Maternal behaviour in the ewe: Consistency in the expression of maternal behaviour during lactation and the effect of variation in dam and sire breed on the development of offspring.

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This thesis has been composed by me and is a record of my own work.

Abstract.

Consistency in the expression of maternal care over a single lactation period was investigated using two breeds of ewe, Scottish Blackface and Suffolk. The two breeds showed differences in their initial maternal behaviour with Blackface ewes showing more affiliative and less negative behaviour towards their newborn lambs than Suffolk ewes. These differences in maternal behaviour continued throughout lactation. Whilst both breeds were able to recognise their lambs at three days post-partum, Blackface ewes were more motivated to associate with their lambs in a maternal choice test, suggesting that they had a stronger ewe-lamb bond than Suffolk ewes. During the rest of lactation Blackface ewes had a closer spatial relationship with their lambs, accepted a higher proportion of suck attempts from their lambs and showed more communication with them via the head-up posture, compared to Suffolk ewes. Blackface ewes actively interacted with their lambs, using the head-up posture to control sucking interactions and to encourage their lambs to remain in close proximity. In contrast Suffolk ewes were not proactive in their relationship with their lambs and appeared to react to the behaviour of their lambs, rather than actively communicating with them.

A factor analysis showed that three dimensions, the willingness of the ewe to interact with her lamb throughout lactation, her response to her lamb's attempts to interact with her and their ewe-lamb bond during later lactation, can be used to describe variation in ovine maternal behaviour. The willingness of the ewe to interact with her lamb accounted for most of the variation between the two breeds, with Blackface ewes showing mostly affiliative behaviour and Suffolk ewes showing more negative behaviour. Within the two breeds variation was mainly due to behaviours associated with the ewe's response to her lamb's attempts to interact with her. Individual Blackface ewes showed consistency in their expression of maternal behaviour throughout lactation, but Suffolk ewes did not. This is likely to result in a stable ewe-lamb relationship in Blackface ewes but an unstable relationship in Suffolk ewes.

The different maternal styles were then used to assess the effect of variation in dam and sire breed on the development of offspring. The lambs' stress response during three tests (social separation, restraint and novel object) and their ability to learn a spatial memory task were assessed. In addition, the ability of day old lambs to recognise their dam, lamb growth rate during lactation and the lambs' own maternal behaviour was examined to assess traits relevant to sheep production. Lambs raised by Blackface ewes showed an active behavioural response during the stress response tests and were quicker to learn the spatial memory task than lambs raised by Suffolk ewes. Suffolk-raised lambs showed a passive response during the stress response tests. Blackface lambs were better able to recognise their dams at 24 hours old than Suffolk lambs. The willingness of the ewe to interact with her lamb throughout lactation showed a positive association with lamb growth. Ewes and their dams were also found to show similarities in their initial maternal behaviour. Therefore, although it was not possible to rule out the possibility of a genetic influence, maternal behaviour in the ewe appears to have the potential to influence offspring development in a variety of ways.

A study on the early maternal behaviour of mares was also conducted to assess the potential for the sheep study findings to be applied to other domestic species. Compared to the ewes, mares showed much less interaction with their young, in particular grooming, during the immediate post-partum period, but also showed much less negative behaviour. However there did appear to be potential for the findings of the sheep study to be applied to the horse.

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Chapter 1: General introduction.

The study of maternal behaviour in domestic species can be of great importance for farming practice and the welfare of farm animals. In animals where litter sizes are small, weaning is relatively late, and man has limited interaction, the behaviour of the dam towards her young is a major influence on their survival and early development. A good mother-young relationship is therefore desirable for both farm productivity and animal welfare, as good maternal behaviour would be expected to maximise an animal's growth and chances of survival. The study of maternal behaviour can help to identify positive and negative traits and, if necessary, aid selection for improved maternal ability.

The domestic sheep, *Ovis aries*, is a good example of a farm animal species where the dam is a major influence on the early life of the young. Sheep generally give birth to single or twin litters, occasionally triplets in some breeds, and the lambs are left with their dam until weaning at around 3 months post-partum. Some breeds are farmed in a hill environment and receive very little or no input from man, for example Scottish Blackface, whereas others are lowland animals, for example Suffolk sheep, and receive more intensive handling, especially during the immediate lambing period. Whilst the maternal behaviour of hill sheep has the most potential to influence the early life and survival of their lambs, maternal care in the lowland breeds is still important, especially once the ewes and lambs are out in the field during later lactation. Previous study of maternal care in the ewe has been wide ranging and much is known about ewe and lamb behaviour and the influence of maternal behaviour on the early life of the lamb, especially during the immediate post-partum period.

1.1. Initial ewe and lamb behaviour.

1.1.1. Pre-parturient behaviour.

Under extensive conditions ewes tend to seek isolation from the flock as parturition becomes imminent. Immediately prior to parturition the ewe becomes restless and begins to lick her lips, making rapid licking or tongue movements in the air and pawing the ground. She also alternates between lying down and straining, and standing, pawing the ground and periodically nosing it. Once the amniotic sac has ruptured she spends much time licking the fluids from the grass (Vince et al. 1985), probably due to her attraction towards amniotic fluid at this time (Levy and Poindron 1987). Ewes nearly always give birth whilst recumbent (Vince et al. 1985).

1.1.2. Post-parturient behaviour.

Immediately after birth ewes stand, turn to face their lambs and begin grooming them (Sharafeldin and Kandeel 1971). During the first 2 hours post-partum grooming is the primary activity of the ewe (Bareham 1976, McGlone and Stobart 1986). Amniotic fluids are important in facilitating the initial contact between the ewe and her lamb and the start of grooming in both experienced and inexperienced ewes (Vince et al. 1985, Levy and Poindron 1987). However, facilitation of grooming via olfactory cues is more important for primiparous than multiparous ewes (Levy and Poindron 1987). Grooming begins with consumption of the remnants of the foetal membranes, gradually moving on to a thorough licking of the lamb (Alexander 1988).

The order of licking the various body regions of the lamb appears to be specific and non-random (McGlone and Stobart 1986). Ewes most frequently start licking the head, working gradually over the body until the lamb is completely dry and free of birth membranes (Hersher et al. 1963, Bareham 1976). Whilst initially intense, grooming becomes spasmodic within approximately 30 minutes of birth (Alexander 1988) and declines to intermittent nosing within a few hours (Bareham 1976). During the first day of life the ewe continues to make contact with her lamb, frequently putting her nose down to touch and lick it (Shillito and Hoyland 1971), however beyond the immediate post-partum period grooming is restricted to brief occasional episodes (Alexander 1988).

The grooming behaviour of the ewe is influenced by litter size. Twin lambs receive less grooming than single lambs during the post-partum period (O'Connor et al. 1992) due to ewes switching to groom the second-born lamb after it is born. The ewe continues to show some grooming attention to both members of the litter, resulting in both lambs receiving less grooming than a single lamb.

1.1.3. The function of grooming.

Grooming of the neonate is considered to play a role in drying and warming the lamb, as well as providing the lamb with tactile stimulation (McGlone and Stobart 1986, O'Connor and Lawrence 1992). There is conflicting evidence as to the influence of tactile stimulation from the ewe on the behaviour of the lamb (Dwyer and Lawrence 1999a). Dwyer and Lawrence (1999a) found that grooming increased the time taken by the lamb to stand and it is possible that grooming initially serves to inhibit the activity of the lamb, conserving energy whilst it is still uncoordinated (Vince et al. 1985, Lynch et al. 1992). McGlone and Stobart (1986) found that licking of the anterior end of the lamb, head and shoulders, frequently led to the lamb attempting to stand and proposed that licking in this area could encourage the lamb to stand. Maternal licking over the head and back may therefore also facilitate righting and standing as the lamb gets stronger. Licking of the face and muzzle is thought to help the lambs to breathe easily (Sharafeldin and Kandeel 1971) and to encourage oral activity (Vince et al. 1984).

Once the lamb has been cleaned, the ewe continues to nose and push the lamb from behind, further encouraging attempts to stand (Sharafeldin and Kandeel 1971). At this stage maternal assistance, in the form of grooming and orientation by the ewe to aid teat seeking by the lamb, facilitates the progress of the lamb toward successful sucking (Alexander and Williams 1964). Grooming behaviour is thought to influence the time taken for the lamb to first stand and suck (Sharafeldin and Kandeel 1971, Bareham 1976, O'Connor et al. 1992).

1.1.4. Initial lamb behaviour.

Within minutes after birth lambs begin to make righting movements (upward and forward head movements, followed by rising on flexed forelimbs and then hind limbs), and attempt to stand. In most breeds they locate the udder within 1 hour post-partum (Vince and Ward 1984, Vince et al. 1984). As described above, the behaviour of the ewe can facilitate this process (e.g. Sharafeldin and Kandeel 1971, Bareham 1976, Vince et al. 1984, O'Connor et al. 1992) but active maternal care is not essential for the lamb to find the udder and suck (Alexander and Williams 1964). However, the visual stimulus provided by the ewe is important for the early behaviour of the lamb. Lambs will direct their initial behaviour towards any large object, especially if it is moving (Vince et al. 1985). Subsequently the lamb will tend to sit or sleep quietly in the presence of a large unmoving object. For example, when ewes move away from the birth site, to graze or drink, their lambs often remain lying quietly next to an alternate large object, such as a fence or a large clump of nettles (Vince et al. 1985).

1.1.5. Teat seeking and initial suck attempts.

After standing, lambs begin searching for the udder, actively nudging their mothers with forceful, pushing head movements (Bareham 1976). They show bunting, munching, licking and sucking movements along the mother's body before the teat is located (Bareham 1975, Vince and Ward 1984). Various features of the ewe's body and the type of stimulation received by the lamb influence its searching behaviour and aid successful location of the teat and sucking (Vince et al. 1984). The lamb initially places its head under any projecting surface at head height (Collias 1956, Smith 1965) and this helps the lamb to begin searching underneath the dam. Stimulation on the top of the muzzle results in forward head movements which facilitate the lamb moving further forward under the dam. Stimulation on top of the face produces forward and upward movements of the head, and stimulation of the lips results in munching and sucking (Vince et al. 1985).

