias of 53% (Figure 6.5; G-test, *G*(31) = 242, *p* < 0.001). Eleven of the 33 horses showed significant individual biases: 8 left and 3 right.

An analysis was also conducted on the absolute values of the vigilance LI-scores. No sex difference was found (Mann-Whitney *U*, *Z* = -0.37, *p* = 0.712) but immature horses (*N* = 50) had stronger absolute vigilance LI-scores than adults (*N* = 28; Mann-Whitney *U*, *Z* = -2.26, *p* = 0.022). Hence, immature horses were more strongly biased than adults (Figure 6.6).

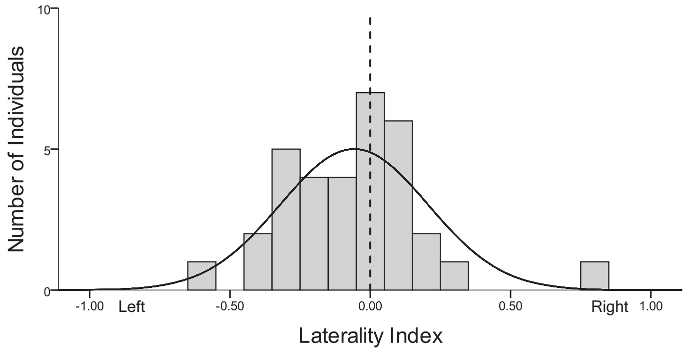


Figure 6.5 Frequency histogram (with normal curve plotted) of laterality indices for vigilance (*N* = 18). The dashed line indicates the zero value (no bias). Values to the left indicate a left laterality and values to the right indicate a right laterality. A weak but significant population bias to the left was detected.

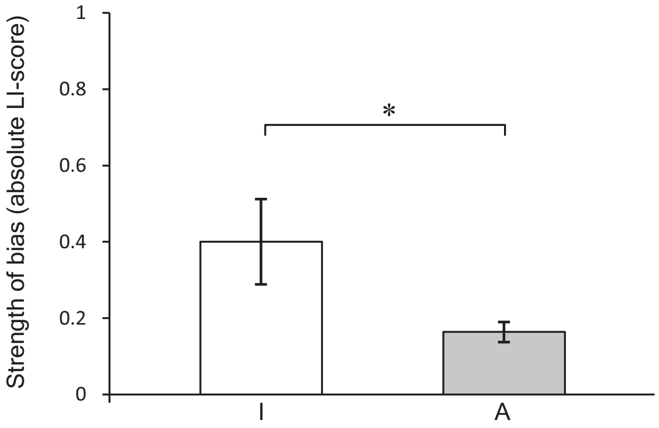


Figure 6.6 The effect of age on the strength of bias for vigilance. The mean strength of laterality and standard error has been plotted. Immature horses (*N* = 5) showed significantly stronger laterality than adults (*N* = 15). White bars represent immature (I) and grey adults (A) * denotes significance at $p = 0.05$

There was no significant association between the total number of scores obtained for each horse and the strength of lateralization ($r(31) = -0.26$, $p = 0.150$).

In summary, a significant but weak left-eye bias was found for vigilance and immature horses were more strongly lateralized than adults.

6.3.4 Side bias in percent reactivity

In total 3710 left and right scores of percent reactivity were obtained with a mean of 112 (*SD* = 51) per horse; 41% of the stimuli horses responded to were other horses. Horses on average raised their head with the poll above wither level (high alert) in 33% of the total number of head lifts. There was no significant difference between immature and adult horses in the height to which the head was raised when looking to the left (Mann-Whitney *U*, $Z = -0.58$, $p = 0.564$) or when looking to the right (Mann-Whitney *U*, $Z = -1.46$, $p = 0.145$). There was also no significant sex difference for the percentage of head lifts above the level of the withers when horses looked to the left (Mann-Whitney *U*, $Z = -1.20$, $p = 0.231$) or to the right (Mann-Whitney *U*, $Z = -0.88$, $p = 0.377$). Hence, all scores were combined and analysis found that looks to the left contained a significantly larger percentage of head lifts higher than wither level ($M = 0.41$, $SE = 0.04$) than did looks to the right ($M = 0.19$, $SE = 0.03$; $t(32) = 11.4$, $p < 0.001$, Cohen's $d = 1.29$, $N = 33$, arcsine transformed data), indicating greater percentage reactivity on the left side than on the right.

6.3.5 Side bias in high alert

Next only head lifts above the level of the withers (high alert), for which a total of 1223 ($M = 37$, $SD = 32$) scores had been attained, were considered. There was a significant leftwards population bias of 73% for this measure in LI-high alert, (G-test, $G(31) = 629$, $p < 0.001$, Figure 6.7). This is the strongest bias reported in this thesis. Twenty-two of the 33 horses showed a significant left-eye bias and zero showed a significant right-eye bias. There was also no significant difference between immature and adult horses for the strength (absolute value) of LI-high alert scores (Mann-Whitney *U*, $Z = -0.18$, $p = 0.860$).

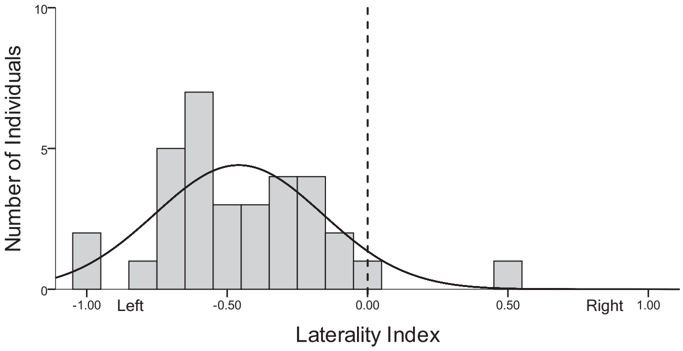


Figure 6.7 Frequency histogram of laterality indices for high alert ($N = 18$). The plot is presented as in the previous figures (e.g. Fig. 6.5).

There was no significant association between the total number of scores obtained for each horse and the strength of LI-scores of high alert (absolute LI-score, $r(31) = -0.31$, $p = 0.076$).

6.3.6 Forelimb preference

Since 100 scores were obtained for each horse, it was not necessary to conduct an analysis to determine the presence of a funnel effect. Since there was no significant difference in forelimb LI-scores between males ($N = 18$) and females ($N = 13$; Mann-Whitney *U*, $Z = -1.06$, $p = 0.289$), the LI-scores of both sexes were combined. Analysis of the entire group found no significant population bias (one-sample t-test, $t(30) = -0.72$, $p = 0.476$; $M = -0.01$, $SE = 0.02$; Figure 6.8). Five out of 31 horses showed significant limb preferences; three left and two right. Absolute LI scores did not differ significantly between immature ($N = 5$) and adult horses ($N = 26$; Mann-Whitney U, $Z = -0.05$, $p = 0.957$). Not only was there little evidence for forelimb preference, individually or as a group, but also there was no effect of age.

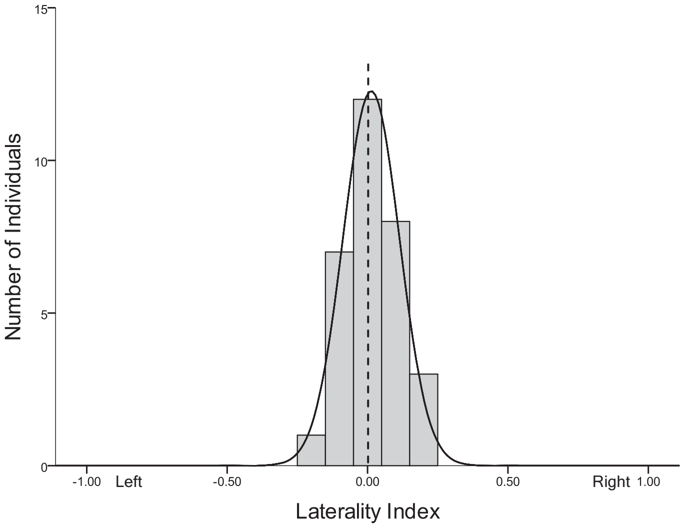


Figure 6.8 Frequency histogram of laterality indices of forelimb preference while grazing ($N = 31$). No significant bias was found. The plot is presented as in the previous figures (e.g. Fig. 6.5).

6.3.7 Comparisons with feral horses

Przewalski horses showed a significantly stronger left-side bias in high alert than did feral horses (73% versus 65% respectively; Mann-Whitney U, $Z = -2.15$, $p = 0.032$) and a trend for stronger leftwards eye bias in attack responses within harem bands (Mann-Whitney U, $Z = -1.9$, $p = 0.055$; Przewalski, 68%; feral, 63%). For all other measures there was less than a 5% difference between feral and Przewalski horses.

Chi-squared analyses were conducted to determine whether the number of individuals showing significant lateralization was different in Przewalski and feral horses. More Przewalski horses displayed an individual bias compared to feral horses for high alert ($N = 94$, $\chi^2(1) = 6.4$, $p = 0.011$), vigilance ($N = 99$, $\chi^2(1) = 6.4$, $p = 0.012$; Table 6.1) and attack responses within harem bands ($N = 87$, $\chi^2(1) = 47.7$, $p < 0.001$). No significant differences were found for the other measures (agonistic threats within harem, $N = 87$, $\chi^2(1) = 0.3$, $p = 0.587$; attacks in stallion fights, $N = 41$, $\chi^2(1) = 0.1$, $p = 0.702$; forelimb preference, $N = 102$, $\chi^2(1) = 0.5$, $p = 0.462$) but there was a trend towards significance of threat during stallion fights ($N = 42$, $\chi^2(1) = 3.7$, $p = 0.054$).

Table 6.1 The number of horses that displayed significant individual biases for high alert, vigilance, and attack responses within harems. For each measure, feral and Przewalski horses differed significantly in the number of individuals showing a significant preference. More Przewalski than feral horses displayed a significant bias.

High alert	Sig. Bias	No bias
Feral	23 L 1 R	37
Przewalski	22 L	11

Vigilance	Sig. Bias	No bias
Feral	5 L 3 R	58
Przewalski	8 L 3 R	22

Harem Attack	Sig. Bias	No bias
Feral	4 L	63
Przewalski	16 L	4

L = significant left bias, R = significant right bias.

6.4 Discussion

Przewalski horses showed a left-eye bias in threat and attack responses during agonistic interactions within harem bands and also in stallion fights. They showed a weak left-eye bias in vigilance, but no population bias of limb preference. Of particular note, a strong left-side bias in high alert (73% leftwards bias) was found in the Przewalski horses. This was the strongest population bias revealed in this project and is important because it suggests that prior to domestication horses may have been more strongly lateralized than their descendants. Hence, Przewalski horses (*Equus ferus przewalski*) show lateralization consistent with the common pattern found in other vertebrates (MacNeilage et al., 2009).

6.4.1 Eye preference in agonistic behaviour

The left-eye bias shown by Przewalski horses in attack was stronger than the left-eye bias displayed in threat. This is consistent with findings from other species showing that more aggressive responses are controlled by the right hemisphere (e.g. chicks Howard et al., 1980; rats, Denenberg, 1981) and confirms similar results in the feral horses (Chapter 4).

Przewalski horses exhibited stronger (i.e. 5% or more difference; Table 6.2, p. 101) left-eye preferences of both threat and attack in stallion fights than they did in interactions within harem bands. Agonistic interactions within harem bands and those during stallion fights differ in that, stallion fights are interactions between rival stallions, whereas interactions between members of a harem bands are concerned with defence of social hierarchy, space and access to food. Stallion fights involve ritualistic fighting as evidenced by postures such as the head bow and faeces sniffing and marking (Feh, 2005). During ritualistic fighting stallions assess their opponent rather than engage in actual aggressive attacks that could result in injury.

Previous reports have stated that stallion fights in the herd of Przewalski horses studied have become more ritualistic since the horses were first released into the reserve (Association pour le cheval de Przewalski: TAKH, 2008). When two stallions challenge each other they perform a ritualistic dominance display that entails smelling faeces, head bowing, squealing with tossing of the head and foreleg lifting or striking, which can sometimes escalate into an actual fight involving pushing, striking, kicking, rearing and biting (Feh, 2005). Most of the threat records obtained in this study of the stallions involved ritualistic dominance displays. A number of agonistic responses categorised as attack (e.g. strike, rear) did not involve actual contact but they were categorised as such because they entailed the same motor actions irrespective of whether or not contact occurred and the intention to harm, had the opponent not moved out of the way, could not be evaluated (these responses were scored as high level threats, *Section 3.3.1.2,* p. 47). The finding of weaker left-side bias in attack in stallion fights than in interactions within harems emphasises the importance of context on the functional significance of behaviour. Although a behavioural response may appear to be similar in two situations, the brain mechanisms controlling it may vary. Use of the left eye to view the opponent may indicate aggression, whereas use of the right eye may indicate ritualistic posturing.

We know that the left hemisphere controls the performance of routine behaviour (MacNeilage, 2007; MacNeilage et al., 2009). Since ritualised fighting involves routine motor sequences, the weaker left-eye biases shown by stallions during fighting and threat displays compared to those found in interactions between members of harem bands could show more use of the left hemisphere. On average, stallion–stallion agonistic interactions contained more looking bouts (Mean 12.7) per interaction than encounters between members of harem bands (Mean 1.3), indicating that stallions changed the eye they used to view their opponent often and, hence, may have switched between use of the left and right hemispheres. This may mean alternation between ritualised threat and actual attack.

Since agonistic responses scored as attack within harem bands usually involved contact and caused harm, if the recipient did not rapidly move out of the way, they were not ritualistic posturing. Of the 20 Przewalski horses belonging to harems, 16 showed a left-eye bias for attack (including all harem stallions), whereas only six of 18 stallions showed significant eye-bias for attack during stallion fights. The stronger lateralization of attack and threat within harem bands compared to stallion fights may reflect greater involvement of the right hemisphere in controlling actual aggression, as shown in other species (e.g. chicks Howard et al., 1980; rats, Denenberg, 1981).

6.4.2 Side bias in vigilance and reactivity

The left-side bias of head-turning in the high alert position, measured using only head lifts that were above the level of the withers, shown by the Przewalski horses was very strong indeed (73% left). This means that horses were more likely to lift their head above the level of the withers when they turned their head to the left than right. Moreover, 22 of 33 horses were lateralized at the individual level, all to the left. In comparison, left-side biases in response to simulated predators have been found in other species, although they were weaker than that found here in Przewalski horses. Toads showed a 60% leftwards bias for jumping sideways from lateral presentation of a model predator (Lippolis et al*.,* 2002), stripe-faced dunnarts displayed a 62% leftwards bias in making a response (e.g. startle, retreat, ears back or orientation; Lippolis et al*.,* 2005) and lizards showed a 65% leftwards bias in direction of turning after escape of a simulated predator attack (Bonati et al., 2010). In dogs, Siniscalchi et al. (2008) found an even stronger 85% leftwards head-turning bias to the sound of a thunderstorm, a stimulus to which they were highly reactive, than that found in horses. This left bias in high alert in Przewalski horses was stronger than that shown by the feral horses (65% left).

Since there were no predators in the reserve, it can be said that experience with predators is not necessary for the left-side bias in high alert to develop. Moreover, there was no age effect on the strength of individual bias in high alert suggesting that experience does not have an influence on the laterality in high alert, at least in horses older than one year of age since no Przewalski horses younger than one-year-old could be observed. Many head-turning responses were elicited by conspecifics (41%). Hence, threat from conspecific rivals or unseen threats may be sufficient for the development of this bias.

6.4.3 Limb preference

Przewalski horses displayed no limb preference at the population level and very few individuals showed a significant preference (16%; 3 left and 2 right). The absence of limb preference at the population level in Przewalski horses suggests that the ancestral horses may not have shown limb preferences in the wild. Individual limb preference may have been a disadvantage to horses living in the wild (discussed in Chapter 5, *Section 5.4.1,* p. 84). Other lateralized functions may be sufficient to maintain cohesion and coordination of the group. Indeed, free-roaming reindeer circle leftwards (anticlockwise) when corralled or when forced into a group in the open but they do not display limb preferences to paw the ground in search of food (Espmark and Kinderås, 2002).

6.4.4 Comparison of lateralization between Przewalski and feral horses

It was an important consideration for this thesis to see whether or not Przewalski horses differ from feral horses. The findings from Przewalski and feral horses are summarised in Table 6.2. Generally, Przewalski horses showed lateralities similar to those of feral horses in direction and strength. Both groups showed left-eye biases in agonistic behaviour, left head-turning responses in high alert and vigilance, and no limb preference. However, the left-side biases in high alert and attack within harem bands were stronger in Przewalski than feral horses. In addition a larger percentage of Przewalski horses showed individual lateralization in high alert, attack and vigilance in comparison to feral horses.

Table 6.2 Strength of group biases (% left) and the percentage of individuals that were significantly lateralized in Przewalski horses ($N = 33$) and feral horses ($N = 76$).

Group		High alert	Vigilance	Harem		Stallion fights		Limb
				Threat	Attack	Threat	Attack	
% leftwards population bias	Feral	**65**	54	57	**63**	55	61	50
	Przewalski	**73**	53	58	**68**	52	63	50
% horses with significant bias	Feral	**39**	**12**	36	**6**	30	41	23
	Przewalski	**67**	**33**	25	**80**	6	33	16

Bolded values represent significant differences at $p < 0.05$ between Przewalski and feral horses.

Social structure can be ruled out as an explanation why feral and Przewalski horses would differ in lateralization of attack and high alert since both groups live under similar natural social conditions. Two further reasons can be given. First, lateralization of aggression and/or high alert may have been weakened by prior domestication of the feral horses. The lack of a difference between Group 1 and Group 2 feral horses in laterality of attack and high alert suggests that selection against aggression and reactivity over many generations of domestication is robust and, therefore, supports this explanation.

Alternatively, selective breeding of Przewalski horses in captivity or the captive environment may have influenced laterality of aggression. In fact, some evidence points to abnormal aggression being a product of previous captivity practices restricting social experience. Feh and Munkhtuya (2008) found that founder stallions from this particular group engaged in infanticide, whereas second generation stallions did not, that is, the stallions had become less aggressive in natural social conditions.

Regarding laterality of high alert, it is unlikely that the absence of predators in the Przewalski reserve and the presence of predators in the habitat of the feral horses contribute to the difference shown by feral and Przewalski horses. Based on previous research by Brown et al. (2004) on fish from high and low regions of predation, the prediction would be that an absence of predators would result in little or no biases and the presence of predators would result in a strong bias. Results from feral and Przewalski horses are contrary to this.

6.4.5 Conclusion

Similar to feral horses, Przewalski horses show preferred right-hemisphere use in agonistic responses within harem bands, high alert and vigilance. Although modification of lateralization during captivity cannot be ruled out completely in Przewalski horses, findings of laterality in these horses suggest that it is likely that the wild ancestor of *Equus caballu*s was lateralized, and perhaps, more strongly than present day *Equus caballus*.

CHAPTER 7 DOMESTIC HORSES

7.1 Introduction

This chapter reports on lateralization in domestic riding horses of various mixed breeds, using the same methodology employed for recording laterality in the feral and Przewalski horses to enable direct comparison of the measured lateralities.

It is believed that horses have been domesticated for 6000 years (Levine, 2005). The earliest evidence of domestication dates back to 3500 BC in the Eneolithic Botai culture of Kazakhstan, (Outram et al., 2009). Evidence suggests that domesticated horses were used for milking.

Since then domestic horses have been employed for a number of purposes throughout history including, meat, transport (riding and cart), farming (pulling ploughs), warfare and industrial use (Hall, 2005). More recently, in the developed world, they are used for recreational riding. Such different uses involve different types of training. Draught horses are handled on both sides and driven from behind, whereas riding horses are predominantly handled on the left side and are subjected to a rider on their back, which has implications for balance. The majority of horses today in developed countries are used for the purpose of recreational riding. Domestic breeds used for the purpose of riding were chosen in this study because they are conventionally handled on the left side and they are ‘broken in’ to the saddle and rider. Being ‘broken in’ may be a traumatic experience (Nicol, 2005) to the horse and it is known that stressful experiences can modify lateralization (Chapter 2, *Section 2.4.3,* p. 24).