Lambs respond more vigorously to surfaces that are warm (Vince 1984, Billing and Vince 1987a), smell of inguinal wax (Vince and Ward 1984) and have an optimal degree of yield to touch (Billing and Vince 1987b). Lambs are also more responsive to bare skin than wool. Wool is naturally cooler than skin but lambs still show more response to bare skin, compared to wool, when both have been heated to the same temperature (Billing and Vince 1987b). All these features relate more specifically to the udder area (warmth, bare skin, smell of inguinal wax, specific yield) than to any other area of the ewe's body and a more active response to them facilitates the location of the teat and successful sucking. As lambs are known to actively respond to maternal, but not alien ewe, inguinal wax, it may also play a part in recognition and maintenance of the ewe-lamb bond (Vince and Ward 1984).

1.1.6. Sucking behaviour.

In the first hour post-partum sucking frequency is high but decreases by the end of the first day (Bareham 1976). Initially the lamb searches the whole of the ewe's body before sucking, even if it has previously successfully sucked. However, within a few hours the lamb learns the location of the udder, moves directly towards it and suckles without prior teat seeking. This process is likely to be reinforced by the milk reward it receives during sucking (Bareham 1975). Prevention of sucking from birth leads to a decrease in the teat-seeking activity of lambs and this is probably due to a failure to receive a reward. The initial high level of teat seeking in lambs is therefore thought to be driven by inherent stimuli within the lamb, as well as by hunger (Alexander and Williams 1966a).

1.1.7. Lamb vocalisation after birth.

Bleating is initially weak but increases in volume rapidly (Bareham 1976). A loud 'distress' call is made if the ewe stops licking for a period of time (Bareham 1976) and maternal licking reduces the bleating rate of lambs (Shillito and Hoyland 1971, Vince et al. 1985). Ewes reply to lamb bleats with low pitched vocalisations (Bareham 1976).

1.1.8. Ewe vocalisations after parturition.

Immediately after parturition vocalisation by both the ewe and the lamb is frequent (Dwyer et al. 1998). Even breeds which are normally silent, for example Soay sheep, show repeated vocalisation after birth (Shillito and Hoyland 1971). Ewes have two types of vocalisation, a low pitched 'rumble' and a high pitched bleat. The low pitched rumble is almost exclusively associated with newborn lambs (Shillito 1972), suggesting that its function is likely to be specific to bonding with, and care giving to, the lamb. The role of low pitched vocalisation in the bonding process is described below.

Ewes also have a high pitched vocalisation which differs in function to the low pitched rumble. High pitched bleats are made when the ewe is separated from the lamb, appearing to be calls for help or alarm (Shillito 1972), and may function to recall the separated lamb (Dwyer et al. 1998). Bleating ewes are more attractive to separated lambs than silent ewes and the bleating may stimulate the lamb to return to the ewe (Pollard 1992). Vince et al. (1985) also found that ewe bleats had an activating effect on isolated newborn lambs. Lambs normally approach the dam after vocalisation and ewes will initially stand after bleating, not moving quickly towards their lambs (Shillito-Walser et al. 1981).

High pitched bleats are rarely made when the lamb is with the ewe and tend to increase in frequency with lamb age. This could be due to lambs becoming more active and subsequently becoming separated from the ewe more frequently (Dwyer et al. 1998). The significance of the ewe's bleat is also thought to change with increasing lamb age. Initially it functions to call the lambs to the ewe but after the first month post-partum the ewe's bleat acts more as a means of location rather than recall (Shillito-Walser et al. 1984).

1.2. The formation of the ewe-lamb bond and development of ewe-lamb recognition.

An exclusive maternal bond is not universal throughout the animal kingdom but is favoured in gregarious species which give birth to precocial young. A gregarious nature increases the chances of mothering alien young, especially if the young are precocial and therefore highly mobile and relatively independent of the dam (Gubernick 1981). Sheep are both gregarious, living in flocks, and produce young which stand rapidly after birth. They also show synchronised breeding, with all the lambs within a flock being born within a short period of time (Lynch et al. 1992). Consequently it is important for the ewe to be able to recognise her lamb soon after birth and to form an exclusive ewe-lamb bond in order to avoid suckling the young of other ewes. This is likely to decrease the survival chances of her own lambs due to a reduced milk supply and an increased chance of separation.

1.2.1. Establishment of the ewe-lamb bond.

The first hour post-partum is the most important in the establishment of an exclusive bond between the ewe and her lamb (Alexander et al. 1986). Grooming of the lamb is important for the development of this bond (Levy and Poindron 1987), as it allows the ewe to learn the smell, taste, appearance and voice of her lamb and consequently reject alien lambs (Shillito-Walser 1978b). Licking for a period of 20-30 minutes appears to be sufficient for the dam to learn the identity of her young (Smith et al. 1966) and ewes will reject alien lambs after 30 to 120 minutes of full contact with their own lamb (Poindron and Le Neindre 1980).

Ewes which have been prevented from suckling or grooming their lambs during the first 12 hours after birth show differing degrees of selectivity, dependent on the state of the lamb: ewes presented with wet lambs are more selective than ewes presented with dry lambs. As amniotic fluid provides important olfactory cues it appears that olfaction by the ewe is essential for the development of selective behaviour (Alexander et al. 1986). Once selective behaviour has been established the odours used for recognition do not appear to be of maternal origin, nor derived from physical contact between the ewe and lamb (Alexander and Stevens 1982, Alexander et al. 1986), but originate from the coat of the lamb (Alexander and Stevens 1982). Olfactory cues emanate from both the head and the tail region, with odours from the anal region being the most attractive for ewes (Alexander 1978).

Vocal communication between the ewe and lamb is also central to the development of the ewe-lamb bond (Nowak 1996). Low pitched rumbles reduce the activity of the newly born lamb, in particular standing attempts (Vince 1986). This potentially allows the ewe greater contact with her lamb and improves her chance to learn its individual characteristics, facilitating the bonding process. Although low pitched vocalisations are expressed by all ewes they appear most frequent in primiparous ewes (Dwyer et al. 1998). It is possible that reduced activity may make the newborn lamb more attractive, or less aversive, to an inexperienced ewe. Some ewes have been known to groom their newborn lamb but to head butt it aggressively when it moved (Lynch et al. 1992).

1.2.2. Cues used by the ewe to recognise her lamb.

The cues used by the dam to recognise her young depend on their physical proximity and age (e.g. Morgan et al. 1975, Alexander 1977, Alexander and Shillito 1977). During the first few days post-partum visual and auditory cues are used in the location and distance recognition of lambs, olfaction when the ewes are in close proximity (less than 25cm) or physical contact with their lambs (Shillito and Alexander 1975, Alexander and Shillito 1977, Alexander 1978). At this time odour is the final criterion used by the ewe to recognise her lamb and allow it to suck (Poindron and Le Neindre 1980), lambs which have an unfamiliar odour will be vigorously rejected by the ewe (Alexander and Stevens 1981). Even ewes which have recently used visual and auditory cues to correctly identify their own lamb at a distance will prevent the same lamb from sucking if it has been washed to remove any familiar odour (Alexander and Stevens 1981). With increasing lamb age, ewes display much less overt sniffing of their young and appear to become more reliant on visual and auditory cues to identify them during sucking interactions (Alexander and Stevens 1981, Ferreira et al. 2000). However olfaction still has an important role in recognition at 1 month post-partum (Ferreira et al. 2000).

1.2.3. Development of recognition in the ewe.

All three types of recognition, visual, olfactory and auditory, develop during the first day post-partum (Terrazas et al. 1999). Although olfactory recognition develops first, within 4 hours of parturition, it is not a necessary prerequisite for the establishment of visual and auditory recognition (Ferreira et al. 2000). Close recognition, via olfaction, is unaffected by housing but long distance recognition does not develop in animals which are housed indoors during the first few weeks post-partum (Poindron and Schmidt 1985). This is unsurprising as housed animals will not have the need, nor opportunity, to learn the cues associated with distance recognition if they are never more than 18m apart.

1.2.4. The cues used in distance recognition.

Visual and auditory cues are both used in distance location and recognition (Alexander 1977, Alexander and Shillito 1977) but their relative importance appears to differ. Visual cues seem to be more reliable than auditory cues and ewes need a visual stimulus to approach a lamb (Alexander 1977, Shillito-Walser 1978a). Manipulation of the physical appearance of the lamb, via colouring the coat, causes ewes to be hesitant in their approach of their lambs and to delay suckling, even when olfactory and auditory cues are unaltered (Alexander and Shilllito 1978). In contrast, lamb bleats have non-specific attractive properties for the ewe (Alexander 1977) and their main function appears to be to attract the dam's attention and to help her orientate towards the separated lamb (Shillito and Alexander 1975, Shillito-Walser 1978a). Ewes appear to look for their lamb once they hear it bleat (Shillito-Walser et al. 1981). However there is some specificity in a lamb's voice (Alexander 1977) and some distance discrimination is based on sound (Shillito and Alexander 1975).

Moving lambs have been found to provide a better visual signal and to be more attractive to the ewe (Alexander and Shillito 1977, Alexander 1978). This could explain why separated lambs walk around when they have lost their dam, rather than remaining at a location that may be familiar to the ewe. Ewes have been shown to have a short-term memory of the last location of their lamb and often return to this site when they are separated from their lamb (Alexander and Shillito 1977, Alexander 1978). However this may be less effective in producing a reunion than the attraction of the ewe to a moving stimulus.

1.2.5. Features of the lamb used in visual recognition.

Alexander and Shillito (1977) investigated the regions of the lamb's body which are important for visual recognition during the first 2 weeks post-partum. They concluded that visual cues are associated mainly with the head but their results also indicate that the appearance of the lamb as a whole (head, trunk and legs), or just its trunk and legs, are also important for recognition. Maternal ewes take 2-3 weeks to learn the faces of their lambs (Kendrick et al. 1996), suggesting that the ewes in Alexander and Shillito's study (1977) would not have been able to recognise their lamb's facial features until the end of the experimental period. Unfortunately the authors do not indicate at which age the various body regions were investigated. However, when considered together, the results of both studies suggest that the ewe may initially use the appearance of her whole lamb for recognition, switching to cues from just the head as her lamb becomes older and she is better able to recognise its face.