Several accounts have been given as to why horses are traditionally handled on the left side. Often, the reason for mounting horses on their left side is attributed to people wearing swords on their left side (i.e. to avoid hitting the horse with the sword while mounting). It is also possible that left-side handling was an attempt to habituate horses on this side because early handlers noticed that horses were more reactive to objects on their left than right side (Larose et al., 2006). Alternatively, handling of horses on their left side could be due to right handedness and footedness in humans; it is much easier for a right handed and footed person to lead and mount a horse on its left side, this being the case particularly before the invention of the stirrups (approximately 100 AD, Hall, 2005). Asymmetrical handling of riding horses on their left side may influence lateralization.

7.2 Methods

All domestic horses in the sample were riding horses. Since breed differences in laterality of domestic horses have been reported (e.g. McGreevy and Thomson, 2005; Larose et al., 2006), breed was also considered as a variable that might influence lateralization. A subgroup of Arab horses was investigated separately because this breed is considered to be the most flighty of all horse breeds (Hausberger, 2004). It was expected the Arab breed might show a group bias for limb preference, as has been found in thoroughbred horses, reflecting their flighty temperament (McGreevy and Rogers, 2005). Before domestication, more than one subspecies of *Equus ferus* existed across Europe and Asia (discussed in Chapter 2, *Section 2.3.2,* p. 21). It is thought the Arab breed may have arisen from one of these subspecies, one of fine build and adapted to living in an open environment (Goodwin, 2002; Hausberger and Richard-Yris, 2005). In an open environment, where very few obstacles are present and the best strategy for dealing with predators is flight, it may be an advantage to show a limb preference as this would ensure members of the group turn in the same direction and thus maintain the integrity of the herd.

Experiential influences on side biases were also examined. One possible explanation put forward in Chapter 4 for the weaker bias shown by adult compared to immature feral horses was the experience of moving over steep unstable terrain. Since one of the groups of domestic horses was kept on rocky, steep and uneven terrain this hypothesis could be tested.

Data were also collected from a subgroup of domestic riding horses that had not been ‘broken in’, and therefore never ridden ($N = 11$), so that these horses could be compared with those that had been ‘broken in’ and ridden. Also a subgroup of horses used for trail riding were chosen because unlike the other riding horses they were usually ridden on a daily basis and they were part of a large herd ($N = 29$), although not a natural social structure, both of which may influence lateralization. Only limb preference and agonistic data were obtained from this latter subgroup.

7.2.1 Subjects

Data were collected from 84 (41 males, 43 females) domestic riding horses: 24 recreational riding horses, 29 working riding horses, eight crossbred Arab horses and 23 purebred Arab horses. Eleven of these domestic riding horses had not been ‘broken in’ to saddle and had, therefore, never been ridden, although they had received asymmetrical left-side handling. Some details of the horses are provided in Chapter 3 *Section 3.2.3* (p. 40) and further details are given in Appendix III. Briefly, each of the ‘broken in’ recreational riding horses was owned and ridden by one person, usually on weekends only, whereas the working riding horses were owned by a trail riding business and usually ridden every day by many people of differing levels of riding ability (all trial riding horses had been ‘broken in’). The subgroup of Arab horses belonged to the Copeland breeding stud in North Queensland (purebred Arab $N = 13$), a group used for recreational and endurance riding (purebred Arab $N = 6$; crossbred Arab $N = 3$) and a smaller group belonged to the Center for Eco-Ethos Research in France (purebred Arab $N = 4$; crossbred Arab $N = 5$). The crossbred Arab horses were at least half-blood Arab (one parent was purebred). The purebred Arab horses used in the present study were trained for endurance and dressage.

7.2.2 Limb preference

Forelimb preference data were collected from 81 domestic horses that were not lame; of these, 73 were each observed for one hour and 8 were observed until 100 scores had been obtained (at the Centre for Eco-Ethos Research and Education). Data were also obtained from one other horse used as a case study because it had been previously injured and was lame (at the Centre for Eco-Ethos Research and Education).

7.2.3 Side bias in vigilance and reactivity

Vigilance, high alert and percentage reactivity scores were obtained from 54 horses. Each horse was observed for at least an accumulated total of five hours to score percentage reactivity and an accumulated three hours to score vigilance behaviour. Scores were not collected from the trail riding horses because they rarely lifted their heads to look at stimuli. In addition, one of the Arab horses (a stallion) from north Queensland was moved before it could be sampled for vigilance and reactivity. The definitions and method of calculating laterality in vigilance, high alert and percentage reactivity are described in Chapter 3 *Section 3.3.1.1,* (p. 45) and on page xii.

7.2.4 Eye bias in agonistic behaviour

Agonistic behaviour was scored from 82 horses. Two Arab breeding stallions were not scored because they were housed alone in separate fields. Each horse was observed for at least an accumulated total of five hours. Agonistic data were scored as described in Chapter 3, *Section 3.3.1.2* (p. 47) and definitions are given on page xii.

7.3 Results

7.3.1 Laterality in domestic riding horses

7.3.1.1 Limb preference

The number of scores obtained from domestic horses for the forelimb placed in front of the other when grazing ranged from 55 to 126 ($M = 102$). There was no association between the total number of scores obtained from each horse and the absolute value of the LI-score of limb preference ($r(79) = -0.09$, $p = 0.416$; Figure 7.1). Hence, no funnel effect was present and all scores were used in further analysis.

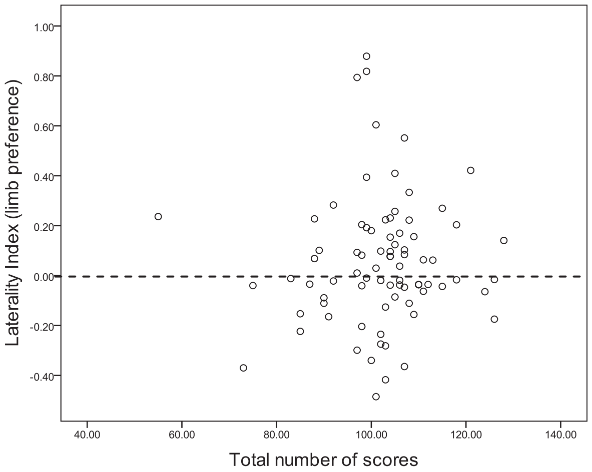


Figure 7.1 Scatterplot of laterality index of forelimb preference during grazing (Y-axis) and the total number of scores obtained from each horse (X-axis). The correlation between absolute values of LI-scores and the total number of scores was not significant, indicating no funnel effect. The dotted line indicates no bias.

No significant population bias of forelimb preference was found (one-sample, $t(80) = 1.91$, $p = 0.060$), although there was a trend for a rightwards bias. There was no significant sex difference in LI-scores of limb preference ($t(79) = -0.16$, $p = 0.876$, Cohen's $d = 0.04$). Limb preference of immature horses ($N = 6$) did not differ significantly from limb preference of adult horses ($N = 75$; Mann-Whitney U, $Z = -0.79$, $p = 0.427$). Also limb preference of non-ridden horses ($N = 10$) did not differ significantly from limb preference of ridden horses ($N = 71$; Mann-Whitney U, $Z = -0.73$, $p = 0.464$).

Following division of the data into purebred Arab versus non-Arab horses (crossbred Arab horses were excluded from this analysis), a significant rightwards population bias was detected in the group of purebred Arab horses (one-sample, $t(21) = 2.89$, $p = 0.009$; Cohen's $d = 1.27$, 61% right-limb preference) but no significant bias was found in the group of non-Arab horses (one-sample, $t(51) = -0.71$, $p = 0.481$, Cohen's $d = 0.05$; Figure 7.2). In addition, purebred Arab horses showed significantly more rightward LI-scores than did non-Arab horses (Mann-Whitney *U*, $Z = -2.96$, $p = 0.003$, $N = 74$).

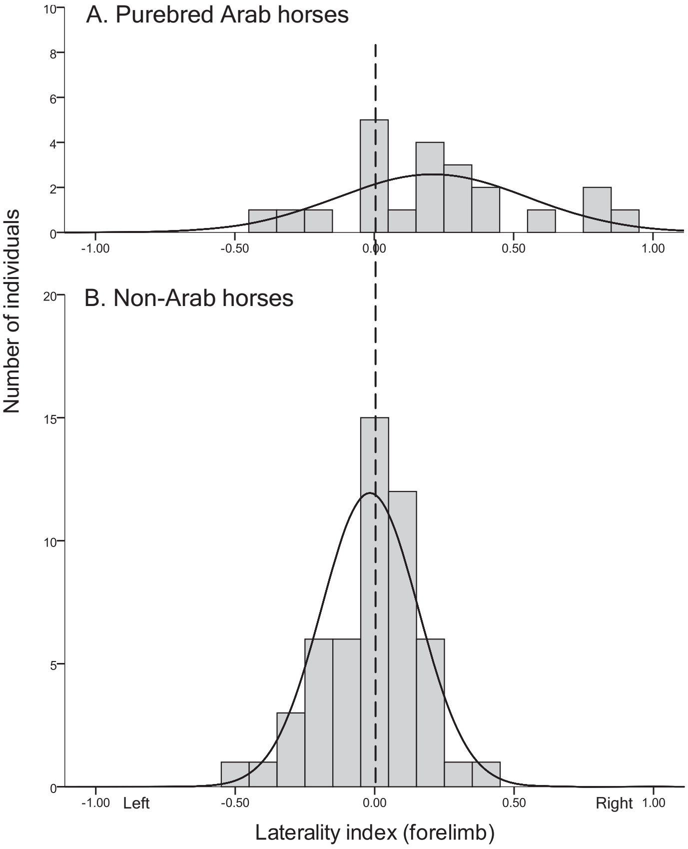


Figure 7.2 Histograms (with normal curve plotted) showing the population distributions of limb preference for A, purebred Arab horses ($N = 22$) and B, non-Arab (excluding crossbred Arab) riding horses ($N = 52$). The purebred Arab horses showed a significant rightwards population bias of large effect size. The purebred Arab horses also showed more variance than the non-Arab horses. No significant bias was present in the non-Arab group (B). The dashed line indicates the zero value (no bias). Values to the left indicate a left laterality and values to the right indicate a right laterality.

Results from each subgroup of domestic riding horses are summarized in Table 7.1. There was a significant difference in LI-scores across the four subgroups of horses (Kruskal-Wallis test, $\chi^2(3, N = 81) = 12.4$, $p = 0.006$). *Post hoc* pair-wise comparisons revealed that purebred Arab horses differed significantly from recreational horses (Mann-Whitney *U*, $Z = -3.09$, $p = 0.002$) and working horses (Mann-Whitney *U*, $Z = -2.17$, $p = 0.030$), but not from crossbred Arab horses (Mann-Whitney *U*, $Z = -0.87$, $p = 0.409$). The LI-scores of crossbred Arab horses did not differ significantly from those of recreational (Mann-Whitney *U*, $Z = -1.55$, $p = 0.131$) or working riding horses (Mann-Whitney *U*, $Z = -0.86$, $p = 0.410$). Recreational riding horses displayed significantly lower, and thus more leftwards, LI-scores than working horses (Mann-Whitney, $Z = -2.07$, $p = 0.038$).

No significant population biases were found in the crossbred Arab (one-sample, $t(6) = 0.87$, $p = 0.416$, Cohen's $d = 0.65$), recreational riding (one-sample, $t(22) = -1.90$, $p = 0.071$, Cohen's $d = 0.81$) or working riding horses (one-sample, $t(28) = 1.08$, $p = 0.288$, Cohen's $d = 0.46$). It should be noted that there was a trend towards significance in the recreational riding horses for a left bias and the effect size of 0.8 was large (Cohen, 1988).

Table 7.1 Limb bias of the different groups of domestic horses.

	LI-score			Absolute LI-score	
	M	*SD*	*N*	*M*	*SE*
Recreational	-0.08	0.19	23	0.15	0.02
Working	0.03	0.14	29	0.12	0.02
Crossbred Arab	0.10	0.30	7	0.23	0.08
Purebred Arab	0.21	0.34	22	0.30	0.06

LI - laterality index limb preference, *M* – mean, *SD* – standard deviation, *N* – sample size, *SE* – standard error.

Thirty out of 81 domestic horses displayed a significant limb preference. Significantly more purebred Arab horses showed an individual preference compared to the other riding horses (Pearson's $\chi^2(1, N = 81) = 3.97$, $p = 0.046$; Table 7.2). In particular, the two youngest Arab horses (less than 6 months of age) showed very strong individual, right-forelimb preferences of 79% and 88%. There were significantly more non-ridden horses than ridden horses that showed an individual preference (Pearson's $\chi^2(1, N = 81) = 12.6$, $p < 0.001$; Table 7.2). As can be seen in Table 7.2, more than half of the non-ridden horses had a significant limb preference, whereas less than 20% of ridden horses had a significant limb preference.

Table 7.2 The number of domestic horses that showed a significant individual limb preference. A, purebred Arab versus other riding horses and B, ridden versus non-ridden horses

A.

	Purebred Arab	Other riding
Significant bias	12	18
No bias	10	41

B.

	Non-ridden	Ridden
Significant bias	7	13
No bias	3	58

Purebred Arab horses (*N* = 22) also showed stronger limb preferences (absolute LI-scores) than all other riding horses (*N* = 59; Mann-Whitney *U*, *Z* = -2.55, *p* = 0.011). However, in comparison to the other riding horses, there were more immature (purebred Arab, *N* = 4; other riding horses, *N* = 2) and non-ridden horses (purebred Arab, *N* = 8; other riding horses, *N* = 2) in the group of purebred Arab horses.

Considering all horses, immature horses (*N* = 6) showed significantly stronger limb preferences (absolute LI-scores) than mature horses (*N* = 75; Mann-Whitney *U*, *Z* = -2.9, *p* = 0.003; Figure 7.3A). Also non-ridden horses (*N* = 10) showed a significantly stronger strength of limb preference than ridden horses (*N* = 71; Mann-Whitney *U*, *Z* = -3.06, *p* = 0.002; Figure 7.3B); four of the non-ridden horses were adult horses.

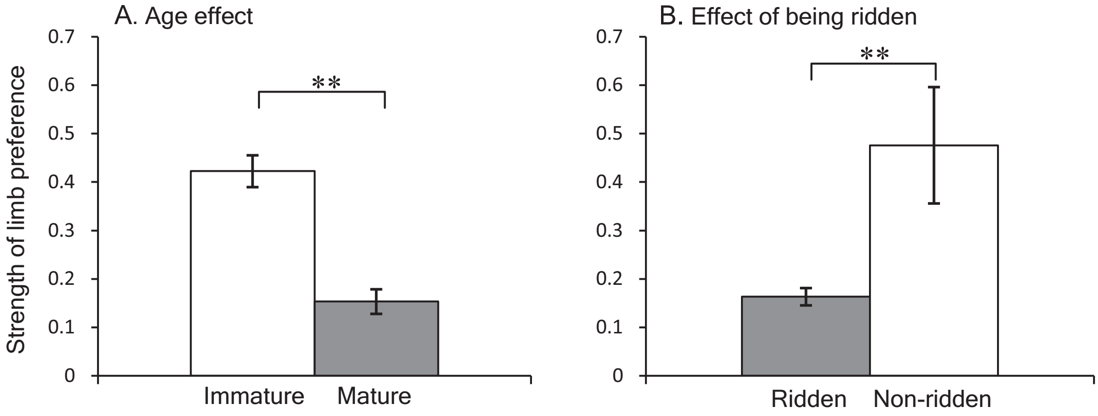


Figure 7.3 The effect of age and being ridden on the strength of limb preference. A, immature horses (*N* = 6) displayed significantly stronger limb preference than adult horses (*N* = 75). B, non-ridden horses (*N* = 10) displayed significantly stronger limb preference than ridden (*N* = 71) horses. Absolute values of laterality scores of forelimb preference are plotted on the Y-axis. Means and standard errors are plotted. ** $p < 0.01$

In addition, a group of Arab and crossbred Arab horses kept on terrain that was particularly steep and rough (*N* = 8), at the Centre for Eco-Ethos Research and Education, was compared to the remaining Arab and crossbred Arab riding horses kept on flat or undulating terrain (*N* = 21) in order to determine whether an effect of walking over rough terrain was present. There was no significant effect of terrain on the strength of limb preference (Mann-Whitney *U*, *Z* = -1.12, *p* = 0.262) or its direction (Mann-Whitney *U*, *Z* = -0.63, *p* = 0.546).

7.3.1.1.1 Case study on lameness and limb preference

Limb preference data were collected for a horse that had had an injury when it was younger and had developed lameness in its left forelimb. A binomial *Z*-score showed this horse to have a significant preference to place the left forelimb in front of the other during grazing, *Z* = -3.08, *p* < 0.05 (two-tailed, *Z* exceeds a value of |1.96|).

7.3.1.2 Side bias in vigilance

The mean number of vigilance scores obtained per horse was 48, but scores ranged from 3 to 77. As mentioned above, no vigilance scores were obtained from the working horses. Since there was no significant funnel effect (*r*(52) = - 0.15, *p* = 0.271; Figure 7.4), the strength of laterality in vigilance was independent of the number of scores obtained for each horse.

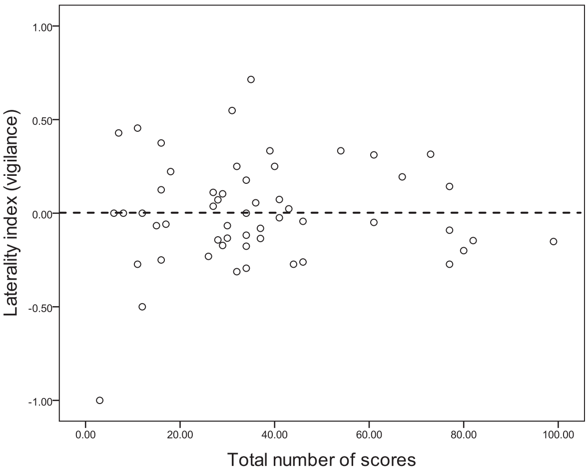


Figure 7.4 Scatterplot of the relationship between laterality index of vigilance and the number of scores (X-axis) obtained for each horse. LI-scores are plotted on the Y-axis. There was no significant funnel effect, i.e. there was no significant association between absolute LI-scores and the total number of scores. The dotted line indicates no bias.

No significant sex (Mann-Whitney *U*, *Z* = -0.68, *p* = 0.495; *N* = 54, 23 males and 31 females) or age (Mann-Whitney *U*, *Z* = -0.12, *p* = 0.908; *N* = 54, 7 immature and 47 adult horses) differences were detected in LI-scores of vigilance. Also, there was no significant difference in the LI-scores of vigilance between ridden and non-ridden horses (Mann-Whitney *U*, *Z* = -0.41, *p* = 0.683).

There was no significant side bias in vigilance (G-test, *G*(52) = 39, *p* > 0.05, *N* = 54). However, there was a significant difference between the LI-scores of vigilance in purebred Arab and non-Arab recreational horses (*t*(44) = -2.12, *p* = 0.040, Cohen's *d* = 0.63; crossbred Arab horses were excluded from this analysis). Non-Arab recreational horses showed more negative LI-scores (and thus a leftwards side bias) for lifting their heads to attend to stimuli detected to the side than did purebred Arab horses (Figure 7.5).

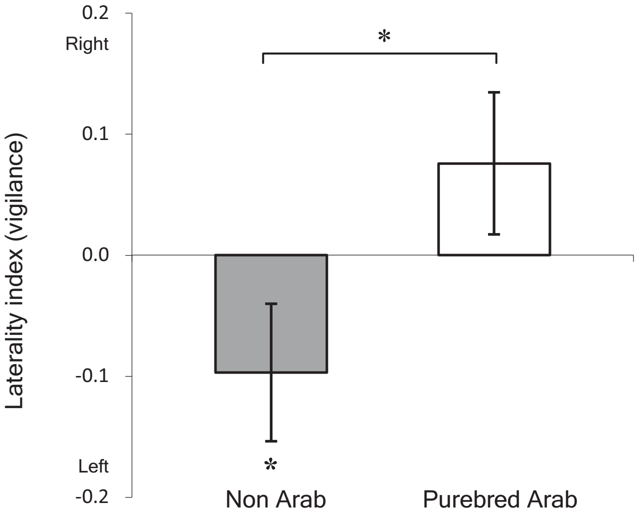


Figure 7.5 Comparison of side bias in vigilance; non-Arab riding horses showed a more leftwards side bias than purebred Arab domestic horses. Mean LI-scores (and standard errors) are plotted on the Y-axis and purebred Arab ($N = 23$) and non-Arab horses ($N = 24$) are represented on the X-axis. * $p < 0.05$

The non-Arab recreational horses showed a significant left-side bias (G-test, $G(22) = 69$, $p < 0.001$, $N = 24$; 55% left-side preference), whereas purebred Arabs did not show a significant side bias (G-test, $G(21) = 9.7$, $p > 0.05$, $N = 23$; Figure 7.6).

There were no significant differences in the strength of side bias in vigilance between ridden and non-ridden horses (Mann-Whitney U, $Z = -0.39$, $p = 0.699$), immature and adult horses (Mann-Whitney U, $Z = -1.3$, $p = 0.210$), or males and females (Mann-Whitney U, $Z = -1.26$, $p = 0.208$).

7.3.1.3 Percentage reactivity

The percentage of heads lifts above the level of the withers was significantly higher when domestic horses looked at stimuli detected on the left side than on the right side (paired t-test, $t(53) = 2.15$, $p = 0.043$, Cohen's $d = 0.33$, arcsine transformed data; Figure 7.7).

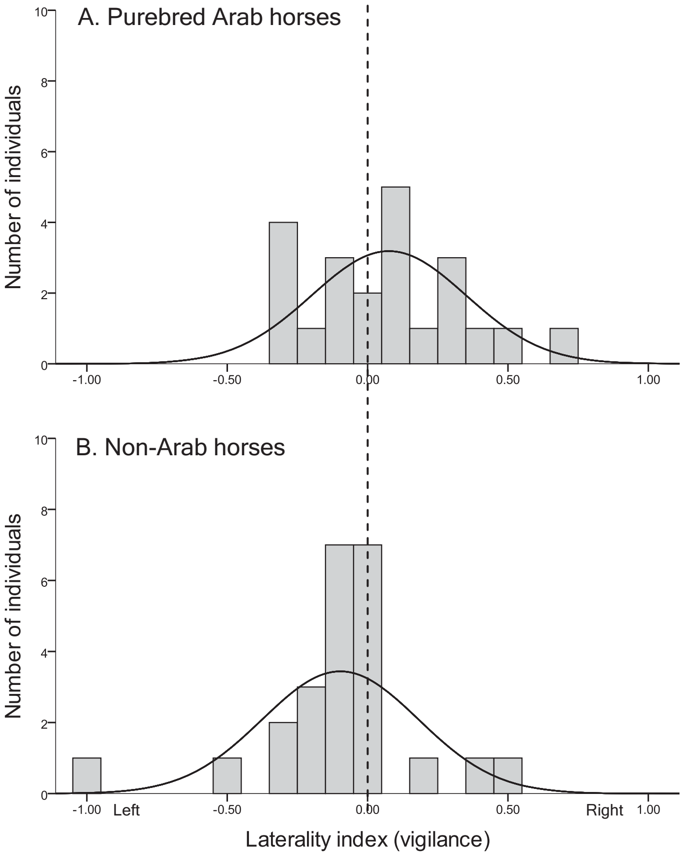


Figure 7.6 Population distributions of side bias in vigilance. LI-scores are plotted on the X-axis and the number of individuals on the Y-axis. A significant left-side bias was shown by the non-Arab recreational riding horses (B) and no significant bias was found in the purebred Arab horses (A). The dashed line indicates the zero value (no bias). Values to the left indicate a left laterality and values to the right indicate a right laterality.

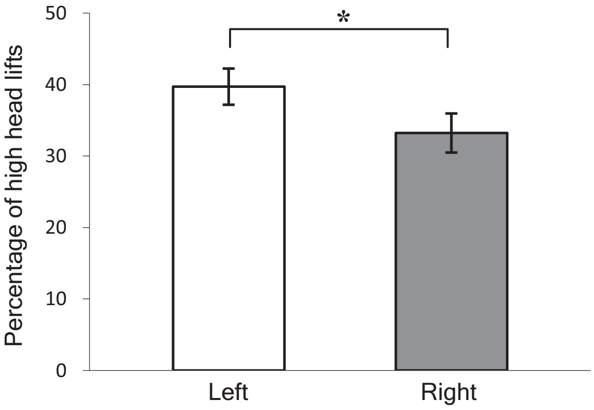


Figure 7.7 The percentage of high head lifts (Y-axis) in order to look to the left or right. Horses raised their head higher when looking to the left ($N = 55$). Means and standard errors are plotted. * $p < 0.05$

No significant sex differences were detected in the scores of looks to the left (Mann-Whitney *U*, $Z = -0.91$, $p = 0.363$) or right (Mann-Whitney *U*, $Z = -1.23$, $p = 0.220$; 23 males, 31 females). There were also no significant differences between immature ($N = 7$) and adult horses ($N = 47$) in percentage of high head lifts for looks to the left (Mann-Whitney *U*, $Z = -0.81$, $p = 0.417$) or right (Mann-Whitney *U*, $Z = -0.25$, $p = 0.821$).

Significant differences were, however, detected between ridden ($N = 43$) and non-ridden horses ($N = 11$) in the percentage of high head lifts for looks to the left (Mann-Whitney *U*, $Z = -2.14$, $p = 0.033$) but not looks to the right (Mann-Whitney *U*, $Z = -1.75$, $p = 0.080$; Figure 7.8). Non-ridden horses showed stronger percent reactivity on their left side than ridden horses. Whereas ridden horses showed a significant asymmetry with a higher percentage of high head lifts when looking to the left than to the right ($t(42) = 2.27$, $p = 0.028$, Cohen's $d = 0.37$, arcsine transformed data), no significant asymmetry was found in the horses that had never been ridden ($t(10) = 0.54$, $p = 0.599$, Cohen's $d = 0.22$, arcsine transformed data).

Next, the same data (arcsine transformed data) were analysed to see whether purebred Arab horses ($N = 22$) differed from non-Arab horses ($N = 24$). A 2x2 factor ANOVA was conducted with side of looking as a within measure and subgroup (purebred Arab and non-Arab recreational horses) as a between measures factor. Crossbred Arab horses were not included in this analysis because there were substantially fewer horses in this subgroup than in the other two groups. There was a significant main effect of subgroup ($F(1,44) = 15.37$, $p < 0.001$, partial $\eta^2 = 0.26$), no significant effect for side of looking ($F(1,44) = 1.72$, $p = 0.197$, partial $\eta^2 = 0.04$) and no significant interaction between side of looking and subgroup ($F(2,44) = 0.61$, $p = 0.440$, partial $\eta^2 = 0.01$; Figure 7.9). Purebred Arab horses lifted their heads above the level of the withers more often than non-Arab horses and they were not lateralized.

A separate analysis was conducted for the crossbred Arab horses and this found that they were significantly more likely to lift their heads high in a greater percentage of times when looking to the left than to the right ($t(7) = 3.08$, $p = 0.018$, Cohen's $d = 0.91$; arcsine transformed data).

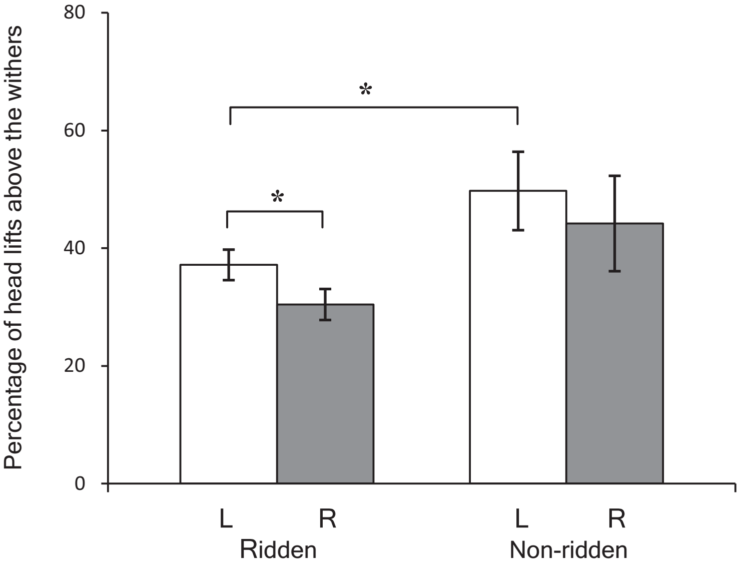


Figure 7.8 Comparison of the percentage of head lifts above the level of the withers for looks to the left and looks to the right between ridden ($N = 11$) and non-ridden horses ($N = 43$). Ridden horses showed a significant left-side bias and a significantly lower percentage of head lifts above the wither level than non-ridden horses when looking leftwards. Means and standard errors are plotted. L Left, R Right, * $p < 0.05$

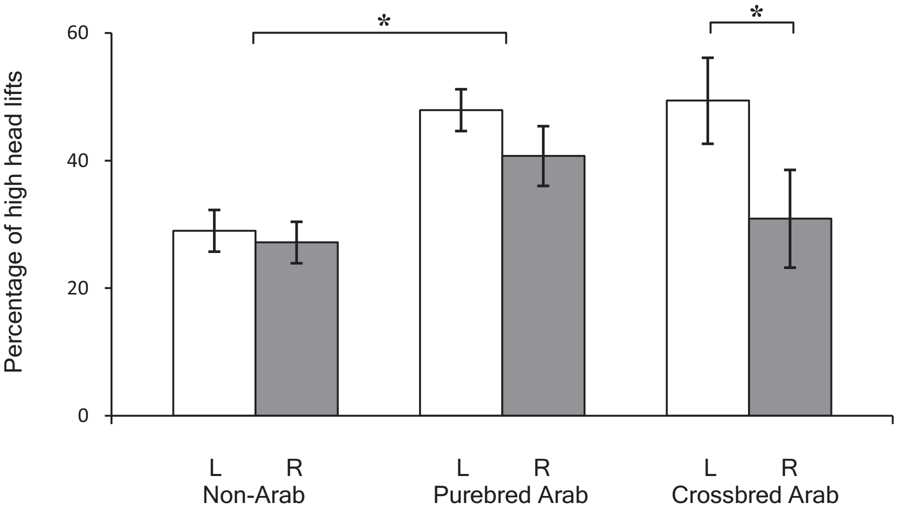


Figure 7.9 Comparison of the percentage of head lifts above the withers for looks to the left and looks to the right between non-Arab recreational ($N = 24$), purebred Arab ($N = 22$) and crossbred Arab riding horses ($N = 8$). Arab horses were significantly more likely to lift their heads higher than non-Arab horses. Only crossbred Arab horses showed a significant left-right difference in the percentage of head lifts above the withers. Means and standard errors are plotted. L Left, R Right ,* $p < 0.05$

7.3.1.4 Side bias in high alert

Analysis of laterality index of high alert versus number of scores per horse showed that there was no funnel effect ($r(52) = -0.22$, $p = 0.114$; Figure 7.10). No significant differences in the LI-scores of high alert were found between males ($N = 23$) and females ($N = 30$; Mann-Whitney U, $Z = -1.2$, $p = 0.246$), between immature ($N = 7$) and adult domestic horses ($N = 46$; Mann-Whitney U, $Z = -0.58$, $p = 0.562$) or between ridden ($N = 11$; 54% left) and non-ridden horses ($N = 44$; 53% left; Mann-Whitney U, $Z = -0.15$, $p = 0.878$).

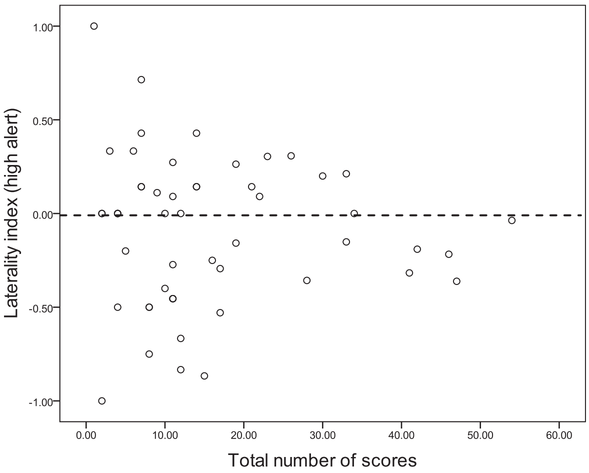


Figure 7.10 Scatterplot of the relationship between laterality index of reactivity (high alert) and the total number of scores (X-axis) obtained for each horse. LI-scores are plotted on the Y-axis. There was no significant funnel effect, i.e. there was no significant association between absolute LI-scores and the total number of scores. The dotted line indicates no bias.

Domestic horses showed a significant left-side bias (G-test, $G(52) = 132$, $p < 0.001$, $N = 54$, 56% left group bias; Figure 7.11). No significant difference was found between purebred Arab ($N = 22$) and non-Arab recreational riding horses ($N = 23$; $t(43) = -0.20$, $p = 0.839$, Cohen's $d = 0.06$). This means that the left-side bias in high alert was not dependent on breed and was, therefore, not related to any difference in emotionality of breed.

Immature horses had stronger LI-scores of high alert than did adult horses (Mann-Whitney U, $Z = -2.42$, $p = 0.015$; Figure 7.12). There were no significant differences in the strength of side bias in high alert between ridden (28% bias) and non-ridden horses (45% bias; Mann-Whitney U, $Z = -1.47$, $p = 0.141$) or between males and females (Mann-Whitney U, $Z = -0.65$, $p = 0.517$).

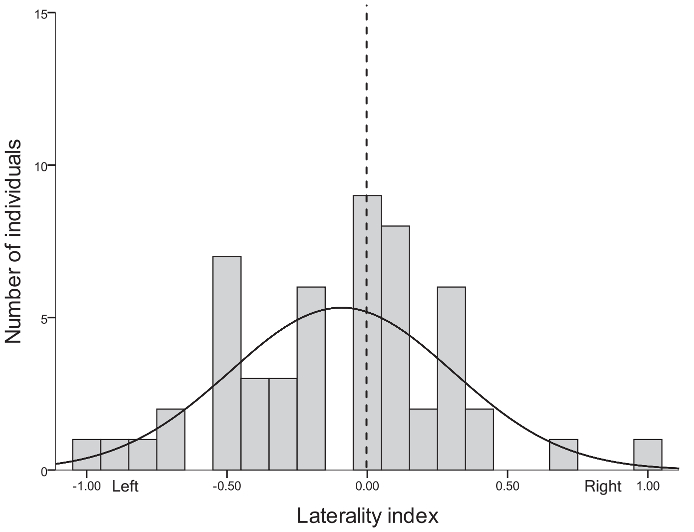


Figure 7.11 Population distribution (with normal curve plotted) of LI scores for high alert shown by domestic horses ($N = 54$). There was a significant leftwards bias of 56%. Laterality index scores are plotted on the X-axis and the number of individuals is plotted on the Y-axis. The dashed line indicates no bias (LI-score of zero), negative scores represent leftwards bias and positive scores represent rightwards bias.

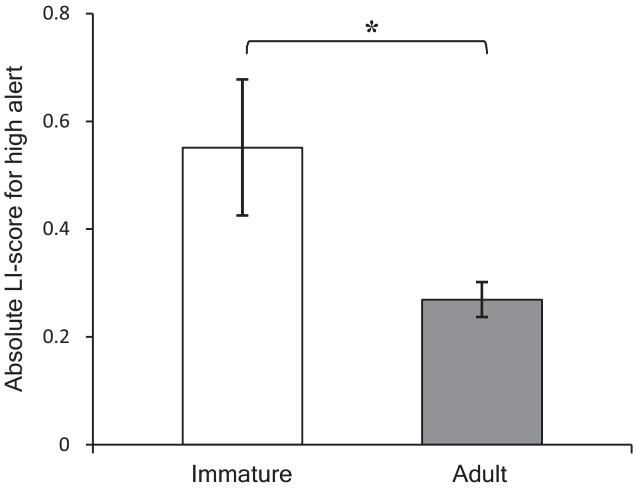


Figure 7.12 Comparison of the strength of side bias in high alert between immature and adult domestic horses. Absolute LI-scores (and standard errors) are plotted on the Y-axis. Immature horses ($N = 7$) showed stronger side biases than adult horses ($N = 46$). * $p < 0.05$.

7.3.1.5 Eye preference in agonistic interactions

There was no funnel effect in the scores of the agonistic looking bouts that involved threats only (Spearman's rho, $r_s(80) = -0.10$, $p = 0.377$) or in the scores of agonistic looking bouts that contained at least one attack (Pearson's $r(74) = -0.05$, $p = 0.671$; Figure 7.13).

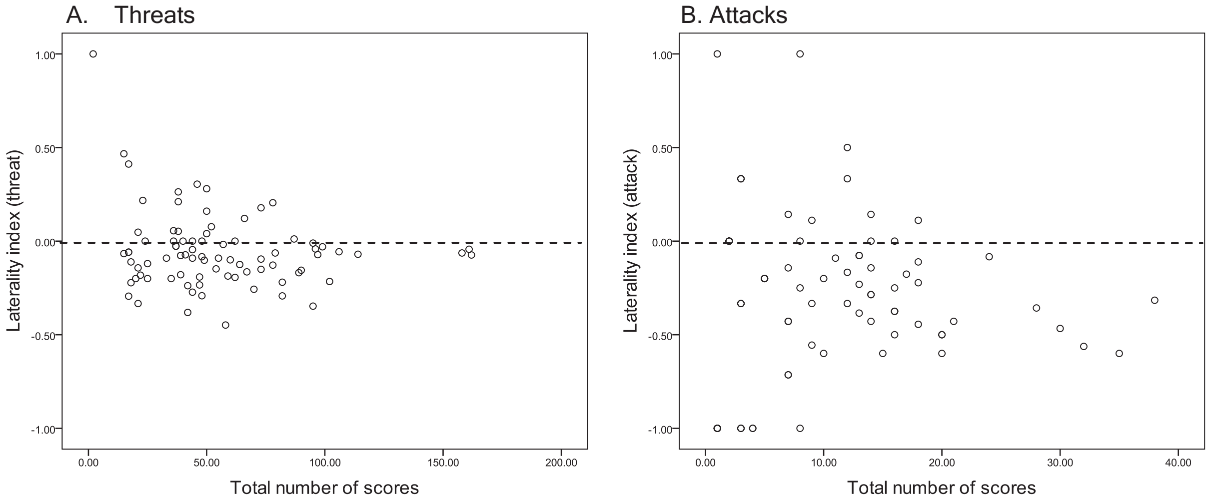


Figure 7.13 Scatterplots of the relationship between the number of scores (X-axis) obtained for each horse and laterality index in A, threats and B, attacks. LI-scores are plotted on the Y-axes. No significant associations between absolute LI-scores and the total number of scores were found. The dotted line indicates no bias. Note the difference in scale on the X-axis.