1.2.6. Recognition of the ewe by the lamb and the formation of attachment to the dam.

The exclusive nature of maternal care in the ewe means that the solicitation of maternal care from other ewes is likely to be unsuccessful (Nowak et al. 1990) and the lamb must rapidly learn to recognise its dam. The lamb therefore plays an active role in the bonding process (Nowak 1990a, Asante et al. 1999) and attachment between the ewe and lamb is dependent on mutual recognition, rather than the ewe alone being able to recognise her lamb (Shillito and Hoyland 1971).

1.2.7. Establishment of the attachment between the lamb and its dam.

The first 12 hours post-partum are thought to represent a critical period in the establishment of a maternal-offspring bond and the behaviour of the ewe at this time is crucial for the formation of a strong attraction between the lamb and its dam (Alexander and Williams 1966a). In particular, the response of the ewe to the lamb's first sucking attempts plays an important role in the establishment of dam recognition by the lamb (Nowak 1996). Suckling allows the lamb to establish a preference for the dam and facilitates the learning of her smell and appearance (Shillito-Walser 1978b, Nowak et al. 1997). During the first few hours the newborn associates suckling with the presence of its mother and motivation to seek proximity to her will depend on whether or not it was able to suck (Nowak et al. 1997). This preference is mediated via the ingestion of colostrum, rather than just the physical process of suckling (Goursaud and Nowak 1999). Newborn lambs which have been prevented from sucking lose interest in their mothers (Alexander and Williams 1966a) and are not attracted to her in a choice test (Nowak et al. 1997). In addition, at 12 hours old well-fed lambs are better able to distinguish their dams from unfamiliar ewes, compared to poorly fed lambs, possibly due to being more motivated to find her (Nowak and Lindsay 1990).

Although unrestricted sucking from birth is required in order for a preferential relationship to develop, maintenance of an established preference for the dam does not rely strongly on sucking (Nowak et al. 1997). At 3 days old, lambs which have been prevented from sucking for the same time period as newborn lambs (6 hours), show no disruption in their preference for their dam.

1.2.8. The role of lamb vocalisation in ewe-lamb bonding.

High bleating activity by the lamb during the immediate post-partum period also improves the quality of the bond between the mother and her lamb. The lamb's bleats attract the ewe, causing her to respond more actively towards it, increasing close contact, nosing, bleating and suckling. This increased maternal care provides the lamb with more stimuli to respond to and so reinforces the opportunity for both animals to learn to recognise each other (Nowak 1990a).

1.2.9. Recognition of the dam.

Lambs begin to be attracted to ewes between 12 and 18 hours post-partum and can recognise their dam at close quarters by 24 hours old (Nowak et al. 1987, Nowak et al. 1990, Asante et al. 1999). Lambs rely on visual and auditory cues to recognise and locate their dam and close-up recognition may occur through low pitched rumbles, olfactory cues, and visual cues only perceptible at close quarters (Shillito 1975, Arnold et al. 1975). However, during the early post-partum period lambs may rely more on the behaviour of their dam for recognition, rather than her physical features (Nowak et al. 1987, Terrazas 2002). Ewes show accepting behaviour towards their own young, including a high rate of low pitched vocalisations, but show rejecting behaviour when approached by alien lambs. Lambs appear to use these behavioural cues to identify their dam whilst they are still learning her physical features (Terrazas 2002).

Initially lamb recognition of the dam is considered to be poor, (Shillito 1975, Nowak et al. 1990), and lambs are not thought to be able to recognise their dams through high pitched bleats alone (Nowak et al. 1990). As these bleats tend to be emitted when the animals are separated, an unlikely event during the first few hours post-partum, this could be due to there being no opportunity for the lambs to learn to recognise their dam's bleating (Nowak et al. 1990). In addition, Nowak et al.'s study

(1990) may have been confounded by the tendency of very young lambs, less than 3 days old, to stand still when separated and to wait for their dam to find them (Shillito 1975). During the tests of dam recognition the ewes were, of necessity, out of sight of their lambs. It is possible that lambs of this age recognise their dam's vocalisations but still need visual cues to be stimulated to move towards her. Consequently it cannot be definitively concluded that young lambs are unable to use high pitched bleating to recognise their dam. Measuring the auditory response of lambs to the voices of their dam and alien ewes could provide more information on this.

1.2.10. Mother seeking behaviour in young lambs.

In very young lambs (36 hours old) mother seeking behaviour is very strongly biased by the location of the dam in previous successful reunions (Shillito 1975, Nowak 1994), possibly indicating that these lambs are relying on spatial location to locate their dam rather than her physical characteristics. In wild and feral sheep the ewe is known to stay in the vicinity of the birth site for several days (Lynch et al. 1992). Therefore attraction to a spatial location would be advantageous for newborn lambs for several reasons, the most obvious being that the ewe is likely to be in that place. In addition lambs are only able to discriminate their dams at close quarters from 12 hours of age (Nowak et al. 1987) and from a short distance at 24 hours old (Nowak 1994). Consequently a separated lamb relying on close-up ewe characteristics would have to approach closely nearby ewes in its attempts to locate its dam, increasing its chances of wandering farther from its mother. By remaining on a site associated with the dam, the lamb will reduce its chances of becoming lost whilst its ability to recognise its dam at a distance is still developing. This will also facilitate the ewe in searching for the lamb as the search area will be greatly reduced.

1.2.11. Development of distance recognition.

Over the first few days postpartum lambs learn the characteristics of their dam used in distance recognition (Nowak 1990b) and this may reflect the rapid change in the spatial relationship between the ewe and her lamb. Initially the ewe will follow and retrieve lambs which wander from the birth site (Bareham 1976) and the ewe and lamb are rarely far apart. Subsequently, at around the second or third day post-partum, mother-young separations begin to occur more frequently as ewes resume grazing activity and lambs develop exploratory behaviour. Maintaining contact with, and following the mother in the paddock, necessitates the learning of ewe characteristics from a distance (Nowak 1990b) and the more frequent separations also provide the opportunity to do this.

During the first 2 weeks lambs use both visual and auditory cues to identify their dam at a distance (Shillito 1975, Alexander 1977). However, the relative importance of visual cues, compared to auditory cues, increases between 2 and 4 weeks post-partum and older lambs rely more on visual cues than auditory cues (Alexander 1977). Visual cues are generally thought to be the most important cues used in lamb recognition of their dam. Lambs which are presented with two alien ewes of different breeds, show a preference for the ewe of their dam's breed if they can see the ewes but make no distinction if they have only auditory contact (Shillito-Walser 1980).

1.2.12. Effect of breed and litter size on the development of dam recognition.

Different breeds have been found to rely differentially on visual and auditory cues. For example, Clun Forest lambs rely more on visual cues whereas other breeds, e.g. Dalesbred, are able to use both visual and auditory cues (Shillito-Walser 1980). This may be related to the natural ecology of the different breeds. Dalesbred sheep normally live in hilly country where the ewe could easily be out of sight, conferring an advantage in being able to recognise the dam by voice as well as by sight (Shillito-Walser 1980). Jacob lambs also show poor dam recognition with auditory cues alone but have a high success rate when the ewes are visible. This may be due to physical appearance rather than ecology, as Jacob ewes are spotted and show a wide variation in physical appearance. This could result in a greater reliance on visual, rather than auditory, cues in this breed (Shillito 1975).

Litter size also appears to influence the development of lamb recognition of the dam in some breeds (e.g. Merino). In these breeds single lambs develop dam recognition more quickly than twin lambs (Nowak and Lindsay 1990, Nowak et al. 1990). It is not clear why this is the case but a number of reasons have been proposed. Examples include: an increased frequency of temporary separations from the dam in twin litters; a delay in attachment to the dam due to reduced tactile stimulation and colostrum intake; the presence of the sibling interfering in the bonding process; or a lesser degree of physiological and behavioural maturation due to the shorter gestation of twin lambs (Nowak et al. 1990). However other breeds do not show this differentiation in the development of dam recognition. The ability of Border Leicester X Merino lambs to recognise their dam is not affected by litter size, although this could be due to hybrid vigour in the Border Leicester breed (Nowak and Lindsay 1990).

Interestingly, the fact that individuals within twin litters behave differently indicates that the establishment of mother discrimination is characteristic of the individual lamb, rather than a result of sharing a common gene pool and/or a common pre- or post-natal environment (Nowak and Lindsay 1990). Nevertheless, it should be considered that twin lambs may also differ in their experience of initial maternal care, especially if the birth interval between the twins is long. This would result in the first born receiving a period of exclusive maternal care and consequently more maternal attention than the second born twin. Differences in behaviour within twin litters may therefore also be due to the maternal care experienced as well as characteristics of the individual lamb.

1.3. Abnormal maternal behaviour during the post-partum period.

Abnormal maternal behaviour at parturition can range from simple disinterest in the lamb to outright aggression and abandonment (Sharafeldin and Kandeel 1971). Disinterest in the lamb is characterised by a failure to groom the lamb properly after birth. These ewes show slower licking than normal mothers and also retreat from standing lambs as if afraid (Sharafeldin and Kandeel 1971). Some ewes show circling behaviour when their lamb attempts to suck, turning around to keep the lamb at their head and so preventing it from reaching the udder. This is thought to be unintentional and caused by the ewe trying to keep her lamb in view (Sharafeldin and Kandeel 1971). Aggression towards the neonate generally occurs when the lamb approaches the udder. It is usually short-lived and soon replaced by normal care giving behaviour, as is reluctance by the ewe to stand still during initial sucking attempts (Alexander 1988). Ewes showing very poor maternal behaviour are rare. These animals desert the lamb immediately after birth and butt it away whenever it attempts to approach (Sharafeldin and Kandeel 1971).

1.3.1. Potential causes of abnormal behaviour.

Factors thought to increase the likelihood of abnormal behaviour include inexperience, dystocia, birth of large litters, poor weather conditions and under-nutrition (reviewed by Alexander 1988). The tendency to perform abnormal behaviour can also be influenced by breed. Suffolk ewes have been shown to perform less neonatal grooming and to have a higher likelihood of behaving aggressively towards their newborn lambs, compared to Scottish Blackface ewes (Dwyer and Lawrence 1998). The two breeds also differ in their reaction toward their lambs' attempts to find the udder and suck. Compared to Blackface ewes, Suffolk ewes show less co-operation with their lambs' initial sucking attempts, performing a higher rate of backing away and walking forward over the top of their lambs as they contact the udder and attempt to suck. In addition, Suffolk ewes are more likely than Blackface ewes to reject at least one lamb and to show negative behaviour such as butting the lamb and withdrawing from it. They also continue to show such negative behaviour for longer than Blackface ewes (Dwyer and Lawrence 1998, Dwyer and Lawrence 1999a).

It is not known how, or if, these breed differences in maternal behaviour influence the development of the ewe-lamb bond. As discussed above, artificial manipulation of ewe-lamb interactions has shown that contact between the ewe and newborn lamb is important for the development of lamb recognition by the ewe (e.g. Alexander et al. 1986), and the behaviour of ewes during initial suck attempts has a strong influence on the development of lamb attachment to its dam (e.g. Nowak et al. 1997). These differences in ewe behaviour may therefore have long-term consequences for the ewe-lamb relationship in the two breeds. This is one area that will be explored further in the current study.

1.4. Regulation of maternal care.

1.4.1. Hormonal control of maternal behaviour.

The onset of maternal behaviour in the ewe is closely associated with parturition and ewes are not attracted to lambs at other times. Ewes are responsive to lambs for a few hours only after birth and this sensitive period fades if a lamb is not present (Poindron and Le Neindre 1980). The onset of maternal care appears to be largely under hormonal control (Poindron and Le Neindre 1980, Levy et al. 1996) with stimulation of the vagina and cervix also being important in facilitating onset (Keverne et al. 1983). Changes in the ovarian steroid hormone balance, together with stimulation of the genital tract, trigger a cascade of neurobiological mechanisms which in turn triggers maternal responsiveness and selectivity. Central release of oxytocin, mainly in the paraventricular nucleus of the hypothalamus, induces an attraction towards the odour of amniotic fluid, facilitating mother-young contact and the immediate onset of maternal care (Levy et al. 1996, Kendrick et al. 1997). Steroid hormones and vaginocervical stimulation are also responsible for neurochemical and electrophysiological changes within the olfactory bulb that are part of the learning mechanisms of individual lamb odour. In particular, the release of noradrenaline, enhanced by oxytocin, appears to be essential for lamb recognition by the ewe (Levy et al. 1996, Kendrick et al. 1997).

Poor maternal behaviour occurs mainly in primiparous ewes (Poindron and Le Neindre 1980, Dwyer and Lawrence 2000b) and inexperienced ewes need more cues to develop maternal behaviour, compared to multiparous ewes (Poindron and Le Neindre 1980). Hormonal facilitation of maternal care is more efficient in experienced ewes (Poindron and Le Neindre 1980) and it is possible that maternal experience increases the sensitivity or number of oxytocin receptors in the brain responding to the feedback action of the sex steroids released at parturition (Kendrick et al. 1997).

In multiparous ewes oestradiol levels are related to the performance of neonatal grooming and low-pitched vocalisations but not to other maternal behaviours such as co-operation with initial suck attempts (Dwyer et al. 1999). The hormonal state of the ewe does not therefore appear to have a large influence on all of the maternal behaviour expressed during the immediate post-partum period. The ewe's response to her lamb's suck attempts has been shown to be strongly affected by previous experience (O'Connor et al. 1992), suggesting a learned component to this particular maternal behaviour (Dwyer et al. 1999). Indeed, the response of ewes to suck attempts within a single parturition improves over time, also suggesting learning. Most ewes which initially fail to stand still when their lamb attempts to find the udder will eventually co-operate and allow their lamb to suck (Alexander 1988, Lynch et al. 1992).

1.4.2. Influence of the lamb on maternal behaviour.

Immediately after parturition the expression of maternal care is unaffected by the behaviour of the lamb (Dwyer and Lawrence 1999a). However, over time, warmth and movement of the lamb appear to become important stimuli for maternal behaviour (Shillito and Hoyland 1971, Vince et al. 1985, Lynch et al. 1992, Garcia Gonzalez and Goddard 1998). Ewes will initially direct equal amounts of grooming towards live and still born lambs but this begins to wane when the ewe does not receive appropriate feedback from the lamb in the form of sucking behaviour (Vince et al. 1985, Dwyer and Lawrence 1999a). The stimuli provided by the lamb rapidly begin to influence subsequent maternal behaviour and beyond the immediate post-partum period maternal behaviour is regulated by sensory information from the lamb, the endocrine state of the ewe being of little importance (Poindron and Le Neindre 1980). Olfactory cues from the lamb are essential to sustain and regulate the mother-young relationship and the ability of the ewe to look after the lamb throughout the lactation period (Poindron and Le Neindre 1980).

1.5. Long-term associations between ewes and lambs and maternal care during later lactation.

Lent (1974) described two types of mother-infant relationship in ungulates during the first few weeks of life: 'Hiders' and 'Followers'. Hiders show extended periods of mother-young separation and the young remain immobile and hidden during these separations. In contrast, Followers maintain a close contact between mother and young and there is frequent communication between the animals. Sheep fall into the second category with ewes having a close spatial relationship with their young and frequent interactions with them from birth.

1.5.1. Following behaviour.

During the first few days post-partum the lamb stays very close to the ewe and shows following behaviour: as the ewe walks with her head down, her lamb runs along side, level with her head and neck (Shillito and Hoyland 1971). Initially, during the first day, the follow response is weak and requires appropriate stimulus from the ewe in the form of frequent naso-nasal contact, pacing and maintaining an optimum speed (Winfield and Kilgour 1976). As their lambs become older ewes attempt to elicit following behaviour by moving a short distance away and then running back to the lamb if it does not approach. By 3 days of age most lambs will follow their dam when disturbed (Morgan 1970, cited in Morgan and Arnold 1974). With increasing lamb age the ewe becomes more reluctant to leave the flock and return to the lamb, and after the first week it becomes the responsibility of the lamb to move to the ewe, rather than vice versa (Shillito-Walser et al. 1983).

The follow response is generalised at first and lambs will follow any ewe during the first few days post-partum. After 10 days of age the response becomes more specific to the dam (Winfield and Kilgour 1976) but a generalised response persists in a stress situation (Lent 1974). Following behaviour in young lambs is facilitated by good mutual recognition with the dam (Oppong-Anane et al. 1989).

1.5.2. Sucking interactions during later lactation.

Over the first few weeks post-partum the ewe produces more milk than her lamb can drink and the lamb's appetite determines how much is consumed (Trivers 1985), the ewe allowing free access to the udder (Ewbank 1967). Twin-bearing ewes will initially allow one twin to suck without the other being present but after the first few weeks of lactation the ewe will only allow sucking if both lambs are present (Ewbank 1967). At approximately 4 weeks post-partum ewes begin to produce less milk and to restrict access to the udder (Trivers 1985). The timing of this change is dependent on environmental conditions, with poorer conditions leading to earlier restriction (Trivers 1985, Festa-Bianchet 1988, Rachlow and Bowyer 1994).

1.5.2.1. Control of sucking interactions.

Descriptions of how the ewe controls her lamb's sucking attempts once she has begun to restrict access to the udder are very vague. Ewbank (1967) and Geist (1971) mention that the ewe appears to signal her readiness to suckle by calling her lamb and Lawrence (1984) observed that lambs become conditioned to the ewe raising her head (head-up posture) as a cue for suckling. O'Connor (1990) also found a relationship between suckling and ewe vocalisation and performance of the head-up posture. This is an area that can still benefit from further investigation, especially as the head-up posture has only been studied in one breed of ewe, the Scottish Blackface. The communication between ewes and their lambs during later lactation, particulariy in relation to sucking interactions, will be further investigated as part of the present study.

1.5.2.2. Duration of sucking bouts.

The duration of an individual sucking bout decreases with increasing lamb age (Ewbank 1967, Festa-Bianchet 1988) and lambs graze more and suck less as they become older and less dependent on the ewe for nutrition (Morgan and Arnold 1974). In a study of the nursing behaviour of Clun Forest sheep Ewbank (1967) found that weight gain in lambs was related to sucking frequency during the first 3 weeks of life but not in the subsequent 3 weeks. This further suggests that the lambs are most reliant on the ewe for nutrition during the first few weeks post-partum.

1.5.2.3. The role of suckling in maintaining the ewe-lamb bond.

Suckling is thought to satisfy emotional as well as nutritional needs in young animals (Cameron 1998). Shackleton and Haywood (1985) identified two types of suckle, long and short, in lambs less than 2 weeks old. Long suckles occurred after a ewe had been lying and were considered to have a major nutritional function. Short suckles were observed when the ewe was active and were thought to act to reinforce the ewe-lamb bond without supplying much nutrition. Ewes often feed their lambs after a disturbance or a period of separation and searching. It is thought that this may act as a comfort behaviour, the close contact between the ewe and lamb providing familiar sensory stimuli and reinforcing their attachment with each other (Shillito and Hoyland 1971, Arnold et al. 1979).

1.5.2.4. Weaning.

In most mammals weaning is a gradual process, involving the progressive decrease in the provision of milk by the dam, accompanied by increasing intake of solid food and profound behavioural changes in the mother-young relationship (Martin 1984). Weaning in the sheep occurs between 3 and 6 months of age. It appears to be controlled by the ewe and is achieved by the rejection of sucking attempts. Weaning happens gradually in some breeds and quite suddenly in others, although all lambs will have been receiving progressively less milk (Arnold et al. 1979). Lambs continue to make sucking attempts after weaning is complete (Arnold et al. 1979) but by 5 months of age they show decreasing interest in the behaviour of their dams (Arnold et al. 1979, Arnold and Pahl 1974).