No significant difference was found between male ($N = 43$) and female domestic horses ($N = 39$) in agonistic looking bouts containing threats only ($t(80) = -0.31$, $p = 0.755$) or agonistic looking bouts containing at least one attack ($t(72) = -0.92$, $p = 0.360$). There were also no significant differences in side bias in agonistic looking bouts between ridden ($N = 71$) and non-ridden horses ($N = 11$; threats, Mann-Whitney U, $Z = -1.76$, $p = 0.079$, $N = 82$; attack, Mann-Whitney U, $Z = -0.47$, $p = 0.636$, $N = 76$) or between immature ($N = 7$) and adult domestic horses ($N = 75$; threat, Mann-Whitney U, $Z = -1.28$, $p = 0.201$, $N = 82$; attack, Mann-Whitney U, $Z = -0.61$, $p = 0.540$, $N = 76$).

Domestic horses showed a significant left-side bias in agonistic looking bouts that contained threats only (G-test, $G(80) = 412$, $p < 0.001$, $N = 82$; 53% leftwards group bias), as well as in agonistic looking bouts that contained at least one attack (G-test, $G(75) = 309$, $p < 0.001$, $N = 77$; 65% leftwards group bias; Figure 7.14). Note that there were substantially more scores in total for threat than attack. Excluding crossbred Arab horses, comparison of purebred Arab and non-Arab riding horses revealed no significant difference in side bias in agonistic looking bouts containing at least one attack (Mann-Whitney U, $Z = -0.03$, $p = 0.979$, $N = 70$) or agonistic looking bouts with threats only (Mann-Whitney U, $Z = -1.91$, $p = 0.056$, $N = 74$), although in the latter analysis there was a close-to-significant trend for non-Arab riding horses to show a stronger leftwards bias (lower LI-scores) than purebred Arab horses.

Social grouping was considered next. There was no significant difference between horses housed as one large herd (trail riding horses, N = 29) and horses housed as pairs or small groups (remaining horses, N = 53) in the strength of the left-eye bias in threat (Mann-Whitney U, Z = -0.30 p = 0.767, N = 82) or that of attack (Mann-Whitney U, Z = -0.65, p = 0.517, N = 76).

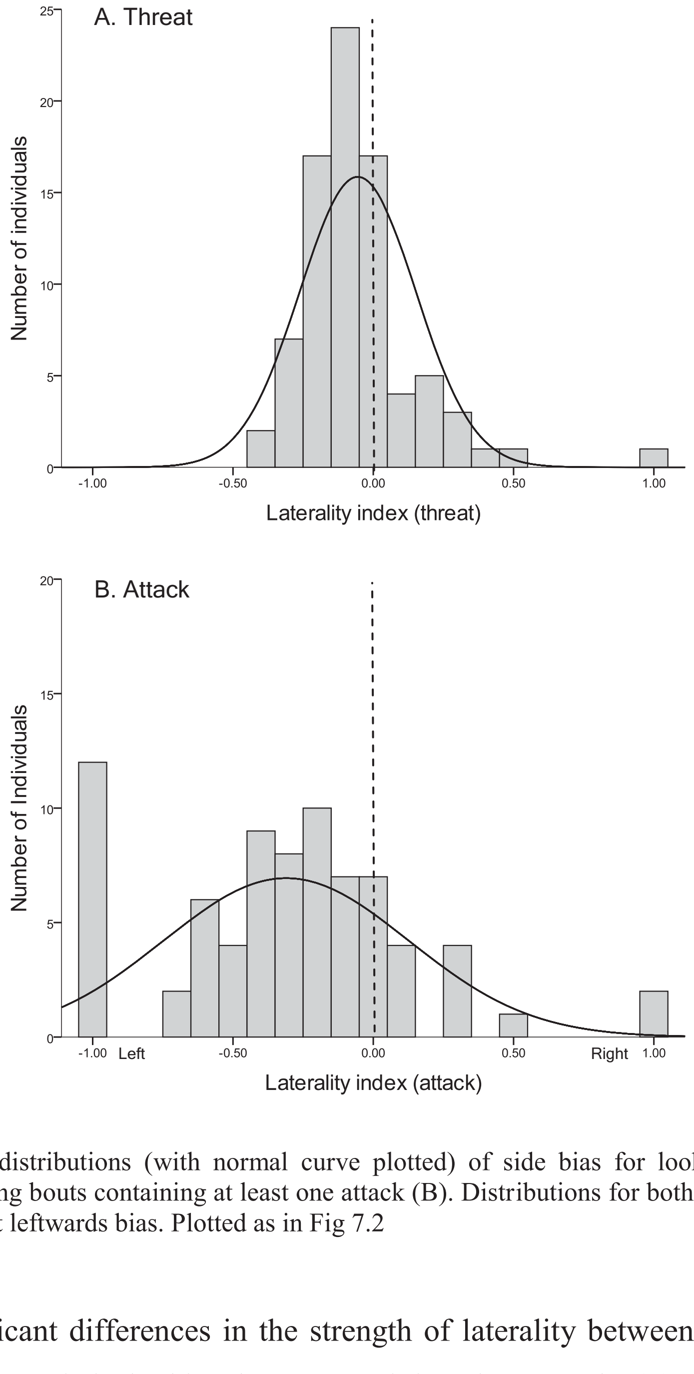


Figure 7.14 Population distributions (with normal curve plotted) of side bias for looking bouts consisting of threats only (A) and looking bouts containing at least one attack (B). Distributions for both threat and attack looking bouts showed a significant leftwards bias. Plotted as in Fig 7.2

There were no significant differences in the strength of laterality between males (N = 39) and females (N = 43) in agonistic looking bouts containing threats only ($t(80)$ = 0.18, p = 0.857, Cohen's d = 0.04) or in looking bouts containing at least one attack ($t(74)$ = -0.15, p = 0.885, Cohen's d = 0.03). No significant difference was found between immature (N = 7) and adult horses (N = 75) in side bias in looking bouts containing at least one attack (Mann-Whitney U, Z

= -0.46, p = 0.645, N = 76) but there was a trend for immature horses to show a weaker side bias in looking bouts that contained threats only (Mann-Whitney U, Z = -1.89, p = 0.058, N = 82).

There was, however, a significant difference between non-ridden (N = 11) and ridden horses (N = 71) in the strength of side bias for looking bouts containing threats only (Mann-Whitney U, Z = -3.14, p = 0.002, N = 82); non-ridden horses (6% bias) showed weaker side biases than ridden horses (17% bias; Figure 7.15). No significant difference was detected between non-ridden and ridden horses in agonistic looking bouts containing at least one attack (Mann-Whitney U, Z = -0.45, p = 0.651, N = 76).

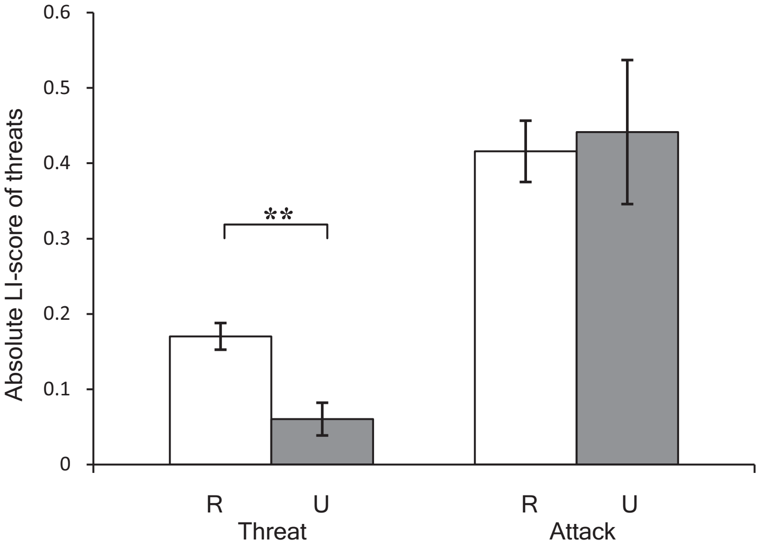


Figure 7.15 Comparison of the strength of side bias (Y-axis) in agonistic looking bouts containing threats and those containing at least one attack between non-ridden (U, N = 11) and ridden (R, N = 71) domestic horses. Horses that had never been ridden showed weaker absolute LI-scores than those that had. Means and standard errors are plotted. ** $p < 0.01$

Since it was thought that ridden horses might show a side bias of similar magnitude to the feral horses a comparison was made between the ridden (N = 73) and feral horses (N = 67). Feral horses showed a significantly stronger side bias in threat (21% bias) than ridden domestic horses (17%; Mann-Whitney U, Z = -2.12, p = 0.034).

7.3.2 Comparisons between groups of horses

7.3.2.1 Eye bias in agonistic responses

The results of laterality scores of agonistic interactions determined from the domestic horses were compared to those from feral and Przewalski horses using the interactions within harems bands. Data from stallion fights were not included because no such interactions had occurred during observations of the domestic horses (i.e. stallions were not housed together).

A significant effect of group (domestic, feral, and Przewalski) was detected in looking bouts containing threats only (Kruskal Wallis, $\chi^2(2, N = 161) = 10.1$, $p = 0.007$). *Post hoc* pairwise comparisons showed that domestic horses had significantly higher LI-scores (i.e. a weaker leftwards bias) than feral horses (Mann-Whitney *U*, $Z = -2.27$, $p = 0.023$, $N = 141$) and Przewalski horses (Mann-Whitney *U*, $Z = -2.91$, $p = 0.004$, $N = 102$). There was no significant difference between feral and Przewalski horses (Mann-Whitney *U*, $Z = -0.68$, $p = 0.495$, $N = 79$).

No significant effect of group on LI-scores of looking bouts containing threats only was found in males (Kruskal Wallis, $\chi^2(2, N = 70) = 4.20$, $p = 0.122$) but there was a significant effect of group in the females (Kruskal Wallis, $\chi^2(2, N = 91) = 6.84$, $p = 0.033$). *Post hoc* pairwise comparisons were then conducted on the females. Domestic horses showed significantly weaker leftwards biases than feral horses (Mann-Whitney *U*, $Z = -2.10$, $p = 0.036$, $N = 78$) and Przewalski horses (Mann-Whitney *U*, $Z = -2.23$, $p = 0.026$, $N = 56$) but there was no significant difference between feral and Przewalski horses (Mann-Whitney *U*, $Z = -0.21$, $p = 0.835$, $N = 48$; Figure 7.16).

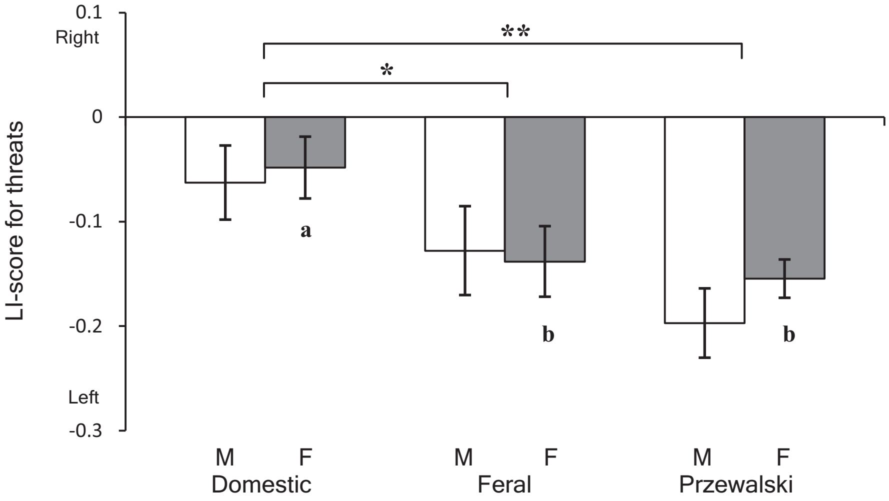


Figure 7.16 Mean side bias in threat for males and females of the three groups of horses. When the entire data set and only females were analysed, significant differences existed between domestic horses and both feral and Przewalski horses; domestic horses showed weaker left-side biases. * $p < 0.05$, **$p < 0.01$, 'a' and 'b' differ at $p < 0.05$. M, Males (white bars); F, Females (grey bars). Means and standard errors are plotted.

By contrast, no significant effect of group was detected in the side bias in looking bouts that contained at least one attack (Kruskal Wallis, $\chi^2(2, N = 158) = 3.90$, $p = 0.142$). Nor was there a significant effect of group when female horses were considered separately (Kruskal Wallis, $\chi^2(2, N = 90) = 0.27, p = 0.875$).

There was, however, a significant effect of group for males (Kruskal Wallis, $\chi^2(2, N = 68) = 7.92, p = 0.019$). Pairwise comparisons revealed that male domestic horses showed significantly less negative LI-scores of than Przewalski horses (Mann-Whitney *U*, $Z = -2.10, p = 0.036, N = 45$) but did not differ significantly from feral horses in LI-scores (Mann-Whitney *U*, $Z = -1.50, p = 0.134, N = 61$). Feral horses showed less negative LI-scores than Przewalski horses (Mann-Whitney *U*, $Z = -2.58, p = 0.010, N = 30$, Figure 7.17). Hence, an effect of group on side bias in looking bouts containing attack was revealed only in male horses; Przewalski horses showed a stronger left-side bias than both feral and domestic horses.

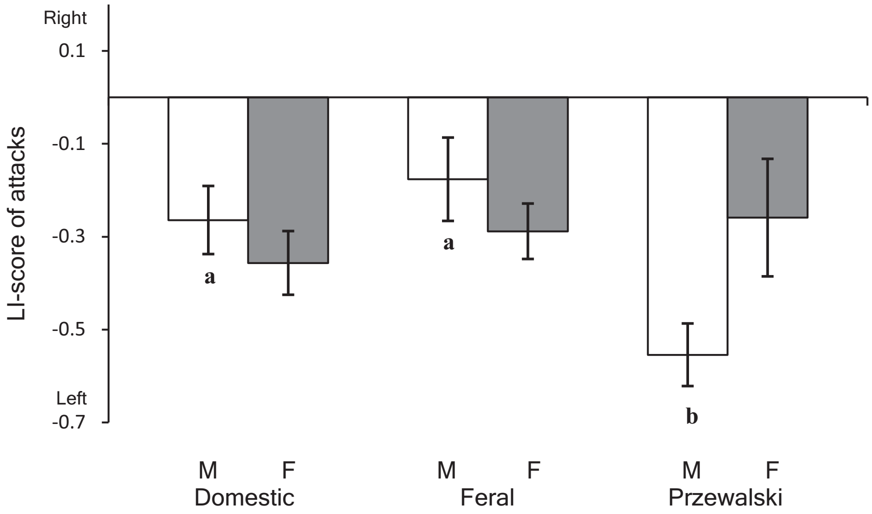


Figure 7.17 Mean side bias in attack scores across the three groups of horses. Male Przewalski horses showed stronger left-side biases than males from the other two groups. 'a' and 'b' differ at $p < 0.05$ M, Males (white bars) F, Females (grey bars). Means and standard errors are plotted.

7.3.2.2 *Side bias in vigilance*

No significant effect of group (domestic, feral and Przewalski) was detected in LI-scores of vigilance, either in all horses (Kruskal Wallis, $\chi^2(2, N = 154) = 2.32, p = 0.314$) or when the scores of males (Kruskal Wallis, $\chi^2(2, N = 76) = 1.05, p = 0.593$) and females (Kruskal Wallis, $\chi^2(2, N = 78) = 1.09, p = 0.580$) were analysed separately.

7.3.2.3 Percentage reactivity

A mixed factor ANOVA to test the presence of an effect of group on side bias in the height to which horses raised their head (i.e. percentage of lifts above the wither level) was unable to be conducted because sample sizes varied. Therefore, percentage difference scores between left and right looks were calculated as (Left% - Right%) / (Left% + Right%) and non-parametric tests conducted on these scores. There was a significant effect of group on percentage difference scores for the percentage of high head lifts for leftwards looks compared to rightwards looks (Kruskal Wallis, $\chi^2(2, N = 145) = 22.2, p < 0.001$). Pairwise comparisons found a significantly smaller difference in domestic horses compared to feral (Mann-Whitney U, $Z = -3.36$, $p = 0.001$, $N = 112$) and Przewalski horses (Mann-Whitney U, $Z = -4.20$, $p < 0.001$, $N = 86$), and feral horses had significantly smaller left-side difference scores than Przewalski horses (Mann-Whitney U, $Z = -2.24$, $p = 0.025$, $N = 92$). Hence, there was an increase in the strength of the left-side bias in the percentage of high head lifts from domestic to feral to Przewalski horses (Figure 7.18).

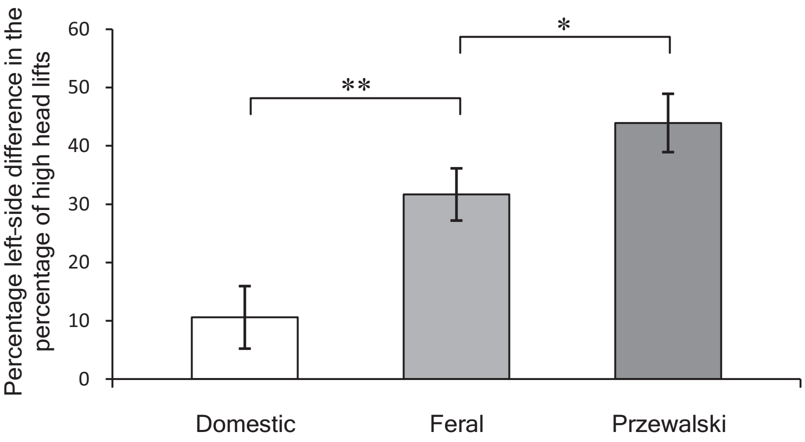


Figure 7.18 Comparison between groups in the percentage left-side difference scores for the percentage of head lifts that were above the wither level (Y-axis), calculated as (Left% - Right%)/(Left% + Right%). The left-side preference significantly increased from domestic to feral to Przewalski horses; Przewalski horses showed the strongest left-side laterality. * $p < 0.05$, ** $p < 0.01$

7.3.2.4 Side bias in high alert

A significant effect of group was detected (Kruskal Wallis, χ^2 $(2, N = 144) = 22.5, p < 0.001$) in left-side bias in high alert. *Post hoc* pairwise comparisons showed that domestic horses had significantly higher LI-scores (i.e. weaker leftwards bias) than feral horses (Mann-Whitney U, $Z = -2.88$, $p = 0.004$, $N = 111$) and Przewalski horses (Mann-Whitney U, $Z = -4.34$, $p < 0.001$, $N = 86$) and feral horses showed significantly weaker leftwards LI-scores than Przewalski horses

(Mann-Whitney *U*, $Z = -2.82$, $p = 0.005$, $N = 91$). Hence, there was an increase from domestic to feral to Przewalski horses in the strength of the leftwards side bias in high alert (Figure 7.19).

There were also significant differences in laterality of high alert between groups in males and females considered separately (males, Kruskal Wallis, χ^2 (2, $N = 72$) = 8.36, $p = 0.015$; females, Kruskal Wallis, χ^2 (2, $N = 72$) = 13.2, $p = 0.001$). Regarding males, domestic horses displayed lower LI-scores of high alert than Przewalski horses (Mann-Whitney *U*, $Z = -2.57$, $p = 0.010$, $N = 43$) but they did not differ significantly from feral horses (Mann-Whitney *U*, $Z = -1.14$, $p = 0.253$, $N = 52$). Przewalski horses displayed significantly lower LI-scores of high alert than feral horses (Mann-Whitney *U*, $Z = -2.27$, $p = 0.023$, $N = 49$). In comparison, female domestic horses displayed significantly lower LI-scores than feral horses (Mann-Whitney *U*, $Z = -2.74$, $p = 0.006$; $N = 59$) and Przewalski horses (Mann-Whitney *U*, $Z = -3.01$, $p = 0.003$, $N = 43$) but there was no significant difference between feral and Przewalski horses (Mann-Whitney *U*, $Z = -1.76$, $p = 0.079$, $N = 42$; Figure 7.19). Hence, male feral and domestic horses showed weaker left-side biases in high alert than male Przewalski horses, and female domestic horses showed weaker left-side biases than female Przewalski and feral horses. The latter two showed similar biases (Figure 7.19).

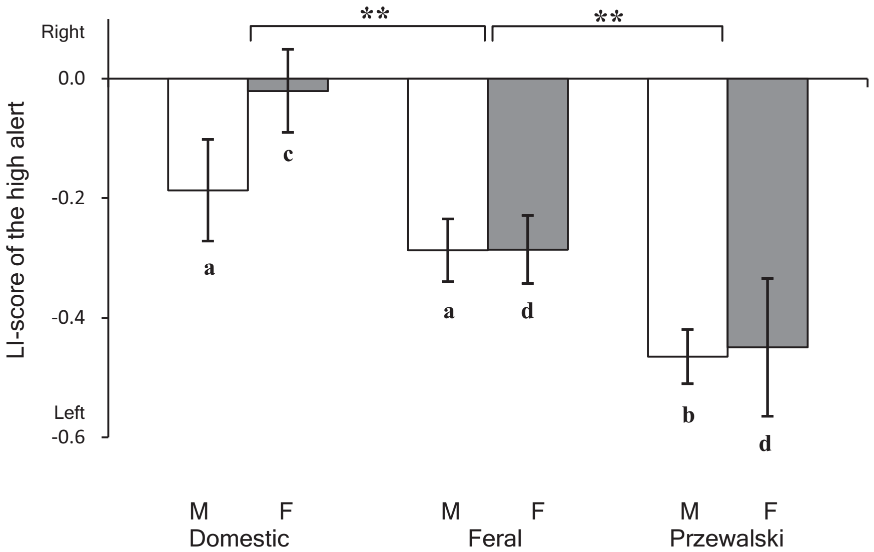


Figure 7.19 Mean side bias in high alert across the three groups of horses. Each group differed significantly from every other. The left-side bias increased from domestic to feral to Przewalski horses. ** $p < .01$. Female feral and Przewalski horses showed stronger left-side biases than female domestic horses. Male Przewalski horses showed stronger left-side biases than feral and domestic horses. Significant differences are present between ‘a’ and ‘b’ at $p < 0.01$ and ‘c’ and ‘d’ at $p < 0.05$. LI-scores of high alert are plotted on the Y-axis. M, Males (white bars); F, Females (grey bars). Means and standard errors are plotted.

7.3.2.5 Limb preference

No significant effect of group was detected in the LI-scores of limb preference, either for all horses (Kruskal Wallis, $\chi^2(2, N = 183) = 1.62$, $p = 0.445$) or when the scores of males (Kruskal Wallis, $\chi^2(2, N = 93) = 1.71$, $p = 0.426$) and females (Kruskal Wallis, $\chi^2(2, N = 90) = 0.49$, $p = 0.782$) were analysed separately.

7.4 Discussion

A summary of the results of laterality in the domestic, feral and Przewalski horses is presented in Table 7.3. Similar to the Przewalski and feral horses, the domestic horses displayed left-side biases in agonistic responses (threat and attack) and reactivity (high alert). However, the strengths of left-side biases in threat and high alert were weaker in the domestic horses than in the feral and Przewalski horses, which perhaps, indicate that experience under domestic conditions may influence lateralization.

Unlike the feral and Przewalski horses, the domestic horses did not show a side bias in vigilance but a difference between purebred Arab and non-Arab horses was revealed; non-Arab horses exhibited a left-side bias, whereas purebred Arab horses did not show a bias. A breed difference was also found in forelimb preference during grazing. Purebred Arab horses displayed a bias to place the right forelimb in front of the left while grazing, whereas the non-Arab horses showed no forelimb preference.

Table 7.3 Findings of side biases in feral horses, Przewalski horses and domestic horses.

Laterality index		Feral	Przewalski	Domestic		
				All	Pure Arab	Non-Arab
Threat	#	57% L	58% L	53% L	—	—
Attack	#	63% L	68% L	65% L	—	—
Vigilance		54% L	53% L	—	—	55% L
High alert	#	65% L	73% L	55% L	—	—
Limb use		—	—	—	61% R	—

Side biases of measures are given for purebred Arab and non-Arab horses when there was a significant difference between these two groups. L – significant left preference, R – significant right preference, $p < 0.05$, % leftwards population biases are given when significant, — not significant, # differences between groups varied with sex.

The findings reported here of a left-eye bias in high alert are consistent with those found in other studies of domestic horses using experimental and quasi-experimental techniques (Larose et al., 2006; Austin and Rogers, 2007). In the Austin and Rogers (2007) study, the flight distances of horses in response to a potentially threatening object presented on the left or right side were recorded. Similar side differences in reactivity when responding to threatening stimuli (a model predator) presented to the left and right side of the animal have been found in toads (Lippolis et al., 2002) and stripe-faced dunnarts (Lippolis et al., 2005). Larose et al. (2006) placed horses in an arena and scored eye preference to look at a novel object and emotionality of each horse so that these two variables could be correlated. In both studies on horses, not only were left-eye biases found but also use of the left eye was associated with greater reactivity.

7.4.1 Differences between the Arab breed and other horses

7.4.1.1 Limb preference

Breed difference was found in several measures, but by far the most unexpected result was that of limb preference in Arab horses, not only because the feral and Przewalski horses showed no biases, but because the bias in the purebred Arab horses was in a direction opposite to that found in previous research on other domesticated breeds of horses (McGreevy and Rogers, 2005; McGreevy and Thompson, 2005). The purebred Arab horses showed a significant right-limb population preference, whereas the non-Arab horses showed no population bias.

Previous studies of domestic horses have also found breed differences in forelimb preference while grazing. In particular, both thoroughbreds and standardbreds showed left-limb preferences and quarterhorses showed no preference (McGreevy and Rogers, 2005; McGreevy and Thompson, 2005). However, the authors of these studies were unable to disentangle breed influence from training effects because horses of the three breed types had been trained for different purposes. Although one study of racehorses did suggest limb preference occurred irrespective of breed (Williams and Norris, 2008), the limb preference measured, leading forelimb in galloping, may not be comparable to forelimb preference exhibited during grazing.

The right-limb bias (or left-supporting limb bias) in the purebred Arabs reported here (61% right) does not appear to be due to human influence. The two youngest foals (aged less than six months), which had been handled to a minimal extent, showed very strong individual right-limb preferences (79% and 88% right); indeed, these preferences were the strongest of all limb preferences measured in this project. Whether this breed difference in direction of limb preference was already present in the original subspecies from which Arabs horse may have evolved or whether it has resulted from selective breeding by humans cannot be ascertained. It has been shown that strains of mice can be selected only for strength of paw preference and not direction of paw preference (Collins, 1985) but there is some evidence to the contrary (Biddle and Eales, 1996). Different strains of mice, for instance, differ in the direction of paw preference (Waters and Denenberg, 1994). There also appears to be a hereditary component in the direction of handedness in chimpanzees (Hopkins et al., 1994, 2001) and humans (Annett, 1979; McManus, 1991).

Arab horses are known (from practical knowledge and previous research) as the most flighty of all breeds of horses (Houpt and Kusunose, 2000; Hausberger 2004) and findings for percentage reactivity reported here confirm this. The purebred Arab horses raised their head above the level of the withers more often than non-Arab horses. Possibly, limb preference is linked to temperament as discussed in Chapter 1, *Section 1.2.1* (p. 6), but thoroughbreds, which are also considered a flighty breed (Houpt and Kusunose, 2000), displayed a left-forelimb preference while grazing (McGreevy and Rogers, 2005). As noted by McGreevy and Rogers (2005), it is unclear which forelimb is important in determining which hemisphere may be dominant. The lame horse studied in this project showed an individual preference to place the left forelimb in front of the right when grazing and it was this left limb which had been injured, suggesting the supporting forelimb corresponds to the hemisphere that is in charge. The finding in the Arab horses of a left-supporting forelimb preference is consistent with the association between limb preference and reactivity shown by the feral horses (Chapter 5, *Section 5.4.2,* p. 86).

One explanation for the difference in the direction of group bias of limb preference between Arab and thoroughbred horses might lie in management practices. The group of Arab horses in this study were housed in groups/pairs, and thus had direct social contact, whereas thoroughbred racing horses are usually housed in separate stables for most of the time and are thus physically isolated from conspecifics. Not only does stabling restrict grazing opportunity, it can lead to abnormal behaviour problems such as stereotypies which are a sign of distress (Kiley-Worthington, 1987). Given that associations have been reported between paw preference and stress reactivity in rodents (Nevue, 1996; Waters et al., 1996) and laterality can be changed by neonatal exposure to stressful environments (Tang and Verstynen, 2002), it is possible management practices causing stress to horses might have some influence on limb preference.

Furthermore, the types of stereotypies engaged in by horses have been associated with the type of work for which the horse is used and may reflect the stressors specific to the type of work (Hausberger et al., 2009). Eventing and jumping horses display mainly licking and biting stereotypies and dressage horses exhibit mainly windsucking and head tossing. Some stereotypies may emerge from avoidance behaviour in a given stressful situation, either from physical restriction or psychological conflicts imposed on the horse (discussed by Hausberger et al., 2009). For example, head tossing is believed to be a response to strong bit restraint, which is prominent in dressage. Different types of stereotypies involve repetition of different motor actions and may themselves influence limb preferences. Those, such as weaving and box walking, which involve locomotor activity, may exaggerate an already present limb bias. Box walking is more prevalent in stabled Arabs than other breeds (review, Mills, 2005) and thoroughbreds are known to engage in weaving (Ninomiya et al., 2007). Perhaps, as is the case of stereotypies, different types of work and training may differentially influence lateralization of limb preferences, either directly through practice or indirectly through stress.

7.4.1.2 Other side biases

There was a left-side bias in high alert in all the horses. In spite of the breed difference in reactive temperament (above), there was no difference in the side bias in high alert. Even though Arab horses are more flighty, they are not necessarily more left-lateralized for reactive responses than non-Arab horses.

Conversely, Larose et al. (2006) found two breeds (trotters and French saddlebreds) with similar emotionality index differed in eye bias to view a novel object and the association between this measure and emotionality. It seems reactivity alone does not predict eye-preference to view a novel object. Larose et al. (2006) argued that breed differences in eye preference to view a novel object could be due to breed-specific training and, therefore, experience.

7.4.2 Effects of experience on laterality

7.4.2.1 Side bias in reactivity

A number of the side biases measured in the domestic horses appear to be affected by experience. Lateralization of percentage reactivity is notable because it seems that asymmetrical handling by humans may have an effect on this side bias. The domestic horses in the present study were riding horses, predominantly handled, saddled and mounted on their left side. Domestic riding horses that had been ridden were less likely than domestic riding horses that had never been ridden, irrespective of age, to raise their head above the level of the withers when looking to their left but not when looking to the right.

It is possible left-side handling may lead to habituation to stimuli seen on the left side, as shown by Larose et al. (2006). French saddlebreds, which are handled predominantly on the left side, used the right eye more often to look at the novel object and trotter horses, which are handled symmetrically, used the left eye more often. Larose et al. (2006) also found no presence of an eye bias in two-year-old French saddlebreds that had never been ridden, whereas ridden horses showed a right-eye bias. Ridden horses in the present study showed a left-side bias for percentage reactivity, whereas domestic horses that had never been ridden did not show a side bias at all. Although left-side handling in both studies appears to habituate horses to stimuli on their left side, there is a difference in the direction of side bias shown by ridden horses in each study.

On closer inspection of Figure 7.8 (p. 114) it can be seen that habituation may also occur on the right side. Although the difference in percentage reactivity on the right side between domestic horses that had been ridden and those that had not been ridden was not significant, there was a trend for non-ridden domestic horses to be more reactive. In spite of this, non-ridden domestic horses did not show a bias and, although it appears they may show a tendency in the same direction as the ridden horses, they showed greater variance and the size of the effect was low. Compared to the ridden horses, non-ridden domestic riding horses were more reactive, as measured by the percentage reactivity for looks to the left and to the right, in general.

The difference in the direction of side bias in reactivity between the results reported here and those found by Larose et al. (2006) illustrates experience of left-side handling is not entirely responsible for side bias in percent reactivity in ridden horses. Perhaps, the extent to which a side bias develops is dependent on the amount and type of handling or work. Alternatively, the context of testing may be important. Horses in the Larose et al. (2006) study were tested under controlled experimental conditions but in the present study data were collected under naturalistic conditions.

An effect of age was detected in the strength of the laterality index in high alert. Immature horses exhibited stronger side bias than adult horses, a result that is in agreement with those from the feral horses (Chapter 3). It is unclear whether the change in strength of laterality with age is caused by maturation and/or experience (discussed in Chapter 4, *Section 4.4*, p. 72). Comparisons between the three groups of horses showed domestic horses had a weaker left-side bias in high alert than feral and Przewalski horses. Since domestic horses differ from the feral and Przewalski horses in how they are managed (e.g. social restraints, restriction of movement, interactions with humans), it could be argued these experiences, rather than maturation, affect lateralization of reactivity. Moreover, it appears females may be more affected by domestic conditions than the males. The laterality index in high alert for the male domestic horses did not differ from that of the feral horses, suggesting lateralization of reactivity in males is unaffected by domestic conditions. Instead lateralization of reactivity in males may have been affected by the process of domestication, since the male Przewalski horses showed stronger side biases than both the feral and domestic horses.

7.4.2.2 Limb preference

Age effects on the strength, but not direction, of limb preference were found in the domestic horses in this study. The strength of preference weakened with age, which is opposite to that reported in a previous study on limb preference in domestic horses (McGreevy and Rogers, 2005). This weakening with age is in line with the results from the feral horses reported in this thesis (Chapter 4). It was proposed in Chapter 4 that the change in the strength in limb preference might be the result of locomotion over unstable and uneven substrate and steep terrain. Limb preference in the domestic horse may also be changed by experience but the types of experiences that may influence limb preference in domestic horses may differ from those important in feral horses.

A significant effect of being ridden was found in the domestic riding horses in the present study. Likewise, a possible effect of riding was found by Wells and Blache (2008) but all the non-ridden horses they tested were under three years of age and all the ridden horses were over three years of age. In the present study, horses that had never been ridden included immature as well as adult horses that were older than two years (4 out of 11). Moreover, the riding effect found by Wells and Blache (2008) was related to the direction not strength of limb bias, whereas in the present study non-ridden domestic riding horses showed stronger limb preferences than ridden horses. Wells and Blache (2008) concluded horses have no inherent limb preference but the present findings from the domestic horses and those reported in Chapter 4 for feral horses appear to refute their conclusion. However, testing of horses soon after birth is required to determine this.

Unexpectedly, the trail horses showed a weaker leftwards bias than the recreational riding horses even though they were ridden substantially more often; usually every day compared to weekends for the recreational horses. Wells and Blache (2008) attributed the rightwards limb bias found in ridden horses to the experience of being ridden because ridden horses trained for dressage and jumping showed stronger right-limb preferences compared to the non-ridden horses. If a rightwards limb bias is due to being ridden, the more rightwards biases in trail riding horses compared to recreational horses could be said to follow this pattern simply because the former are ridden more often. However, other research has shown a leftwards increase in forelimb preference with riding experience; standardbred horses that had not been ridden showed a weaker leftwards group bias than thoroughbred horses which were ridden (McGreevy and Thompson, 2005) and older thoroughbreds showed stronger and more leftwards limb preferences than younger horses (McGreevy and Rogers, 2005). It does not appear that the type of training is involved because, unlike the horses studied by Wells and Blache (2008), the trail horses were not trained for dressage and jumping. Instead, the recreational riding horses in the present study underwent dressage and jumping training similar to the horses in the study of Wells and Blache (2008) and they showed a more leftwards bias in comparison to the trail horses.

An alternative explanation for the results reported here may be that balance is accentuated in the trail horses and this may contribute to the weaker left-limb preference shown by trail riding horses in comparison to recreational riding horses. The trail riding horses were ridden along winding uneven bush trails and need to remain balanced beneath very inexperienced (usually novice) riders, whereas the recreational riding horses were predominantly ridden on open flat areas by riders with experience and balance. Results showing locomotion in a habitat with steep and rough terrain is not related to the strength or direction of limb preference are in partial disagreement with the above explanation but the horses that inhabited the extremely rough terrain (Centre for Eco-ethos Research and Education, France) were either crossbred or purebred Arab horses and, as shown above, Arab horses differed from other domestic horses, as well as feral and Przewalski horses, in both the direction and strength of limb preference. The trail riding and recreational riding subgroups of horses used here did not include Arab horses and locomotion over rough terrain may or may not affect these horses similarly. Nevertheless, maintenance of balance while being ridden by an inexperienced rider cannot be ruled out as a factor that may modify limb preference.

7.4.2.3 Eye preference in agonistic responses

Ridden domestic riding horses showed stronger laterality in threat, but not attack, responses than non-ridden domestic riding horses. It would be expected that laterality of agonistic responses would decrease with asymmetrical handling because horses would become less sensitive to stimuli on their left, as has been found for eye bias in emotional responses in domestic horses

(Larose et al., 2006), but this was not the case. This is a new finding in domestic horses and shows that some biases such as attack are resistant to entrainment by humans while others such as threat and reactivity are not. Since there was no effect of age on eye bias in threat, it is unlikely the stronger eye biases of threats shown by ridden horses in comparison to non-ridden domestic riding horses was maturational or the product of early experiences. One possible experience specific to ridden horses that might account for these findings is the process of 'breaking in' at 2-3 years of age. This is a stressful event to the horse and occurs at a time that may correspond to a sensitive period (as described in other species by Bateson, 1979; 1987); the time period in which young feral horses leave their natal band (Boyd and Kieper, 2005). Such stress during a period of increased brain plasticity might produce changes in brain lateralization, as has been shown by exposing neonatal rats to a stressful handling procedure (Denenberg, 1981).

Domestic horses showed a weaker left-eye bias in threat than did the feral and Przewalski horses, although it should be noted that this difference was more prominent in the females. Female domestic horses displayed a weaker left-side bias in threats during agonistic interactions than female feral and Przewalski horses, suggesting lateralization in female horses may be sensitive to social structure or management practices under domestic conditions. The absence of a significant effect for the same measure in males, although the tendency was in the same direction as females, may be due to the majority of male domestic horses in this study being gelded (castrated). Gonadectomy has been shown to affect motor laterality of gerbils (Clark, 1998), and this might have a similar effect in horses. This could be tested in future research by comparing lateralization in groups of stallions with the same in groups of geldings.

7.4.3 Conclusion

Left-side biases were found in domestic horses in agonistic behaviour, vigilance and reactivity. Hence, in domestic horses, like feral and Przewalski horses, the right hemisphere controls agonistic behaviour and responses to potential threats. However, these biases were weaker than those in feral and Przewalski horses. Since domestic horses differ from both these groups in social structure and management by humans, it is likely that experience in the domestic environment plays a role in the expression of lateralization. One experience that appears to influence lateralization of agonistic threat, percent reactivity, and limb preference in domestic riding horses is whether or not a horse has been ridden (which involves 'breaking in' to the saddle). Another explanation for the difference between feral and domestic horses in the strength of laterality is that they have undergone different selective pressures; feral horses undergo natural selection. This is unlikely, however, given that there was no difference between Group 1 and 2 feral horses in laterality. Finally, it appears that selective breeding, perhaps for flightiness, may have led to limb preference in the Arab domestic horses.

CHAPTER 8 GENERAL DISCUSSION

8.1 Introduction

Measurement of laterality in horses revealed significant left-eye biases, at the population level, in threat, attack, high alert and vigilance. These left-eye biases are consistent with the pattern of lateralization for reactivity to model predators, attending to novelty and agonistic behaviour documented in other vertebrate species (Chapter 1, *Section 1.2,* p. 2). Notably, all three groups of horses, feral, domestic and Przewalski, showed leftwards biases even though they had undergone different selection pressures and were living in different environmental conditions. This would suggest that right hemisphere control of these functions is resilient to domestication and entrainment by humans and is, therefore, a characteristic of the two species of horse (*Equus caballus* and *Equus ferus przewalski*), as it is of other vertebrate species.