1.5.3. Changes in the spatial relationship between the ewe and her lamb.

Lambs stay close to their dam at all times during the first week post-partum (Arnold and Grassia 1985) and the ewe is the lamb's most frequent nearest neighbour (Hinch et al. 1987). This tendency for the ewe and lamb to be nearest neighbours persists in later lactation (Dwyer and Lawrence 1997). Ewes and lambs continue to remain in close contact during the first month of life (Morgan and Arnold 1974) and ewes actively seek their lambs when separated (Arnold and Grassia 1985, Hinch et al. 1987). Thereafter they increasingly stop prior to reaching their lamb, bleat loudly and return to the flock (Hinch et al. 1987, Hinch et al. 1990). The distance the dam is willing to move toward the lamb declines gradually, reaching a minimum just prior to weaning (Hinch et al. 1987). Lambs also begin to seek their mothers at around 1 month of age. The transition in responsibility for maintaining spatial proximity is likely to be gradual, reflected in the gradual increase in ewe-lamb distance as the lamb becomes older and/or the maternal role becomes less, followed by a decline in distance as the lamb's role increases (Hinch et al. 1987). The time-scale of this change varies in different breeds (Shillito Walser et al. 1981, Hinch et al. 1987).

After 6 weeks of age the lamb is responsible for the majority of approaches and contacts with its dam (O'Connor 1990), but the ewe continues to control the dynamics of their relationship (Lawrence 1984, Dwyer and Lawrence 1997). A head-up posture is used to signal when the lamb may approach its dam (Lawrence 1984).

1.5.4. Behavioural synchrony and associations between ewes and lambs.

Lying and walking tend to be synchronised in ewes and lambs during the first 4 weeks but when the ewe is grazing the lamb can be involved in a variety of activities, including playing in peer groups. Their frequency of play, gambolling alone or in a group, increases over the first 2 weeks and is then gradually replaced by grazing. Play most frequently occurs away from the dam (Morgan and Arnold 1974). Lambs develop social relationships with peers whilst still largely dependent on milk for nutrition and gradually begin to associate with other lambs whilst the ewe is grazing. At 1 month post-partum peer groups are an important feature in the lamb's life but this importance decreases over the following months as they resume close contact with their dam, grazing by her side (Arnold and Grassia 1985).

1.5.5. Changes in the ewe-lamb bond during later lactation.

The nature of the social bond between the ewe and her lamb changes with increasing lamb age (Arnold and Grassia 1985), as the lamb becomes less dependent on the ewe for protection and nutrition. The lamb's decreasing dependence is shown by an increased ewe-lamb distance, increased association with peers, decreased sucking and increased grazing by the lamb. However, the ewe-lamb association continues to be the primary social relationship for both the ewe and her lamb (Morgan and Arnold 1974).

Initially the ewe exhibits a very strong interest in her lamb which then decreases as the maintenance of the bond gradually becomes the lamb's responsibility (Arnold et al. 1979, Arnold and Grassia 1985). Ewes also show greatest maternal protection in response to disturbance during the first week post-partum (Morgan and Arnold 1974). Despite independent activities over the first 4 weeks it is apparent that ewes and their lambs maintain a close contact (Morgan and Arnold 1974), and although lambs spend much time with their peers their behaviour always conforms with that of the adult ewes (Shillito and Hoyland 1971). During later lactation maternal care involves not only suckling the lamb and staying close to ensure the lamb does not become lost, but also ensuring that if the flock moves the lamb can maintain its spatial relationship with the dam (Morgan and Arnold 1974).

1.5.6. Ewe-lamb associations after weaning.

There is conflicting evidence of the persistence of ewe-lamb associations after weaning is complete. Hinch et al. (1990) found associations between Merino ewes and their offspring 2 years after birth, although these associations weakened over time, especially after the birth of the next offspring. However Lawrence and Wood-Gush 1988) and Geist (1971) found no evidence of associations at this time. This may be due to the different experimental set-ups in the various studies. In Hinch et al.'s study (1990) the animals were maintained in small paddocks, whereas in the other studies the animals were observed on the open hill. The lack of potential for dispersal in Hinch et al.'s study (1990) will have resulted in more contact between the ewe and her offspring. This is likely to have increased their propensity to associate with each other, compared to the free-ranging animals in the studies by Lawrence and Wood-Gush (1988) and Geist (1971), and may account for the different conclusions.

1.6. The role of maternal care in lamb survival.

Mammalian young cannot survive in the absence of maternal care, or a substitute that provides sustenance and protection (Alexander 1988). Poor maternal care in the ewe has been linked to lamb death (Arnold and Morgan 1975, Bareham 1976) and most lamb deaths occur during the first few days post-partum (Nowak and Lindsay 1992). There are a number of reasons for maternally related neonatal death, the most obvious being desertion by the dam (Arnold and Morgan 1975). However, this is rare and other deficiencies in maternal care can reduce the chances of lamb survival and may also ultimately result in the death of young. In a review of maternal care in ungulates Alexander (1988) compiled a list of ewe behaviours that increased the chances of their young surviving. These included: seeking isolation for birth, selection of a safe sheltered birth site, absence of interference with, or by, other parturient ewes, intense persistent grooming of all members of the litter, absence of aggression, co-operation with the first suck attempts, remaining at the birth site for at least 5 hours, concern at the absence of the lamb, ability to keep the litter together after leaving the birth site and active defence of the young.

The majority of ewe behaviours listed by Alexander (1988) are concerned with the establishment of the ewe-lamb bond, and a strong association between the ewe and her young could therefore be considered to be the most important factor influencing lamb survival. The development of mutual recognition between the ewe and her lambs very soon after birth is a prerequisite for the formation of a stable relationship and is crucial for the survival of the young (Nowak 1996). The influence of maternal behaviour, in particular grooming and co-operation with sucking attempts, on the development of mutual recognition between the ewe and her lamb has been discussed in previous sections.

1.6.1. Isolation during parturition.

Pre-parturient ewes can show interest in the newborn young of other ewes and may even steal them away from the dam, disturbing the bonding process (Lynch et al. 1992). Seeking isolation from the flock at birth reduces the possibility of other ewes interfering with the lamb and subsequently disrupting the development of mutual recognition (Lynch et al. 1992). In Soay sheep, lambs whose dam remained separate from the flock for 2 days responded more specifically to their dams than to other ewes (Shillito and Hoyland 1971). In contrast lambs which had not been kept away from the flock did not orientate so definitely towards their mother.

1.6.2. Sheltered birth site.

Climate is a major factor in lamb mortality (Arnold and Morgan 1975) and Alexander (1988) suggested that selection of a sheltered birth site is likely to improve survival chances. However, maternal behaviour in the ewe does not appear to be directed towards the provision of shelter for the newborn lamb (Alexander and

Williams 1966b) and shelter seeking before birth has only been observed in recently shorn ewes (Lynch et al. 1992).

1.6.3. Time spent at the birth site.

The time spent at the birth site is also an important factor in the establishment of maternal behaviour, influencing the strength of bonding between a ewe and her lambs and her awareness of the size of her litter (reviewed by Alexander 1988). A ewe remaining there for at least 6 hours is more likely to show excellent maternal care and lamb survival chances are improved (Nowak 1996). Time spent at the birth site varies between breeds, ranging from several days in Soay ewes to 4 hours or less in Merino ewes (Lynch et al. 1992).

1.6.4. Co-operation with initial suck attempts.

The neonate is solely dependent on the ewe for nutrition (Alexander and Williams 1966a). In extreme cases a lack of co-operation with initial suck attempts can do more than disrupt the bonding process and may directly result in the lamb's death through insufficient intake of colostrum and starvation (Nowak and Lindsay 1992). Even temporary failure to co-operate with initial suck attempts can decrease chances of survival as lambs which do not receive successful outcomes to early suck attempts show a decline in teat seeking, decreasing their chances of ever sucking successfully (Alexander and Williams 1966a).

1.7. Other factors influenced by maternal behaviour.

From the above discussion it can be seen how maternal behaviour in the ewe influences the behaviour of her young and its chances of survival. Maternal behaviour is also known to influence the development of offspring, although this has not been widely studied in sheep. Extensive studies in the rat have shown that maternal care can influence the young's stress response in later life (e.g. Liu et al. 1997). Rats receiving high levels of positive maternal traits, such as licking and grooming and arched back nursing (LG-ABN), subsequently show a reduced stress response, compared to animals receiving low levels of LG-ABN. These maternal behaviours have also been found to influence learning ability (e.g. Anisman et al.

1998) and the young's own maternal behaviour (Francis et al. 1999b) in the rat. The maternal behaviour of primates is influenced by the maternal style of the dam, as is the young's personality (reviewed by Fairbanks 1996).

There are also examples of differences in maternal care influencing the development of farm animals. Brahman calves which have been subject to restricted nursing and access to their dam have a greater plasma cortisol concentration and heart rate in response to restraint, compared to calves which remained with their dam throughout lactation (Lay et al. 1998). In addition, pullets which have been brooded by a maternal hen are less neophobic, more likely to seek the proximity of a conspecific and interact more agonistically with unfamiliar individuals, compared to unbrooded pullets (Perre et al. 2002). The influence of maternal behaviour on offspring development is discussed more extensively in Chapter 7.

1.8. Aims of the current study.

1.8.1. Maternal behaviour in later lactation.

From the above review it can be seen that although the study of maternal behaviour in the ewe has been extensive, there are still areas which could benefit from further investigation. The initial post-partum period has been studied in great detail, in particular the establishment of the ewe-lamb bond and development of mutual recognition between ewes and their lambs. However the later lactation period has received less attention and information about the behaviour of ewes and the persistence of the ewe-lamb bond at this time is limited. It is known that ewes and lambs retain a strong association (e.g. Morgan and Arnold 1974) but this is generally described via their spatial relationship, rather than the behavioural interactions between them. More detailed study of the relationship between ewes and their lambs during later lactation would help complete the picture of maternal behaviour in the ewe, providing further information on how the ewe-lamb bond is manifest during later lactation and how this changes over time.