The left-side bias in high alert was particularly strong and will be discussed further in *Section 8.3*. The left-eye bias in attack was also strong and robust to the effects of management and handling. Even though Przewalski horses have been shown to be more aggressive than feral horses (Feh, 1988), this appears to have had no effect on the neural organisation of brain lateralization since, when feral and Przewalski horses engage in an attack response, both show a left-eye bias. There may, however, be some evidence that genetic selection that took place during domestication has had some influence on the strength of the left-side bias of attack in males (discussed further in *Section 8.2.1*).

Although all three groups exhibit left-eye bias in agonistic behaviour and in their responses to potential threats from the environment, they do differ in the strength of the left-eye biases. Since Przewalski horses exhibited strong lateralization, it is likely that the ancestral horses of *Equus caballus* were also lateralized, and perhaps, evenly more strongly than their domestic descendents. It could be argued that breeding in captivity for a century may have produced some changes in lateralization of captive Przewalski from that of wild ancestors. However, such changes would have had to occur over a short period of time compared to domestic horses and for different reasons than in the domestic horses. The major selection criterion for breeding Przewalski horses was not tameness, as in the case of domestic horses, but genetic diversity, a priority for an endangered species (Geyer et al., 1989). Since 1991, however, the herd studied has been allowed to form natural social groups and allowed to breed naturally (Association pour le cheval de Przewalski: TAKH, 2008). Nevertheless, breeding in captivity may have influenced lateralized behaviour in a different way than domestication may have. Given that it may take generations of breeding for tameness until changes in behaviour manifest, as found in foxes (documented to have taken six generations; Trut 2000), it would be worth investigating laterality of Przewalski horses six generations after re-introduction to the wild to see whether behavioural lateralization changes over this period of time.

It could also be argued that feral horses, given their recent domestic origins, have retained the lateralization of their domestic ancestors that may have been entrained. If this were the case, it may be expected that lateralization would be stronger in the group of horses tested that had been feral for only two to five generations (Group 1) than in the group that had been feral for 10 to 20 generations (Group 2). However, since the strength of these left-eye biases did not differ between the two groups, it is rather unlikely that handling effects had been passed on to subsequent, unhandled generations of the feral horses.

8.2 Eye bias in agonistic responses

The left-eye bias in agonistic behaviour was evident in all three groups of horses, feral (threat 57%; attack 63%), Przewalski (threat 58%; attack 68%) and domestic horses (threat 53%; attack 65%). Comparisons made between the agonistic responses of domestic, feral and Przewalski horses were based solely on harem interactions because no stallion fights were recorded in domestic horses. Domestic stallions are rarely housed together. When agonistic events clearly involved high levels of aggression, such as during attacks within harems and attacks between domestic horses, a stronger left-eye bias was shown. This is consistent with research on other species showing right-hemisphere control of aggression (Chapter 1, *Section 1.2.2,* p. 8).

Generally, left-eye bias of threats was weaker than that of attack. In the feral horses the strength of laterality of threat decreased with age but this was not the case for eye-bias of attack, suggesting that the two patterns of behaviour may be lateralized independently (discussed in Chapter 4, *Section 4.4.1,* p. 73). In fact, there was no association found between laterality index of threat and laterality index of attack in the feral horses.

The left-eye bias found in agonistic responses also varied according to context. One problem that emerged in scoring of agonistic responses was the difficulty in determining whether the response was intended to harm the opponent or not. Agonistic responses were scored according to motor actions (Table 3.2, p. 49) without any attempt being made to imply intent. Therefore, responses categorised as attack included those that may have been a high level threat and/or actual attack response with contact. For example, the motor action of boxing (two stallions rearing and striking out at each other) was scored the same regardless of whether or not contact occurred because the outcome of the fight could not be predicted, i.e. one horse might move out of the way or halt its approach. Equally, pushing of another horse clearly involved contact but not always doing harm. As a result some responses scored as attack in stallion fights may or may not have involved a high level of aggression. On the other hand, agonistic responses categorised as threat only included responses typically considered to be low level threats, such as head threats, tail swishing, hindquarter movement and lifting of limb (Table 3.2, p. 49). When scoring behaviour, it was important to record objectively measurable responses so as to avoid making subjective judgements, and to consider the context in which they occur (i.e. stallion fights or interactions within harem bands).

The left-eye bias in threats (52 – *55*%) and attacks (61 – *63*%) during stallion fights was generally weaker than that shown during interactions within harems (threat 57 – *58*%; attack 63 – *68*%), particularly in Przewalski horses (percentages for the Przewalski horses are in italics). During fights, stallions engage in ritualised motor actions. One such response is the head bow (Feh, 2005) and this was present in all video-taped stallion fights. In addition, stallions engaged in mutual sniffing of faeces in most of these agonistic interactions, which is also seen in ritualised fighting displays in feral and Przewalski horses (Feh, 2005). The stallion fights scored in this project involved such ritualised behaviour. Since the left hemisphere controls routine motor actions (Chapter 1, *Section 1.2,* p. 2), it may be specifically involved in ritualised displays. Hence, in stallion fights there may be some involvement of the left hemisphere (discussed previously in Chapter 5, *Section 5.4.1,* p. 84).

In feral horses, during stallion fights, stallions belonging to a harem band and bachelor stallions differed in strength of left-eye preference of agonistic responses. This suggests that during the period of time spent in a bachelor band, stallions may not only learn to fight as stated by Khalil and Kaseda (1998), but their laterality may also be influenced. In feral horses, a weaker left-eye bias in stallions from harem bands compared to bachelor stallions may indicate that bachelor stallions have not yet refined ritualised displays and are using their right hemisphere more than harem stallions. This is consistent with left-hemisphere control of routine motor patterns, such as ritualised displays, and with greater experience of ritualised displays in stallions from harem bands (as was discussed in Chapter 4, *Section 4.4.1,* p. 73). As also suggested, gonadal hormones may play a role in eye-preference difference between harem and bachelor stallions (as was discussed in Chapter 4, *Section 4.4.1,* p.73). However, a difference between bachelor and harem stallions was not detected in Przewalski horses, as would be expected if laterality was affected by levels of testosterone.

8.2.1 Laterality in attack

Feral and domestic horses do not differ in the strength of left-eye bias of attack even though they differ in social structure, management and training, suggesting that lateralization of attack is resilient to experience. It is unlikely that selection in the natural habitat has influenced lateralization of attack because Group 1 and 2 feral horses, of differing generations in the feral habitat, do not differ in this respect (Chapter 4, *Section 4.4.3*, p. 75). Furthermore, the strength of eye-bias of attack in domestic horses did not differ according to whether or not horses had been 'broken in' to saddle and ridden (Chapter 7, *Section 7.4.2.3*, p. 130) and, in the feral horses, it did not change with age (Chapter 4, *Section 4.4.1*, p. 73). Since, there were very few immature Przewalski ($N = 5$) and domestic horses ($N = 6$), caution should be exercised when interpreting analysis of age effects in these two groups (Chapter 6, *Section 6.3.1.1,* p. 90 and Chapter 7, *Section 7.3.1.5,* p. 116). Although results of strength of laterality in attack from domestic horses were in line with those from the feral horses, the same was not true of the Przewalski horses.

Comparison of feral and domestic horses with Przewalski horses suggests that lateralization of attack may be affected by selective breeding. Male Przewalski horses exhibited a stronger left-eye bias in attack than feral and domestic horses. Given that Przewalski and feral horses live under the same natural social structure, this difference in eye preference cannot be attributed to social influence. Instead, it could reflect selection against aggressive individuals via the process of domestication. Przewalski horses are resistant to being tamed and are known to be more aggressive than feral Camargue horses (Chapter 2, *Section 2.3.2,* p. 21). It is likely that the ancestors of domestic horses were also more aggressive than modern domestic horses and that selection of less aggressive individuals during domestication may have led to decrease in the strength of lateralization in aggression. Since aggression is a function of the right hemisphere, the more aggressive individuals may be more strongly right-hemisphere lateralized for attack than are less aggressive individuals.

8.2.2 Laterality in threats

Eye-preference in threat, on the other hand, appears to be influenced by experience; domestic horses differed from Przewalski and feral horses in the strength of left-eye preference in threat.

Such experiences may include social structure, asymmetrical handling, training, early weaning and restriction from grazing and socialisation. Training appears to be one experiential factor that affects laterality of threat since ridden domestic riding horses showed stronger eye preferences than domestic horses that had never been ridden. Also, in the feral horses the strength of eye preference of threat decreased with age, which may indicate that social interactions also have an effect on laterality of threat. Under natural conditions horses form bachelor and harem bands, whereas domestic horses are restricted in or prevented from forming natural social groupings. Natural social structure may be important in maintaining lateralization of threat responses. Indeed, intraspecies social interactions are the basis of the model put forward by Ghirlanda et al. (2009) and summarised by Tommasi (2009) to explain maintenance of bias at the population level. The strength of the population bias is predicted by the proportion of competitive and cooperative social interactions within a group.

Perhaps social experience is essential for the development of lateralization of threats, as it is for development of a complete social behavioural repertoire (Bourjade et al., 2008). There may be mechanisms by which social experience might allow the expression and alignment of individual asymmetries so that animals coordinate their behaviour with each other. According to a review by Schaafsma et al. (2009) social experience does influence laterality. Groothuis et al. (2010) have recently presented evidence that social experience influences lateralization by aligning laterality of individuals within a group; they found greater variation between groups of chicks than within groups of chicks reared together. On the other hand, there may be management practices (e.g. early weaning in horses) under domestic conditions that inhibit or interfere with the development of laterality. Although Phillips et al. (2003) found laterality of motor behaviour was actually stronger in cows that had undergone stressful intensive management practices compared to cows that were minimally handled and allowed to form a natural herd, the direction of the bias was not affected. It would be interesting to test domestic horses with varying degrees of social experience to see whether they differ in lateralization of agonistic responses.

8.3 Side bias in vigilance and reactive responses

This study found that left head-turning biases of reactivity and vigilance are consistent with right-hemisphere control of responses to unexpected events and emergency responses (MacNeilage et al., 2009 and discussed in Chapter 1, *Section 1.2,* p. 2). They are also consistent with previous findings from domestic horses showing the same bias in attending to novelty and reactions to fear-inducing objects (discussed in detail in Chapter 2, *Section 2.2,* p. 16).

The strongest bias found in this research project was that of left-side bias in high alert, particularly so in Przewalski horses (73%). Notably there were no predators in the Przewalski reserve suggesting that experience with predators is not necessary for the development of this bias in horses. In contrast, it has been argued by Brown et al. (2007) that direct experience with predators is necessary for the development of eye bias in fish. For instance, if a fish has had no experience with a predator it may not perceive it as a threat and would therefore process it differently in the hemispheres than if it were perceived as a threat. Brown et al. (2004) found that fish taken from high predation areas show eye preference to view a novel object (left-eye preference) and a predator (right-eye preference), whereas those taken from low predation areas do not. Although there are no predators in the reserve in which the Przewalski horses were kept, there is threat from conspecific rivals, which may be sufficient for the development of side bias in high alert.

Considering lateralization of high alert, there was an increasing left-side bias from Przewalski to feral to domestic horses. It is unlikely that any difference between Przewalski and feral horses is due to social factors because both groups live under the same natural social structure. The stronger left-side bias in Przewalski compared to feral horses may be accounted for by selective breeding in feral horses prior to becoming feral (i.e. breeding against reactive individuals). It is also possible that side bias in high alert is influenced by context, whether management practices in the domestic environment or specifically social structure, given that these are the variables in which feral and domestic horses differ. Experience of a natural social structure may contribute to alignment of biases in high alert which might lead to cohesion of the group in escape behaviour.

There were no differences between feral, domestic (excluding purebred Arab horses) and Przewalski horses with regard to left-side bias in vigilance; all showed a weak left-side bias. In contrast, purebred Arab horses showed no side bias in vigilance. It is possible that selective breeding based on reactivity is a factor contributing to an absence of bias in the Arab horses given that the conditions under which they were housed did not differ in visibility of the surroundings or occurrence of potential threats from those of other domestic horses.

8.4 Limb preference

There was little evidence of limb preference in any of the horses, with the exception of left-supporting forelimb preference in purebred Arab horses. As discussed in Chapter 5, *Section 5.4.1* (p. 84), it is unlikely the limb preference in Arab horses was due to training because the two youngest untrained horses exhibited very strong limb preferences, although more young Arab horses would need to be tested to be sure of this. Therefore, it seems that the left-supporting forelimb preference in purebred Arab horses most likely reflects this breed's reactive temperament and is consistent with an association between reactivity and limb preference in the feral horses. Left-supporting limb preference was associated with increasing reactivity. Not only are these findings consistent with each other but they are consistent with findings obtained in race horses for stride pattern in galloping (Williams and Norris, 2007). A right-leading limb preference during racing corresponds to initiation of the gallop with the left hind-limb and may indicate right-hemisphere activation during racing (or flight response). This is most likely a task-specific limb preference, because racing is a flight response and may, therefore, be associated with greater activation of the right than the left hemisphere. Such activation could be associated with the greater use of the left hind limb in bearing weight and propelling the horse forward into each stride.

The absence of limb preference at the population level, in contrast to eye and side preferences in threat and attack responses, as well as reactivity, indicates that limb preference is not a reliable indicator of brain lateralization (discussed in Chapter 5, *Section 5.4.1,* p. 84). Limb preference may, instead, be a better indicator of temperament but, notably, the significant association between limb preference and reactivity was found only in immature horses. In addition, changes with age were found in the strength of limb preference in feral horses. Immature feral horses showed stronger limb preference than adult horses, perhaps indicating that experience of locomotion over unstable substrate may modify it (discussed in Chapter 5, *Section 5.4.1,* p. 84). The current findings on the domestic horses show that being ridden may also be a factor that modifies limb preference (Chapter 7, *Section 7.4.2.2,* p. 129). Considered together with previous studies on domestic horses (e.g., McGreevy and Rogers, 2005; McGreevy and Thompson, 2005; Wells and Blache, 2008), it appears that limb preference may be malleable and may be influenced by different types of experience in feral and domestic horses. Since horses that have been 'broken in' to saddle showed stronger limb preferences than horses that had never been ridden it is possible that stress in early life or at certain points in the horse's development (e.g. early weaning and breaking in) may also influence limb preference in domestic horses (discussed in Chapter 2, *Section 2.4.2,* p. 23 and in Chapter 7). Maturation may also be a factor in the weakening of limb preference with age, considering that immature horses have longer legs than adults and having longer legs has been associated with stronger limb preference (van Heel et al., 2006).

8.5 Lateralization in the natural habitat

Until recently there have been few reported examples of laterality in species observed in the wild. Most studies of lateralization have been conducted under laboratory conditions in order to control the effects of extraneous factors. It is important to conduct such naturalistic studies because evidence of side biases in the wild would suggest that it confers an advantage to survival.

Examples of lateralization in the wild include the report of more aggression directed by gelada baboons to conspecifics seen in their left visual field than in their right (Casperd and Dunbar, 1996) and the observation of eye preferences for vigilance in one of two species of wild sparrows (*Junco hyemalis*) studied by Franklin and Lima (2001). Also, wild black-winged stilts were found to be more likely to engage in courtship displays when a female was viewed on their left side and more likely strike, and with more success, at prey seen on their right side (Ventolini et al., 2005). Grace and Graig (2008) found a preference for the rightwards orientation of prey in the bill of adult wild Caspian terns (*Sterna caspia*) when feeding chicks. A study on Australian magpies found that juveniles birds showed a bias to beg for food on the right side of the parent birds, and birds that used their left eye more often than their right to view an approaching human gave more alarm calls than those that used their right eye more often (Hoffman et al., 2006). More recently, it has been found that Australian magpies exhibit a right-eye preference before approaching a model predator and a left-eye bias before withdrawal (Koboroff et al., 2008).

Moreover, some research has been conducted in an ecological context. Humans have been observed to express a right-ear bias to listen to another person in a noisy setting, and are more likely to grant a request when it is spoken into the right than left ear (Marzoli and Tommasi, 2009). The researchers attribute the latter result to the specialisation of the left and right hemispheres for approach and avoidance behaviour, respectively. This study could be added to the list of observational data showing evidence of lateralization in animals in their natural habitat. The current findings on laterality in feral and Przewalski horses provide further examples showing that animals express eye/ear lateralization in their natural habitat.

There is some evidence that limb preference is present in wild species but it appears to be task specific. Chimpanzees display a left handedness when termite fishing and right handedness when nut-cracking and wadge-dipping (review, Lonsdorf and Hopkins, 2005). Several species of parrots predominantly use their left foot to manipulate food (Rogers, 1980). Free-roaming reindeer herds circle leftwards when corralled or when forced into a group in the open which the authors conclude to be a motor response to stress (Espmark and Kinderås, 2002); reindeer do not display limb preferences to paw the ground in search of food.

The absence of limb preferences in feral horses suggests it may be functionally disadvantageous in the natural habitat (Chapter 5, *Section 5.4.1,* p. 84); any benefit that a herd of horses might gain by having a limb preference, such as turning in the same direction and staying together, could be outweighed by possible detrimental effects, such as reduction in agility during flight and a proneness to injury on one side. Instead, other lateralized behaviour, may ensure that members of the band/herd turn in the same direction when threatened. In the horse, one such side bias might be that of high alert, which was particularly strong.

8.6 Domestication

A greater percentage of Przewalski horses, in comparison to feral and domestic horses, showed individual laterality in the high alert response, vigilance and attack, suggesting selective breeding or the absence of natural selective pressures may have resulted in a reduction of lateralization in domestic horses (Table 8.1). These three measures are involved in the fight and flight responses fundamental to the survival of a social prey species. The ability to keep one eye/hemisphere alert and attuned to detecting predators, or to monitor conspecifics, while simultaneously using the other eye/hemisphere to carry out routine functions may have been a cognitive advantage present in the ancestors of the domestic horse. As discussed in Chapter 1, *Section 1.3.1* (p. 12), lateralized individuals are better than weakly or non-lateralized individuals in performing at two simultaneous tasks (e.g. Rogers et al., 2004; Dharmaretnam and Rogers, 2005; Dadda and Bisazza (2006). It appears that under domestication these forms of laterality may have weakened due to relative lack of need to perform more than one task simultaneously. It is also known that domestic animals have smaller brain size than their wild counterparts (Chapter 2, *Section 2.3.1,* p. 20).

The results suggest that individual laterality in high alert, percent reactivity and threat may have been strengthened in the feral populations, whereas that of vigilance and attack has remained largely unchanged. Interestingly, left-eye bias of high alert and threat are influenced by experience, whereas left-eye bias of attack is not. Feral horses showed stronger left-side biases in high alert, percent reactivity and threat than domestic horses. Given that feral and domestic horses have both undergone selective breeding over 6000 years of domestication, these findings suggest that it is management practices, not selective breeding, that may weaken lateralization in domestic horses or that factors present in the natural environment strengthen side bias in feral horses. Such a finding has relevance to management practices and training on the welfare of domestic horses and raises the question of what types of management practices might influence lateralization in high alert, percent reactivity and threat in domestic horses.

Table 8.1 Percentage of horses exhibiting significant individual lateralization.

Behaviour	Feral		Przewalski		Domestic	
	Number of horses	%	Number of horses	%	Number of horses	%
Threat	21	36	5	25	8	10
Attack	4	6	16	80	11	19
High alert	23	40	22	66	5	9
Vigilance	8	12	11	33	4	9

Significance at $p = 0.05$

It is possible that conventional left-side handling may habituate domestic riding horses to stimuli that they see on their left side and, therefore, weaken any left-side bias that may be present, particularly in percent reactivity and high alert. The absence of such asymmetrical habituation in feral horses could account for them displaying stronger left-side biases than the domestic horses.

One clear effect on lateralization shown in domestic riding horses was that horses that had been ‘broken in’ to the saddle, and hence ridden, differed in side bias of threat and percent reactivity compared to domestic riding horses that had never been ridden (i.e. not ‘broken in’). Horses that had been ‘broken in’ to the saddle showed stronger left-side than right-side percent reactivity compared to no left-right side difference in unbroken horses. Considering that ‘broken in’ and unbroken riding horses are both handled predominantly on the left side it is unlikely that the stronger left than right-side percent reactivity in ‘broken in’ horses is due to conventional left-side handling. ‘Broken in’ horses also showed stronger eye-bias in threat, irrespective of direction of bias, than unbroken riding horses. Together these results suggest that ‘breaking in’, a stressful event, may increase lateralization. The process of ‘breaking in’ a horse to being ridden essentially entails securing a saddle on the horse’s back by tightening a girth strap around its belly, putting a metal bit into its mouth to control movement, which may cause pain until the horse learns to respond, and being on its back which is unnatural to the horse. All of these acts restrict the horse and, depending on the method of training used may evoke fear and frustration in the horse (Waran et al, 2002). Indeed, bucking is a common response.

There was no difference, however, between 'broken in' (55% left) and unbroken horses (54% left) in the strength of left-side bias in high alert. In comparison, feral horses showed a left-side bias of 65% in high alert and are, therefore, considerably more strongly lateralized for high alert than domestic riding horses. Although 'broken in' horses show stronger bias in threat (17% bias) than unbroken horses (6%), they are nevertheless more weakly lateralized than feral horses (21%), although they appear to show laterality that is closer in strength to the feral horses.

Given that exposure to a stressful experience appears to increase lateralization in the domestic horses, as has been found in other species (cows, Phillips et al., 2003, rats, Denenberg, 2005), it is possible that feral horses show stronger lateralization than domestic horses in high alert, percent reactivity and threat at the individual and population levels because they may experience some form of stress in early life. Such stress might arise from encounters with predators or from interactions with conspecifics. On comparison of Figure 7.8 (p. 114) and Figure 4.8 (p. 66), it seems that 'broken in' domestic riding horses display a side bias in percent reactivity in line with Group 1 feral horses, whereas Group 2 feral horses appear to be the most strongly left-lateralized group. The stress of being 'broken in' in domestic riding horses and possible stress from early experience with, for example, predators in Group 1 horses may have similar effects on lateralization in both these groups.

Feral, Przewalski and domestic horses all show left-side bias in agonistic interactions and in reactivity, which suggests that right-hemisphere control over these functions is robust to the effects of domestication, although modification of strength of the left-side biases may occur. The left-side biases in high alert and attack were particularly strong, which raises the question of why humans handle horses on their left side considering that horses are more reactive and aggressive on this side. The tradition of handling horses on their left side might have arisen from human handedness (i.e. wearing of a sword on the left side) and/or footedness (i.e. ease of mounting on the horses left side) and may not have been the best practice. Future research could look at side bias in horses that have been trained on their right side and compare this with horses that are trained on their left side.

8.7 Implications for animal welfare

The findings reported here add to the knowledge of lateralization in domestic animals and could be used to improve livestock management and handling, as also discussed by Morgante and Vallortigara (2007). Left-side biases in reactivity and aggression are particularly relevant to the handling of domestic animals. Left-side bias has been shown in another species of domesticated livestock for attending to novelty (cows, Robins and Phillips, 2009), and da Costa et al. (2004) have deduced from their research on sheep that reactivity is under right-hemisphere control. Apart from the findings reported here, no studies have yet investigated eye bias in aggression of livestock. In order to avoid injury, handlers of livestock could approach animals to capture them and to perform routine procedures on the right instead of left side, at least initially, so that animals are less stressed and the likelihood of aggression is reduced. It may be easier to capture an animal when doing so on its right side, since this side may be less prone to flight responses. Moreover, studies with other species show that the right side/left hemisphere is associated with motivation to approach (e.g. dogs, Quaranta et al., 2007). Therefore, the animal may be more likely to approach and follow when a handler is on its right side than on its left side. This would be a much better method of handling them than chasing and/or cornering them, which more than likely produces stress.

Knowledge of eye use by horses, and other domestic farm animals, may give insight into whether an animal is fearful of an object or is intending to react with aggression. Use of the left eye to view a stimulus may indicate high reactivity and/or aggression. In both cases, injury to animal or human could be avoided by the handler being aware of an animal's eye use and taking precautions.

It is known of horses that reactivity interferes with learning ability (Houpt and Kusunose, 2000). Hence, if horses display a species-typical left-side bias of fear responses, it may be wiser to train them initially by introducing novel objects on their right side. This would enable left hemisphere inhibition of flight response, thereby reducing reactivity and stress.

Although little evidence for population biases of limb preference were found, limb preference was found to be associated with reactivity, and importantly, it was argued that the supporting limb during grazing indicates the hemisphere in charge of behaviour. Since left-supporting limb preference was associated with increased reactivity, the reactivity of an individual horse could be predicted by its limb preference. Given that the association between level of reactivity and limb preference was found in immature horses only, it would be best to determine limb preference while the animal is young before it may be changed by experience or training. To avoid injury, less reactive horses might be chosen for children based on a preference to use their right forelimb for support. As discussed by Rogers (2010), limb preference may reflect cognitive bias and coping style and might be employed by livestock handlers as a basis by which livestock are chosen for different types of management regimes. Animals with a left-supporting limb preference may have a reactive coping style and may, therefore not cope well with intensive farming involving confinement. Horses with a right-supporting limb preference (and therefore low reactivity) might be suited to pursuits such as trail riding and dressage, whereas horses with a left-supporting limb preference might be suited to racing and endurance.

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Appendix I. Number of left and right scores per horse recorded by observing the feral horses: head-turning bias and eye preference

				Head-turning bias										Eye bias in agonistic interactions in harem groups							
				Vigilance				Percentage reactivity		High alert				Threat				Attack			
	Horse	Sex	Age	L	R	LI		L	R	L	R	LI		L	R	LI		L	R	LI	
Group 1	**CU**^{h}	M	A	14	23	0.24		88	80	42	66	0.22	*	156	99	-0.22	*	13	12	-0.04	
	BB	F	A	5	3	-0.25		50	57	10	12	0.09		118	108	-0.04		21	8	-0.45	*
	BU	F	A	7	15	0.36		74	52	28	33	0.08		177	126	-0.17	*	22	23	0.02	
	ZC^{b}	M	I/A	34	19	-0.28	*	0	0	39	12	-0.53	*	51	37	-0.16		6	10	0.25	
	BBC	M	I	4	1	-0.60		40	25	6	3	-0.33		60	46	-0.13		35	24	-0.19	
	BC$_{1}$	M	I	6	7	0.08		37	40	7	12	0.26		112	68	-0.24	*	15	10	-0.20	
	SR	F	I											5	3	-0.25		1	1	0.00	
	CR	F	I											3	7	0.40		2	1	-0.33	
	BS^{h}	M	A	34	16	-0.36	*	70	69	37	20	-0.30	*	163	146	-0.06		10	8	-0.11	
	ZM	F	A	14	12	-0.08		60	50	24	15	-0.23		98	60	-0.24	*	20	6	-0.54	*
	BM	F	A	6	3	-0.33		67	40	6	4	-0.20		33	29	-0.06		1	2	0.33	
	TI	F	I/A	16	14	-0.07		0	0	20	21	0.02		79	65	-0.10		13	6	-0.37	
	PI	M	I	8	16	0.33		48	42	10	15	0.20		18	16	-0.06		1	6	0.71	
	DI^{h}	M	A	37	20	-0.30	*	57	68	32	26	-0.10		95	109	0.07		3	3	0.00	
	GM	F	A	26	24	-0.04		67	58	32	35	0.04		46	34	-0.15		4	1	-0.60	
	DG^{bh}	M	A	19	27	0.17		59	54	27	26	-0.02		50	76	0.21	*	5	4	-0.11	
	LI	F	I/A	24	11	-0.37	*	0	0	32	21	-0.21		10	8	-0.11		6	3	-0.33	
	OM	F	A	10	13	0.13		34	32	11	10	-0.05		54	43	-0.11		17	16	-0.03	
	C^{b}	M	A	13	10	-0.13		50	32	14	6	-0.40									
	SH^{b}	M	A																		
Group 2	**SS**^{h}	M	A	25	19	-0.14		63	38	17	13	-0.13		34	17	-0.33	*	5	2	-0.43	
	GM	F	A	20	16	-0.11		45	11	9	2	-0.64	*	44	32	-0.16		5	2	-0.43	
	CB	F	A	30	25	-0.09		75	67	34	35	0.01		22	19	-0.07		2	1	-0.33	
	DM	F	A	11	13	0.08		50	58	6	11	0.29		25	29	0.07		6	4	-0.20	

LB	F	A	20	12	-0.25		64	56	16	9	-0.28		33	22	-0.20		2	2	0.00	
BC$_2$^{b}	M	I	19	12	-0.23		40	9	7	1	-0.75	*	10	9	-0.05		3	2	-0.20	
NF	F	I/A	13	112	0.79	*	0	0	9	4	-0.38		103	81	-0.12		23	9	-0.44	*
BK	F	I	23	15	-0.21		52	26	16	5	-0.52	*	12	18	0.20		6	1	-0.71	
SC	M	I	16	28	0.27		33	20	8	7	-0.07		14	23	0.24		2	3	0.20	
CK	F	I	20	21	0.02		42	26	6	8	0.14		4	11	0.47		4	4	0.00	
LS^h	M	A	40	26	-0.21		62	20	37	10	-0.57	*	149	96	-0.22	*	17	11	-0.21	
GB	F	A	11	10	-0.05		58	36	11	5	-0.38		133	72	-0.30	*	10	5	-0.33	
BL	F	I	12	7	-0.26		42	0	5	0	-1.00	*	65	39	-0.25	*	2	1	-0.33	
BC$_3$	M	I	20	27	0.15		65	15	17	6	-0.48	*	68	30	-0.39	*	5	2	-0.43	
MO^h	M	A	32	24	-0.14		55	32	24	10	-0.41	*	75	74	-0.01		8	2	-0.60	
CH	F	A	40	33	-0.10		66	37	35	19	-0.30	*	125	67	-0.30	*	7	4	-0.27	
BY	F	A	19	30	0.22		50	37	18	14	-0.13		61	59	-0.02		3	2	-0.20	
YB	M	I	17	21	0.11		75	17	15	4	-0.58	*	57	38	-0.20		1	0	-1.00	
YC	M	I	23	5	-0.64	*	57	29	13	2	-0.73	*	72	44	-0.24	*	7	7	0.00	
OS	F	I	13	8	-0.24		36	0	4	0	-1.00		30	10	-0.50	*	1	2	0.33	
WT	M	I	14	19	0.15		71	37	10	7	-0.18		20	10	-0.33		0	0		
DG^h	M	A	78	71	-0.05		55	18	53	18	-0.49	*	119	124	0.02		8	15	0.30	
EB	F	A	45	41	-0.05		50	21	27	10	-0.46	*	132	78	-0.26	*	36	8	-0.64	*
FB	F	I	11	20	0.29		42	14	5	3	-0.25		51	23	-0.38	*	1	3	0.50	
CS^h	M	A	96	56	-0.26	*	63	41	66	24	-0.47	*	113	90	-0.11		2	4	0.33	
FF	F	A	44	32	-0.16		54	24	25	8	-0.52	*	58	54	-0.04		3	3	0.00	
ST^h	M	A	20	30	0.20		57	16	13	5	-0.44		42	26	-0.24		2	0	-1.00	
SB	F	A	29	23	-0.12		45	11	15	3	-0.67	*	42	19	-0.38	*	2	1	-0.33	
SR	F	A	12	15	0.11		57	6	8	1	-0.78	*	57	26	-0.37	*	3	2	-0.20	
EX	F	I	24	28	0.08		52	11	12	3	-0.60	*	28	16	-0.27		3	1	-0.50	
EO	F	I	13	11	-0.08		31	9	4	1	-0.60		26	9	-0.49	*	8	2	-0.60	
BI^h	M	A	24	27	0.06		48	42	13	13	0.00		23	25	0.04		3	2	-0.20	
WH	F	A	10	10	0.00		80	50	8	6	-0.14		45	22	-0.34	*	3	1	-0.50	

	MG	F	A	4	9	0.38		75	22	3	2	-0.20		21	27	0.13		4	1	-0.60
	FI	F	I	1	2	0.33		0	50	0	1	1.00		24	17	-0.17		2	2	0.00
	CZ	F	A	6	6	0.00		20	0	1	0	-1.00		24	20	-0.09		1	0	-1.00
	BZ	M	I	6	5	-0.09		83	40	5	2	-0.43		15	4	-0.58	*	1	1	0.00
	BF^{h}	M	A						missing an eye											
	OG	F	A	1	0	-1.00		0	0	0	0			8	10	0.11		3	0	-1.00
	GD	F	A	7	8	0.07		0	0	0	0			12	8	-0.20		5	1	-0.67
	BA	M	I	4	1	-0.60		0	0	0	0			1	2	0.33		2	0	-1.00
	RO	M	I	5	2	-0.43		40	0	2	0	-1.00		2	2	0.00		0	0	
	SO^{h}	M	A											9	14	0.22		4	3	-0.14
	RB	F	A											8	3	-0.45		2	0	-1.00
	NB	F	A											6	5	-0.09		1	1	0.00
	DB	F	A											10	10	0.00		1	1	0.00
	PG^{bh}	M	A	26	25	-0.02		48	57	15	16	0.03		22	9	-0.42	*	0	0	
	MC	F	A	31	23	-0.15		61	35	22	8	-0.47	*	38	51	0.15		2	6	0.50
	CC	M	I	8	5	-0.23		50	0	4	0	-1.00		5	3	-0.25		0	0	
	TI^{b}	M	A	26	29	0.05		73	35	24	11	-0.37	*							
	TS^{b}	M	A	38	35	-0.04		80	35	37	15	-0.42	*							
	CO^{b}	M	A	41	19	-0.37	*	67	29	31	7	-0.63	*							
	GR^{b}	M	A	4	3	-0.14		60	60	3	3	0.00								
	HS^{b}	M	A	18	24	0.14		84	50	21	14	-0.20								

L – left, R – right, LI – laterality index calculated as R-L/R+L, * significant individual preference $p < 0.05$, A – more than 2-years of age, I – less than 2-years of age, M – male, F – female. bold – stallion, b - bachelor stallion, h – harem stallion, boxed sections in the column second from the left indicate each band, the first band in Group two was at Halls Peak, 1, 2 and 3 refer to different horses that were given the same initials in the field but were observed at different sites.

Appendix II. Number of left and right scores per horse recorded by observing the Przewalski horses.

			Limb preference				Head-turning bias										Eye preference in agonistic interactions in harem bands							
							Vigilance				Percentage reactivity		High alert				Threat				Attack			
Horse	Sex	Age	L	R	LI		L	R	LI		L	R	L	R	LI		L	R	LI		L	R	LI	
AT^{h}	M	A	54	52	-0.02		56	53	-0.03		41	23	28	20	-0.17		125	74	-0.26	*	23	6	-0.59	*
SB	F	A	60	44	-0.15	*	25	39	0.22		12	3	4	2	-0.33		66	48	-0.16		32	15	-0.36	*
NU	M	A	52	50	-0.02		38	35	-0.04		27	4	12	2	-0.71	*	89	50	-0.28	*	16	6	-0.45	*
SZ^{h}	M	A	47	56	0.09		45	39	-0.07		20	7	10	4	-0.43		106	59	-0.28	*	12	4	-0.50	*
EG	F	A	55	52	-0.03		54	66	0.10		80	49	67	43	-0.22	*	153	126	-0.10		47	23	-0.34	*
CV	F	A	47	58	0.10		6	6	0.00		27	0	3	0	-1.00		20	11	-0.29		4	5	0.11	
BN^{h}	M	A	62	48	-0.13	*	100	48	-0.35	*	73	33	112	20	-0.70	*	105	94	-0.06		29	15	-0.32	*
ST	F	A	55	50	-0.05		27	27	0.00		26	7	12	3	-0.60	*	66	43	-0.21	*	19	5	-0.58	*
SV	F	A	49	62	0.12		45	44	-0.01		42	9	25	5	-0.67	*	109	86	-0.12		30	15	-0.33	*
SJ	M	I	56	46	-0.10		27	13	-0.35	*	71	32	24	7	-0.55	*	17	12	-0.17		6	2	-0.50	
CS^{h}	M	A	55	66	0.09		75	42	-0.28	*	24	13	25	10	-0.43	*	88	70	-0.11		40	9	-0.63	*
AL	F	A	50	61	0.10		45	10	-0.64	*	19	0	10	0	-1.00	*	74	63	-0.08		33	6	-0.69	*
SK	F	A	54	52	-0.02		27	36	0.14		50	24	19	11	-0.27		95	59	-0.23	*	44	16	-0.47	*
AA	F	A	48	52	0.04		12	8	-0.20		6	14	1	1	0.00		65	49	-0.14		30	12	-0.43	*
AM	F	I	50	57	0.07		1	9	0.80	*	20	27	1	3	0.50		17	12	-0.17		0	3	1.00	
AQ	M	I	57	54	-0.03		29	38	0.13		49	13	19	6	-0.52	*	45	29	-0.22		17	1	-0.89	*
AS	F	I	50	58	0.07		23	13	-0.28		32	26	10	6	-0.25		52	36	-0.18		12	4	-0.50	*
SE	F	A	53	50	-0.03		37	18	-0.35	*	48	17	22	4	-0.69	*	68	49	-0.16		2	3	0.20	
SF	F	I	45	58	0.13		36	14	-0.44	*	62	24	38	9	-0.62	*	66	52	-0.12		22	6	-0.57	*
SU	F	A	53	53	0.00		30	26	-0.07		39	7	17	3	-0.70	*	34	31	-0.05		21	9	-0.40	*
BH^{b}	M	A	49	51	0.02		57	42	-0.15		49	29	35	14	-0.43	*								
CT^{b}	M	A	54	48	-0.06		46	50	0.04		36	10	21	6	-0.56	*								
SX^{b}	M	A	42	61	0.18	*	72	45	-0.23	*	31	10	28	6	-0.65	*								

EN^b	M	A	50	54	0.04		68	49	-0.16		80	51	65	39	-0.25	*
AO^b	M	A	62	58	-0.03		64	34	-0.31	*	30	15	26	9	-0.49	*
AV^b	M	A	42	62	0.19		44	58	0.14		72	39	36	30	-0.09	
PR^b	M	A	56	49	-0.07		45	83	0.30	*	32	5	19	5	-0.58	*
AN^b	M	A	41	66	0.23	*	89	71	-0.11		39	11	43	11	-0.59	*
AD^b	M	A					54	41	-0.14		33	9	21	5	-0.62	*
ES^b	M	A	60	45	-0.14	*	46	71	0.21	*	72	51	51	41	-0.11	
EC^b	M	A	59	47	-0.11		53	49	-0.04		73	35	45	22	-0.34	*
EO^b	M	A					23	29	0.12		34	15	11	6	-0.29	
SA^b	M	A	57	48	-0.09		45	60	0.14		16	1	9	1	-0.80	*

L – left, R – right, LI – laterality index calculated as R-L/R+L, * significant individual preference $p < 0.05$, bold – stallion, b - bachelor stallion, h – harem stallion, boxed sections in the column on the left indicate each band, the last four bands were stable bachelor bands, M – more than 2-years of age, I – less than 2-years of age, M – male, F – female.

Appendix III. Number of left and right scores per horse recorded by observing the domestic riding horses.