1.8.2. The influence of ewe behaviour on the development and behaviour of her lamb.

Related to this is a lack of information on the influence of ewe behaviour on older lamb behaviour, both in the immediate and long term. The behaviour of the ewe during the post-partum period is known to affect the behaviour of her young, for example the development of dam recognition by the lamb (Nowak 1996) and its motivation to seek proximity to her (Nowak et al. 1997). As the relationship develops the ewe's behaviour continues to have potential to influence her lamb's behaviour. The behaviour of the ewe during sucking interactions would be expected to be especially important as this is the main interaction between the animals during later lactation and suckling is known to reinforce the ewe-lamb bond at this time (Shillito and Hoyland 1971, Shackleton and Haywood 1985). As discussed above, studies in other animals, for example primates (e.g. Fairbanks 1996), have shown that the style of maternal care received in infancy has an effect on the behavioural development of individuals, influencing their behaviour after weaning. The behaviour of the ewe therefore also has the potential to influence her lamb's behaviour in the long term.

1.8.3. Genetic differences in the expression of maternal behaviour.

An interesting, and relatively recent, area of research is the study of genetic differences in the expression of maternal behaviour. Suffolk ewes have been shown to be more likely to behave negatively toward their newborn lambs, to show less grooming and to make fewer low pitched vocalisations than Blackface ewes (Dwyer and Lawrence 1998, Dwyer et al. 1998). It is not known if these breed differences in maternal behaviour are confined to the immediate post-partum period or persist during later lactation. Additionally, the full implications of these behavioural differences for the growing lambs are not known. The survival rate of lambs born to Suffolk dams has been found to be significantly lower than lambs born to Blackface dams (Dwyer and Lawrence 1998). However, the influence of differences in maternal behaviour on other factors, such as the strength of the ewe-lamb bond and the development of lamb recognition of the dam, can only be inferred and requires further study.

1.8.4. Outline of the study.

To address these questions the current study will focus on three related areas: general maternal behaviour during later lactation, breed differences in maternal behaviour and the implications of the nature of maternal behaviour and the ewe-lamb relationship for lamb development throughout lactation and in later life. The aim is to address all three areas using observations of the maternal behaviour of Suffolk and Blackface ewes between birth and weaning, followed by continued observation of their growing offspring after weaning. These observations will provide information about general maternal behaviour during later lactation and also determine whether or not breed differences in maternal behaviour persist throughout lactation. Differences in maternal behaviour in the two breeds can then be used to look at the consequences of maternal behaviour and the nature of the ewe-lamb relationship for the growing lamb.

1.8.5. Application to other domestic animals.

The sheep is not the only domestic animal where the dam has the potential to be a major influence on her offspring's behaviour and development. Like sheep, goats, (beef) cattle and horses also produce small litters and their young are weaned relatively late. The results of this study may therefore be applicable to other farm species. Goats and cattle are Hider species' (Lent 1974), resulting in their mother-young relationship differing considerably from that of sheep. However the horse is also a Follower species and shows similarities with the sheep in its maternal behaviour. The results of this study therefore have the potential for application to equine behaviour. A pilot study looking at the maternal behaviour of horses and its potential for influencing offspring behaviour is included in the present study.

Chapter 2: General materials and methods.

Figure 2.1 shows a timeline of the observations and tests conducted throughout the 3 year study period. Details of the animals and general husbandry methods are given below. The detailed methods for each set of observations or tests are described in the relevant chapters.

2.1. Animals.

2.1.1. Main flock.

A core flock of 50 primiparous ewes, 25 Scottish Blackface and 25 Suffolk, was established in 1999. The ewes were acquired at approximately 1 year old and managed as a single flock thereafter. All Suffolk ewes were bought in from a Terminal Sire Breeder based in the Borders. Ten Blackface ewes were bought in from a hill farm in Orchill Moor, the other 15 came from SAC's Castlelaw flock (also hill-based). The animals were selected for genetic diversity by using a range of sires (6 in the Suffolk ewes, 10+ in the Blackface ewes: sire information was unavailable for the Orchill Moor ewes). This flock was used in the study of maternal behaviour throughout lactation, reported in Chapters 3, 4, 5 and 6.

2.1.2. Study animals used in the observation of maternal behaviour.

The ewes in the main flock were synchronised in oestrus using progesterone sponges (Veramix, Upjohn, Crawley, UK) and naturally mated to 1 of 4 rams (2 Suffolk and 2 Blackface) to give pure bred lambs and reciprocal crosses. The inclusion of cross bred lambs controlled for the potentially confounding effect of lamb behaviour during observations on ewe behaviour. Eighteen Suffolk and 14 Blackface ewes gave birth to 51 lambs. Litter sizes were unbalanced within the 2 groups with 4 Suffolk and 9 Blackface ewes giving birth to single lambs and 12 Suffolk and 5 Blackface ewes giving birth to twins. Two Suffolk ewes gave birth to triplets but 1 lamb was removed from each of these ewes within 24 hours of birth. The behaviour of these ewes was not found to differ from twin-bearing ewes and they were treated as twin-bearing ewes for the rest of the study. They were not included in the observations of initial maternal care (Chapter 3) as all 3 lambs were still present with the ewe for the

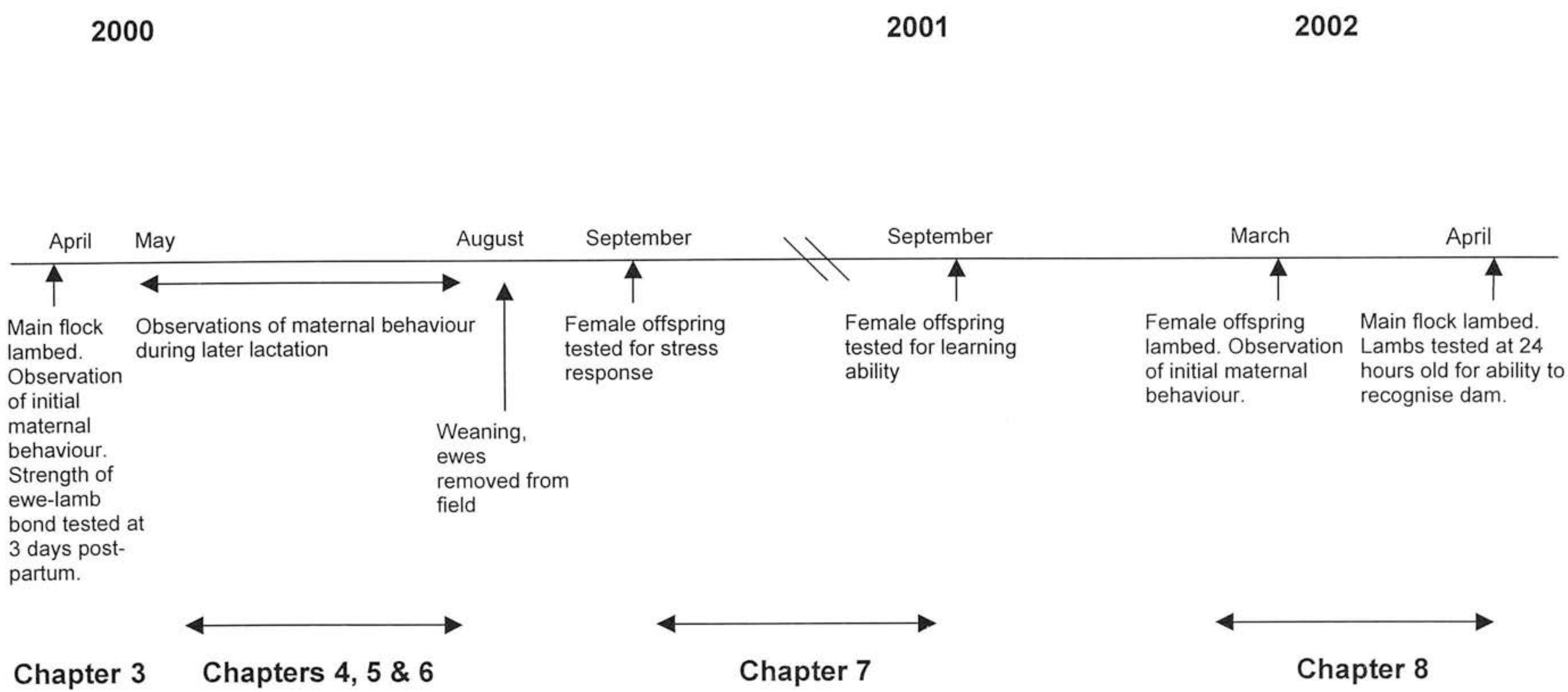

Figure 2.1. A timeline of the observations and tests conducted during the 3 year study period.

majority of these observations. Table 2.1 shows the numbers of each type of lamb included in the study.

Table 2.1.
The number of lambs of each type born during the 2000 lambing.

	Pure bred lambs				Cross bred lambs			
	Male		Female		Male		Female	
Dam breed	Single	Twin	Single	Twin	Single	Twin	Single	Twin
Blackface	4	1	2	3	3	1	0	5
Suffolk	1	5	1	9	1	7	1	7

2.1.3. Study animals used in the investigation of the influence of maternal care on offspring development.

After weaning male offspring were sold and female offspring were retained for further study. These animals were the study subjects in Chapters 7 and 8, investigating the influence of maternal care on offspring development. Respective numbers are given in the relevant chapters.

2.2. Housing and husbandry procedures.

2.2.1. Lambing and the first 3 days post-partum.

Ewes were lambed indoors in a standard sheep shed (30x20m) at Woodhouse Lea farm, Easter Howgate. The shed had natural ventilation via Yorkshire boarding and natural daylight. This was supplemented as necessary using overhead electric lighting.

Ewes were brought indoors in week 8 of gestation and given ad libitum access to hay and water. They were scanned for litter size at approximately 80 days gestation and then fed to maintain body condition and support foetal growth. Ewes were vaccinated with a clostridial vaccine (Covexi-8, Mallinckrodt, Middlesex, UK) in week 17. Ewes were maintained and lambed in groups, in large straw-bedded pens (approx. 7mx7m). Groups were chosen according to breed and litter size to ensure correct feeding. One week before lambing was due to start ewes were marked with an identification number on both sides, using sheep marker spray.