									Head-turning bias									Eye preference in agonistic interactions in harem bands							
					Limb preference				Vigilance			Percent reactivity		High alert				Threat				Attack			
			age	sex	L	R	LI		L	R	LI	L	R	L	R	LI		L	R	LI		L	R	LI	
Recreational Riding Horses	BE	R	A	F	55	51	-0.04		20	14	-0.18	27	35	6	8	0.14		28	19	-0.19					
	PO	R	A	M^{G}	65	37	-0.27	*	20	17	-0.08	44	35	11	6	-0.29		48	47	-0.01		5	2	-0.43	
	TI	R	A	F	66	37	-0.28	*	21	20	-0.02	48	35	11	8	-0.16		36	28	-0.13		1	2	0.33	
	TO	R	A	F	57	48	-0.09		21	16	-0.14	21	41	4	7	0.27		12	12	0.00		1	1	0.00	
	VE	R	A	F	57	53	-0.04		17	12	-0.17	44	40	7	4	-0.27		12	8	-0.20					
	DO	R	A	M^{G}	60	48	-0.11		9	8	-0.06	20	25	2	2	0.00		10	8	-0.11		0	1	1.00	
	BA	R	A	M^{G}	57	53	-0.04		3	0	-1.00	20	50	1	1	0.00		26	16	-0.24		10	7	-0.18	
	SD	U	I	M	47	71	0.20	*	4	4	0.00	40	0	2	0	-1.00		19	18	-0.03		5	2	-0.43	
	CH	R	A	F	50	40	-0.11		24	22	-0.04	29	55	7	12	0.26		44	26	-0.26	*	13	11	-0.08	
	MA	R	A	M^{G}	44	60	0.15		9	3	-0.50	54	14	7	1	-0.75	*	26	22	-0.08		3	9	0.50	
	ST	R	A	F	42	41	-0.01		17	17	0.00	38	42	6	8	0.14		24	26	0.04		9	5	-0.29	
	NI	R	A	M^{G}	75	26	-0.49	*	17	13	-0.13	6	30	1	6	0.71		87	75	-0.07		12	3	-0.60	*
	M	R	A	F	46	63	0.16		7	4	-0.27	25	33	3	1	-0.50		30	43	0.18		6	3	-0.33	
	TM	R	A	M^{G}	33	59	0.28	*	14	20	0.18	20	19	3	4	0.14		29	18	-0.23		16	4	-0.60	*
	PA	R	A	F	52	33	-0.22	*	2	5	0.43							18	20	0.05		8	2	-0.60	
	PY	R	A	M^{G}	64	62	-0.02		21	11	-0.31	50	7	11	1	-0.83	*	37	25	-0.19		6	1	-0.71	
	AS	R	A	M^{G}	63	34	-0.30	*	16	14	-0.07	32	13	6	2	-0.50		52	38	-0.16		28	7	-0.60	*
	RU	R	A	M^{G}	58	45	-0.13		6	6	0.00	29	50	2	4	0.33		61	53	-0.07		4	0	-1.00	
	GK	R	A	M^{S}	51	55	0.04		8	7	-0.07	25	25	2	2	0.00		35	24	-0.19		6	5	-0.09	
	AP	R	A	M^{G}	52	59	0.06		32	29	-0.05	30	6	10	2	-0.67	*	21	29	0.16		0	8	1.00	*
	BE	R	A	F	56	51	-0.05		57	42	-0.15	56	35	32	15	-0.36	*	40	33	-0.10		3	4	0.14	
	FL	R	A	F	57	53	-0.04		16	12	-0.14	6	17	1	2	0.33		11	7	-0.22		0	0		
	PL^{N}	R	A	F					3	8	0.45	33	13	1	1	0.00		14	7	-0.33		1	0	-1.00	
	YC	U	I	F	73	30	-0.42	*	3	3	0.00	0	33	0	1	1.00		15	23	0.21		1	2	0.33	

Trail Riding Horses	BA	R	A	M^{G}	48	49	0.01		43	44	0.01		10	8	-0.11	
	CM	R	A	F	47	57	0.10		50	46	-0.04		19	9	-0.36	
	GM	R	A	F	44	53	0.09		62	40	-0.22	*	6	1	-0.71	
	WT	R	A	M^{G}	49	41	-0.09		29	13	-0.38	*	2	1	-0.33	
	GG	R	A	M^{G}	40	49	0.10		53	29	-0.29	*	8	4	-0.33	
	DL	R	A	F	35	86	0.42	*	5	12	0.41					
	CL	R	A	M^{G}	48	59	0.10		42	31	-0.15		11	5	-0.38	
	CS	R	A	M^{G}	41	47	0.07		22	19	-0.07		4	3	-0.14	
	BS	R	A	M^{G}	48	56	0.08		30	25	-0.09		8	5	-0.23	
	TF	R	A	F	63	39	-0.24	*	56	50	-0.06		11	5	-0.38	
	DC	R	A	M^{G}	46	56	0.10		17	19	0.06		7	6	-0.08	
	LG	R	A	F	60	55	-0.04		84	74	-0.06		6	8	0.14	
	SK	R	A	M^{G}	59	39	-0.20	*	28	16	-0.27		5	3	-0.25	
	SO	R	A	M^{G}	59	52	-0.06		29	37	0.12		4	8	0.33	
	SP	R	A	M^{G}	55	73	0.14		9	8	-0.06		1	1	0.00	
	MB	R	A	F	49	58	0.08		9	14	0.22		1	0	-1.00	
	PB	R	A	F	47	45	-0.02		20	20	0.00		8	0	-1.00	*
	PI	R	A	M^{G}	74	52	-0.17	*	14	24	0.26		25	7	-0.56	*
	RB	R	A	M^{G}	53	60	0.06		39	28	-0.16		9	5	-0.29	
	CZ	R	A	F	63	46	-0.16		9	8	-0.06		4	0	-1.00	
	GD	R	A	M^{G}	45	42	-0.03		14	11	-0.12		3	0	-1.00	
	RO	R	A	F	54	50	-0.04		51	48	-0.03		3	2	-0.20	
	FG	R	A	F	42	66	0.22	*	21	18	-0.08		3	0	-1.00	
	TS	R	A	M^{G}	34	54	0.23	*	44	34	-0.13		1	1	0.00	
	LW	R	A	M	53	38	-0.16		31	23	-0.15		8	6	-0.14	
	DA	R	A	F	48	56	0.08		15	10	-0.20		1	0	-1.00	
	PT	R	A	M^{G}	40	63	0.22	*	27	22	-0.10		11	7	-0.22	
	SG	R	A	M^{G}	54	52	-0.02		23	16	-0.18		2	1	-0.33	
	PG	R	A	M^{G}	50	49	-0.01		21	14	-0.20		10	6	-0.25	

Cross bred Arab Horses	TK	R	A	F	66	58	-0.06		10	6	-0.25	45	50	5	5	0.00		64	31	-0.35	*	7	7	0.00	
	BZ	R	A	F	41	59	0.18		27	40	0.19	63	41	17	17	0.00		4	11	0.47					
	CR	R	A	F					19	22	0.07	30	9	6	2	-0.50		50	32	-0.22	*	7	6	-0.08	
	GR	R	A	F	52	50	-0.02		15	25	0.25	42	11	8	3	-0.45		18	15	-0.09		10	4	-0.43	
	SH	R	A	F	20	81	0.60	*	16	10	-0.23	41	27	7	3	-0.40		19	18	-0.03		13	5	-0.44	
	OX	R	A	M^S	40	64	0.23	*	13	26	0.33	86	69	12	18	0.20		11	6	-0.29		2	1	-0.33	
	SI	R	A	M^G	46	59	0.12		13	16	0.10	29	29	4	5	0.11		0	2	1.00					
	SE	R	A	F	73	34	-0.36	*	21	40	0.31	59	10	13	4	-0.53	*	31	17	-0.29	*	1	0	-1.00	
Purebred Arab Horses	*WH*	R	A	F	49	52	0.03		22	12	-0.29	42	35	10	6	-0.25		52	37	-0.17		25	13	-0.32	
	RY^{LCS}	U	I	F	66	35	-0.307	*	19	15	-0.12	74	6	14	1	-0.87	*	13	9	-0.18		3	2	-0.20	
	RO	R	A	F	58	54	-0.04		13	14	0.04	43	35	6	6	0.00		24	28	0.08		7	2	-0.56	
	OB	R	A	M^S	39	66	0.26	*	5	30	0.71	60	7	3	2	-0.20		31	47	0.21		7	5	-0.17	
	ML	R	A	M^G	36	72	0.33	*	42	35	-0.09	41	30	19	14	-0.15		18	18	0.00		3	2	-0.20	
	ME	R	A	M^G	51	47	-0.04		17	19	0.06	48	57	10	12	0.09		12	9	-0.14		15	5	-0.50	*
	YB	U	I	M	40	59	0.19		21	22	0.02	35	14	8	3	-0.45		42	37	-0.06		12	4	-0.50	*
	FR	R	A	M^G	24	83	0.55	*	33	44	0.14	24	7	8	3	-0.45		10	11	0.05		3	0	-1.00	
	RE	R	A	F	39	59	0.20	*	12	20	0.25	15	21	2	5	0.43		33	27	-0.10		1	2	0.33	
	SA	R	A	F	31	74	0.41	*	25	48	0.32	33	31	8	15	0.30		16	30	0.30	*	4	4	0.00	
	CA	R	A	F	60	58	-0.02		5	11	0.38	50	29	3	4	0.14		18	32	0.28		6	4	-0.20	
	LF	U	I	M	10	87	0.79	*	7	11	0.22	63	50	5	6	0.09		23	21	-0.05		4	5	0.11	
	DF	U	I	F	6	93	0.88	*	12	15	0.11	33	71	4	10	0.43		22	22	0.00		8	8	0.00	
	FI	U	A	F	39	36	-0.04		18	36	0.33	72	61	13	20	0.21		31	31	0.00		22	8	-0.47	*
	CO	U	I	M^G	50	23	-0.37	*	28	16	-0.27	66	50	19	9	-0.36		29	28	-0.02		1	0	-1.00	
	GM	U	A	F	44	62	0.17		29	17	-0.26	68	74	25	17	-0.19		84	77	-0.04		9	4	-0.38	
	BM	U	A	F	67	33	-0.34	*	7	24	0.55	47	61	9	17	0.31		52	45	-0.07		15	6	-0.43	*
	WF^{N}	R	A	M^S	30	69	0.39	*	7	9	0.13	33	29	2	2	0.00									
	NL	R	A	F	42	73	0.27	*	13	15	0.07	53	71	9	12	0.14		42	16	-0.45	*	8	10	0.11	
	RH	R	A	M^S	49	36	-0.15		49	28	-0.27	56	50	27	14	-0.32	*								

	NM	R	A	F	21	34	0.24										8	7	-0.07	1	1	0.00	
	YM	U	A	F	9	90	0.82	*	47	35	-0.15	50	67	28	26	-0.04	24	24	0.00	1	0	-1.00	
	OM	R	A	F	45	53	0.08		48	32	-0.20	48	42	28	18	-0.22	24	20	-0.09	15	5	-0.50	*

L – left, R – right, LI – laterality index calculated as R-L/R+L, * significant individual preference at $p < 0.05$, M^s – stallion, M^G – gelding, A – more than 2-years of age, I – less than 2-years of age, M – male, F – female, L – lame, CS – limb preference case study, *N* – the horse was moved and further records could not be obtained.

Appendix IV. Comparison of 30- and 60-second-interval sampling data with scores of the percentage of time spent with the left forelimb in front of the right (%time = L/(L+R)*100).

Horse	Percentage time		
	Continuous sampling	60-sec-interval sampling	30-sec-interval sampling
1	57	45	64
2	62	83	74
3	48	62	46
4	50	30	43
5	39	22	25
6	62	58	56
7	43	14	24
8	45	47	52
9	21	27	21
10	51	67	54
11	50	38	46
12	51	57	60
13	49	47	44
14	49	62	60
15	48	31	52
16	52	67	45
17	70	67	69
18	63	64	57
19	51	67	43
20	51	47	46

Appendix V. Number of left and right scores per horse recorded by observing the feral and Przewalski horses during stallion fights.

		Eye bias in agonistic interactions in stallion fights							
		Threat				Attack			
	Horse	L	R	LI		L	R	LI	
Feral Group 1	SH^{b}	25	23	-0.04		23	18	-0.12	
	BS^{h}	35	33	-0.03		12	15	0.11	
	ZC^{b}	73	41	-0.28	*	46	43	-0.03	
	C^{b}	47	46	-0.01		17	6	-0.48	*
	DI^{h}	52	44	-0.08		51	35	-0.19	
	DG$_{1}^{bh}$	55	54	-0.01		68	44	-0.21	*
	CU^{h}	37	46	0.11		34	18	-0.31	*
Feral Group 2	PG^{bh}	48	45	-0.03		29	18	-0.23	
	TI^{b}	166	115	-0.18	*	51	35	-0.19	
	GR^{b}	54	32	-0.26	*	11	6	-0.29	
	TS^{b}	133	83	-0.23	*	24	15	-0.23	
	CO^{b}	217	195	-0.05		54	29	-0.30	*
	HS^{b}	246	206	-0.09		106	59	-0.28	*
	BI^{h}	52	58	0.05		29	14	-0.35	*
	SO^{h}	94	89	-0.03		43	20	-0.37	*
	CS^{h}	120	90	-0.14	*	42	25	-0.25	*
	LS^{h}	124	110	-0.06		34	22	-0.21	
	DG$_{2}^{h}$	116	95	-0.10		50	34	-0.19	
	ST^{h}	45	45	0.00		32	25	-0.12	
	MO^{h}	50	50	0.00		22	19	-0.07	
	AB	7	5	-0.17		1	2	0.33	
	YS^{b}	34	16	-0.36	*	10	3	-0.54	
	SS^{h}	34	42	0.11		14	5	-0.47	*
	BC$_{2}^{b}$	16	6	-0.45	*				

Przewalski	EN^b	311	268	-0.07		77	43	-0.28	*
	AV^b	153	134	-0.07		27	16	-0.26	
	AO^b	140	150	0.03		50	24	-0.35	*
	SX^b	107	116	0.04		29	25	-0.07	
	BH^b	61	45	-0.15		9	4	-0.38	
	CT^b	63	52	-0.10		10	9	-0.05	
	PR^b	113	104	-0.04		18	5	-0.57	*
	AD^b	126	108	-0.08		32	29	-0.05	
	AN^b	200	182	-0.05		91	61	-0.20	*
	SA^b	29	27	-0.04		4	1	-0.60	
	EO^b	11	13	0.08		2	1	-0.33	
	ES^b	74	62	-0.09		15	13	-0.07	
	EC^b	83	74	-0.06		14	5	-0.47	*
	AT^h	213	198	-0.04		92	88	-0.02	
	SZ^h	90	86	-0.02		54	27	-0.33	
	CS^h	142	142	0.00		107	60	-0.28	*
	BN^h	149	106	-0.17	*	52	36	-0.18	
	NU	17	15	-0.06		2	1	-0.33	

L – left, R – right, LI – laterality index calculated as R-L/R+L, * significant individual preference $p < 0.05$, b - bachelor stallion, h – harem stallion, b and h – stallion was both a bachelor and a harem stallion within research period.

Appendix VI. Limb preference: Number of left and right scores per horse recorded by observing the feral horses.

	Horse	Sex	Age	Limb preference L	R	LI		Level of reactivity	Level of aggression
Group 1	**CU**	M	A	35	45	0.13		8.9	5.0
	BB	F	A	36	44	0.10		1.5	3.7
	BU	F	A	32	48	0.20		5.2	3.2
	ZC	M	A	44	42	0.05		10.1	12.6
	BBC	M	I	37	36	-0.10		1.2	0.5
	BC$_1$	M	I	38	43	0.08		2.8	2.6
	SR	F	I	34	46	0.15			
	CR	F	I	35	45	0.13			
	BS	M	A	41	39	-0.03		18.4	3.7
	ZM	F	A	38	42	0.05		11.2	4.7
	BM	F	A	41	39	-0.03		7.8	
	TI	F	A	39	41	0.03		8.0	5.4
	PI	M	I	28	52	0.30	*	17.4	0.4
	DI	M	A	47	33	-0.18		14.6	13.0
	GM	F	A	40	40	0.00		12.4	4.0
	DG	M	A	42	38	-0.05		11.0	7.0
	LI	F	I	43	37	-0.08		6.5	2.5
	OM	F	A	40	40	0.00		8.9	0.5
	C	M	A	45	35	-0.13		5.8	5.1
	SH	M	A	44	36	-0.10			
Group 2	**SS**	M	A	44	36	-0.10		11.0	2.1
	GM	F	A	42	38	-0.05		7.0	5.1
	CB	F	A	40	40	0.00		14.4	4.5
	DM	F	A	46	34	-0.15		4.9	3.2
	LB	F	A	38	42	0.05		7.7	1.6
	BC$_2$	M	I	34	46	0.15		8.8	1.5

	NF	F	I	56	24	-0.40	*	5.3	2.8
	BK	F	I	29	51	0.28	*	10.1	2.4
	SC	M	I	30	50	0.25	*	9.8	1.7
	CK	F	I	18	62	0.55	*	13.6	2.4
	LS	M	A	46	34	-0.15		7.0	5.0
	GB	F	A	43	37	-0.08		1.4	3.3
	BL	F	I	27	53	0.33	*	1.0	1.8
	BC$_3$	M	I	38	42	0.05		4.6	1.0
	MO	M	A	43	37	-0.08		2.9	1.7
	CH	F	A	41	39	-0.03		4.7	3.8
	BY	F	A	35	45	0.13		5.7	2.3
	YB	M	I	50	30	-0.25	*	2.1	0.9
	YC	M	I	51	29	-0.28	*	2.0	1.9
	OS	F	I	50	30	-0.25	*	5.3	
	WT	M	I	33	47	0.18		7.1	
	DG	M	A	35	45	0.13		16.6	8.0
	EB	F	A	46	34	-0.15		7.5	6.2
	FB	F	I	53	27	-0.33	*	5.8	0.9
	CS	M	A	38	42	0.05		13.5	5.7
	FF	F	A	30	50	0.25	*	6.3	1.7
	ST	M	A	42	38	-0.05		4.6	3.5
	SB	F	A	43	37	-0.08		7.4	2.1
	SR	F	A	42	38	-0.05		2.4	3.3
	EX	F	I	23	57	0.43	*	12.4	
	EO	F	I	42	37	-0.06		13.8	
	BI	M	A	36	44	0.10		5.4	2.9
	WH	F	A	40	40	0.00		6.1	5.4
	MG	F	A	49	31	-0.23		3.6	3.8
	FI	F	I	34	46	0.15			0.5
	CZ	F	A	48	31	-0.22		4.2	2.1

	BZ	M	I	26	54	0.35	*		
	BF	M	A	44	36	-0.10			
	OG	F	A	38	42	0.05			
	GD	F	A	45	35	-0.13			
	BA	M	I	27	52	0.32	*		
	RO	M	I	39	41	0.03			
	SO	M	A	50	27	-0.30	*		
	RB	F	A						
	NB	F	A						
	DB	F	A						
	PG	M	A	42	38	-0.05		5.4	4.1
	MC	F	A	38	42	0.05		6.3	2.5
	CC	M	I	51	29	-0.28	*	6.8	
	TI	M	A	41	39	-0.03		8.6	4.9
	TS	M	A	45	35	-0.13		7.2	5.4
	CO	M	A	45	35	-0.13		4.6	4.9
	GR	M	A	40	40	0.00			2.1
	HS	M	A	39	40	0.01		4.8	4.0

L – left, R – right, LI – laterality index calculated as R-L/R+L, * significant individual preference $p < 0.05$, bold – stallion, boxed sections in the column second from the left indicate each band, M – more than 2-years of age, I – less than 2-years of age, M – male, F – female. Level of reactivity – number of times a horse lifted its head from grazing to look at its surroundings per hour. Level of aggression – number of times a horse engaged in an agonistic interaction per hour. 1, 2 and 3 refer to different horses that were given the same initials in the field but were observed at different sites.