The ewes lambed over a 17 day period in April 2000. Ewes lambed in their group pens. A handler was present in the lambing shed 24 hours per day to make observations and aid the ewes where necessary. Lambing assistance and interference between ewes and their lambs was kept to a minimum and only given when necessary for the survival of the animal (after Dwyer and Lawrence (1998)). Lambing assistance was given to a ewe 1 hour after the appearance of fluids, with no parts of the lamb and/or 2 hours after parts of the lamb were seen at the vulva with no obvious progress being made. At approximately 30 minutes post-partum all lambs were caught, oral antibiotics given (to prevent watery mouth) and their navels dipped in iodine (to prevent infection). The lambs of multiple litters were marked by birth order using a tape loop around the right hind leg of the first born. In the triplet litters the second born triplet was also marked with a tape loop around the left hind leg. This allowed identification of individual animals, enabling their progress to be accurately followed throughout the study.

After the post-partum observations (Chapter 3), the ewe and her lamb(s) were moved from their group pen into a pen for lambed ewes. This helped prevent mis-mothering and interference by pre-parturient ewes during the early post-partum period. There were 4 'lambed' pens, one for each of the group pens. They were the same size as the group pens (approx. 7mx7m) and also straw bedded. Once in the 'lambed' pen the ewe and lambs were checked to ensure that the lambs had sucked. The ewes' teats were examined for removal of the wax plug and the lambs' abdomens were checked to make sure their stomachs were full. Any lambs that had not sucked or did not have a full stomach were helped to suck. Ewes that were not showing full co-operation with sucking attempts were housed in small individual pens (1.5mx1.5m) within the lambed pen. This aided assistance with sucking attempts. Ewes were released back into the lambed pen as soon as the lambs were seen to be sucking successfully with full co-operation from the ewe. This was usually less than 12 hours after parturition but in some cases took more than 24 hours. Five Suffolk ewes were penned for over 24 hours.

In extreme cases of lamb rejection and aggressive behaviour by the ewe, the animals were housed in a small pen and the ewe contained in a foster crate. This allowed the lambs access to the udder but prevented the ewe from butting her lambs. The ewe was kept in the foster crate for 24 hours and then released. If she still showed aggression towards her lamb(s) the foster crate was replaced for a further 24 hours. Only 1 twin-bearing Suffolk ewe had to be restrained in a foster crate, she accepted her lambs after 48 hours.

All lambs were weighed, tail docked and ear tagged at approximately 24 hours post-partum, male lambs were left entire. The lambs were also marked with their ewe's identification number, and birth order in twins, using sheep marker spray. The number was sprayed on both sides of the lamb. First born twins were marked with a stripe across the shoulders, second born twins with a stripe across the rump.

At approximately 3 days post-partum, dependent on the weather and health of the animals, ewes and their lambs were moved outside. The identification numbers on the Suffolk ewes were checked and re-sprayed as necessary. Blackface ewes were re-marked using a unique combination of coloured tape on both horns. This was due to the length of their fleece making sprayed numbers illegible in outdoor conditions (wind and rain).

2.2.2. Later lactation.

Observations on maternal behaviour after the immediate post-partum period took place in a 6.4 ha field. The field was located at the foot of the Pentland Hills, behind Glencorse Barracks, approximately 1km from Milton Bridge and 1.5km from the Bush Estate. Ewes received supplementary feed for 4 weeks. Pelleted feed (350g/ewe) was given to the ewes each morning. Ewes were checked twice daily by the shepherd and all ewes and lambs were gathered once every 4 weeks to be weighed. Animals seen to be limping or showing signs of illness were caught when seen and treated appropriately. Animals received worming treatment every 4 weeks and ewes were shorn at approximately 8 weeks post-partum. The ewes were removed from the field to be shorn but the lambs were left in the field. Shearing took approximately 3 hours and the ewes and lambs were separated for the minimum period possible.

2.2.3. Weaning.

Lambs were weaned when the youngest lambs were 12 weeks old (average age=15 weeks). The ewes were removed from the field and the lambs left as a group. The male lambs were separated from the female lambs after approximately 1 month and sold at market. The female lambs remained in the field until they were brought inside for the first set of post-weaning tests at approximately 5 months old. These took place in the same shed as that used for lambing.

2.3. Post-weaning maintenance and observations.

The ewes from the main flock were maintained separately from their female offspring throughout the following 2 years. The main flock was used in other studies and extra primiparous ewes were added in 2001. Lambs from the 2002 lambing of the main flock were used to test the ability of lambs of the two breeds to recognise their dam at 24 hours old. This is described in Chapter 8.

The stress response of the female offspring was tested at 5 months post-partum and their learning ability at 16 months post-partum (Chapter 7). Their own maternal behaviour was studied in 2002 (Chapter 8). The ewes were synchronised in oestrus using progesterone sponges (Veramix, Upjohn, Crawley, UK) and mated to a single Blackface ram using artificial insemination. As the aim of this part of the study was examination of the ewes' maternal behaviour, a single sire was used to produce lambs that were as genetically similar to one another as possible. The ewes lambed over a 10 day period in March 2002, the procedures and husbandry methods were as described for the main flock.

2.4. Statistical analysis.

2.4.1. Comparisons of groups.

Most of the analysis in this study involved the comparison of groups, for example Suffolk and Blackface ewes. These comparisons were not simple as the groups differed in more than one factor, e.g. their breed, the size of their litters and the genotype of their lambs. Some of these factors were also unbalanced between the groups, for example 5 Blackface ewes and 14 Suffolk ewes had twin litters, 9 Blackface and 4 Suffolk ewes had single lambs. It was therefore necessary to use a multifactorial analysis method that was able to cope with unbalanced data sets.

Multifactorial analysis partitions the variance within the data in such a way that the variance caused by the different factors can be identified. A test statistic is calculated for each of the factors which can then be compared with a table value to assess its significance. A significant test statistic indicates that the factor makes a significant contribution to variation within the data. Analysis of Variance (ANOVA) is one of the most widely used multifactorial analysis techniques. However it cannot cope with unbalanced data sets. The Residual Maximum Likelihood procedure (REML, Patterson and Thompson (1971)) is similar to ANOVA but can cope with unbalanced data. The REML procedure was therefore used to analyse the data in this study.

During REML analysis factors are entered into a fixed model. The sequence of the factors in the model is important as the test statistic (Wald) for each factor is calculated ignoring the terms fitted later in the model. Accordingly the factor of most interest is fitted last in the model. A Wald statistic is generated for each factor, along with the associated degrees of freedom. The distribution of the Wald statistic approximates to that of a χ^2 and this table is used to assess its significance.

In order for the REML procedure to be used data have to be normally distributed. Where data were skewed transformation was attempted using either square root, or log (base 10) transformations. In the majority of cases this was successful but it was not possible to transform some of the data. In these cases non-parametric tests were used and the different factors examined individually. It was not possible to fully incorporate all the factors in these tests but they did give a measure of individual effects and the possible confounding factors.

2.4.2. Exploration of data sets.

In several instances during the investigation of maternal behaviour and its influence on offspring development it was useful to examine relationships between several different behaviours. Principle Components Analysis (PCA) and factor analysis are two exploratory techniques which can be used to describe a set of variables in a reduced number of dimensions. Factor analysis was used to analyse the data in this study.

Factor analysis is based on the idea that measured values are a function of a systematic component (influence of the factor) plus a random component. Its goal is to identify the systematic components and to give simple names to them. By using factor analysis to explore some of the data sets in this study it was possible to examine unobserved variables, for example the maternal motivation of the ewes or the reactivity of their offspring in putatively stressful situations.

Before running a factor analysis a PCA is run on the data, to generate a set of provisional factors from which to start the factor analysis. During a PCA eigenvalues are generated for each principle component. An eigenvalue greater than 1 indicates a substantial contribution to variance within the data. Consequently, the number of components with an eigenvalue greater than 1 is taken as the number of factors to generate in the factor analysis. A factor analysis is then run with the appropriate number of factors and followed by a factor rotation, in this case an orthogonal Varimax rotation. Rotation is used to make the provisional factors more interpretable, by simplifying the patterns of loadings of the original variables onto the extracted factors. The loading of the variable describes its correlation with the factor, a large loading (close to 1) indicates a high correlation and a small loading (close to 0) indicates a low correlation. A Varimax rotation tries to maximise the variance of loadings within a factor, resulting in polarisation of the pattern of original variables on each factor, towards 1 and 0. This aims to remove, as far as possible, loadings in the mid range (e.g. 0.3-0.7) and thus simplify the interpretation of the factor.

The output of the factor analysis gives the loadings for each variable (behaviour) on each of the factors and also gives a score for the individual sets of variables (in this case individual animals) on each factor. The order of the factors is important as the first factor accounts for the largest amount of variation in the data set, the second factor accounts for the second largest amount and so on. The loadings of the variables on each factor are then used to interpret the factor and assign a name to it. The individual scores on each factor are used to identify how the variables for that individual relate to the factor. This can then be used to describe the individual's variables (i.e. the animal's behaviour) in terms of the underlying factor.

Chapter 3: Maternal behaviour during the first three days post-partum.

3.1. Introduction.

3.1.1. Observation of initial maternal behaviour.

The aims of this first experimental chapter are three-fold. Primarily, it is necessary to confirm that the breed differences in early maternal behaviour observed in previous studies (e.g. Dwyer and Lawrence 1998, Dwyer et al. 1998, Dwyer and Lawrence 1999a) are present in the current flock. As discussed in Chapter 1, one aim of the project as a whole is to investigate the development of maternal behaviour in Blackface and Suffolk ewes, and to ascertain whether the differences in maternal care seen at birth persist throughout lactation. It is therefore important that the experimental animals show the same behavioural differences as those seen previously. As the purpose of these observations of early maternal behaviour is only to confirm, rather than establish, behavioural differences between the ewes, they are not as complex as those made in the previous studies.

3.1.2. The role of the ewes' reaction to novelty in breed differences in maternal behaviour.

A second aim of this chapter is to investigate a possible explanation for breed differences in initial maternal behaviour. Whilst affiliative maternal behaviours (such as grooming) are consistently expressed across parities in individual animals, negative behaviours are more frequent during the first parity, i.e. they are more prevalent in primiparous ewes (Dwyer and Lawrence 2000b). Dwyer and Lawrence (1998) proposed that the higher level of negative behaviour in primiparous Suffolk ewes, compared to primiparous Blackface ewes, may not result from a lower motivation to be maternal but from a greater fear of novelty. Under normal husbandry practice ewes are maintained in groups of similarly aged animals and are therefore unlikely to encounter newborn lambs before their own parturition. The presence of the neonate will consequently be a novel situation, which may cause a fear reaction. This could be manifest as avoidance of the lamb (Boissy 1995), reducing the ewe's interaction with it, or active defence (Boissy 1995), resulting in aggression directed towards the lamb. Both of these behaviours are seen more frequently in primiparous Suffolk ewes, compared to primiparous Blackface ewes.

A standard test for fear of novelty is the novel object test (e.g. Romeyer and Bouissou 1992). In this test the animal is exposed to an unfamiliar object and a large number of behavioural elements recorded. These behaviours are then interpreted in terms of the presence or absence of fear. The novel object test does have limitations as the novel object generally only provides visual stimulation and there is also potential for olfactory and auditory stimuli to be aversive. In addition the novel object is inanimate and movement may also contribute to a fear reaction towards novelty. However the test does give a basic indication of the animal's reaction to novelty.

As the ewes' maternal behaviour was recorded from birth onwards it was not possible to test their reaction to novelty during the initial post-partum period, when they would normally first encounter a lamb. Accordingly they were tested as close to lambing as possible, without risking early onset of labour (at 3.5 months into gestation, normal gestation lasts for 5 months). Barren ewes were also tested to examine the effect of pregnancy on reaction to novelty. Pregnancy induces hormonal changes in the ewe and it is possible that these changes may influence the fear response of ewes. The two hypotheses for this test were that Suffolk ewes would show a greater fear reaction towards the novel object than Blackface ewes, and that pregnant and barren ewes would differ in their reaction towards the novel object.

3.1.3. Ability of ewes in the two breeds to recognise their lambs.

The third aim is to examine the consequences of differences in maternal behaviour for the development of maternal recognition of the lamb. As described in Chapter 1, early maternal behaviour is instrumental in the establishment of an exclusive ewe-lamb bond and ewe recognition of her lamb. The differences in early maternal behaviour found in Suffolk and Blackface ewes may therefore affect the ewes' ability to recognise their young lambs.

Maternal recognition of the lamb consists of two distinct systems, olfaction during sucking interactions and at close proximity (less than 0.25m), and visual and auditory cues when the animals are farther apart (Alexander 1977, Alexander and Shillito

1977, Ferreira et al. 2000). An exclusive olfactory memory of the lamb is formed whilst the ewe is grooming her neonate during the post-partum period (Smith et al. 1966, Shillito-Walser 1978b, Poindron and Le Neindre 1980, Alexander et al. 1986, Levy and Poindron 1987). The lower rate of grooming in Suffolk ewes may therefore result in these ewes having less opportunity to learn the olfactory identity of their young, compared to Blackface ewes. In addition, the Suffolk ewes' lower motivation to interact with their lambs, characterised by lower co-operation with sucking interactions and more frequent negative behaviour, may affect their ability to learn the physical characteristics of their lambs. Overall this could lead to the Suffolk ewes being less able than Blackface ewes to recognise their lambs.

The ability of the ewes to recognise their lambs was examined in a maternal choice test at 3 days post-partum, a time when the bond is be expected to be well-established and ewes able to identify their lambs at close proximity and at a distance (Alexander and Shillito 1977). By giving the ewes a choice between their own and a similar alien lamb it was possible to assess their ability to recognise their own lamb. It was hypothesised that, as Blackface ewes show more grooming and less negative behaviour than Suffolk ewes, Blackface ewes would be better able to recognise their lambs compared to Suffolk ewes.

3.2. Methods.

3.2.1. Experiment 1, novel object test.

3.2.1.1. Animals.

Thirty ewes (5 barren Suffolk, 5 barren Blackface, 10 pregnant Suffolk, 10 pregnant Blackface) were tested at 3.5 months into the gestation of the pregnant ewes. Barren ewes were animals from the same group (i.e. same age and previous experience) which had not been successfully mated. The groups of pregnant ewes were balanced as far as possible according to litter size, detected during an ultrasound scan. Blackface ewes had equal numbers (5 in each) of single- and twin-bearing ewes. Four Suffolk ewes were thought to be carrying a single lamb at the time of testing. However one of these ewes subsequently gave birth to twins, giving 3 single-bearing and 7 twin-bearing ewes in the pregnant Suffolk ewe group. Ewes were tested individually but maintained in small groups of like animals (e.g. barren Blackface ewes, twin-bearing Suffolk ewes) throughout the test period. The majority of groups contained 5 animals but the group of Suffolk singles contained 4 animals and the Suffolk twins 6 animals respectively.

3.2.1.2. Test apparatus.

Ewes were tested individually in the apparatus shown in Figure 3.1. Ewes had visual, olfactory and auditory contact with their flock mates during the test to minimise the effects of social isolation. Social isolation is known to induce stress in social animals such as sheep (Romeyer and Bouissou 1992) and a ewe's behaviour in a novel object test differs according to whether or not she has sight of her flock mates (H. Erhard, personal communication). As the aim of the test was to assess the ewes' reaction toward novelty only, it was necessary to remove the potentially confounding factor of social isolation.

The novel object was a 45cm high traffic cone, which was placed in the centre of the 1m area in front of the holding pen. This meant that the ewes had to stand in close proximity to the cone if they wished to be near their flock mates. As social

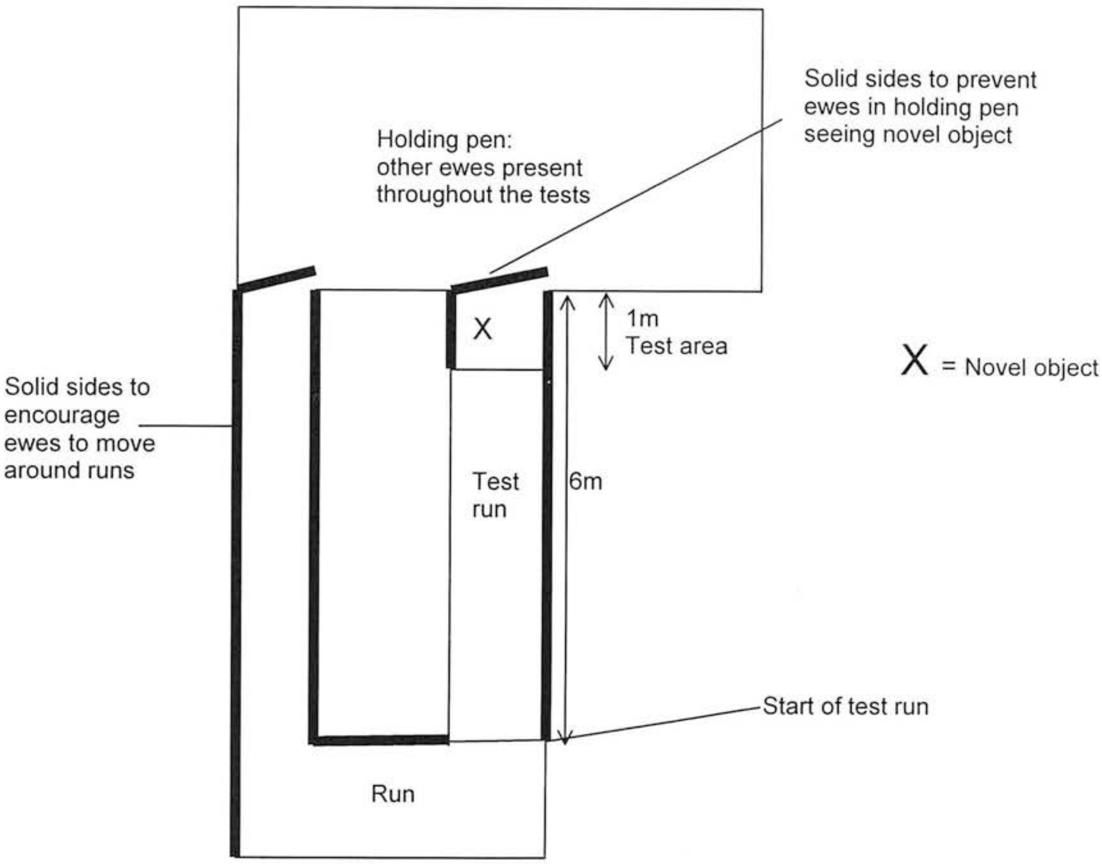

Figure 3.1. Diagram of novel object test apparatus (not to scale).

motivation varies between breeds and individuals, a control test (without the novel object) was also run to provide a measure of the ewes' normal behaviour in the test apparatus. A test area extending for 1m in front of the holding pen was chosen as this is the approximate length of an adult ewe. The ewe was considered to be present in this area if at least two legs and half her body length were inside the area. The area was marked using white tape markers placed on the top of the hurdles forming the run.

3.2.1.3. Training.

Training and testing took place over a 5 day period. Training was given to familiarise the animals with the test apparatus. In the small groups described above, the animals were penned in the apparatus for 20 minutes on 2 consecutive days. During this time all gates were open, giving the ewes access to all areas of the apparatus. The ewes were slowly walked round the apparatus 5 times during the 20 minute period, to ensure they visited each part. On the third day the ewes were trained to walk around the apparatus individually. The group of ewes was penned in the holding pen. An individual ewe was then released into the start of the run and left to walk round to other end. If she did not voluntarily walk round within 30 seconds she was gently walked round by the handler. Once she reached the end of the run the gate was opened, allowing her to return to her flock mates. The ewes were therefore trained to associate walking round the run with access to their social group. This was repeated for each ewe until she voluntarily walked round the run and rejoined her flock mates.

3.2.1.4. Testing.

Testing took place on the fourth and fifth day. On the first day of testing the control test was run, without the novel object. Each ewe was tested for 5 minutes and the following measures taken: latency to reach the test area in front of the holding pen; duration of the test spent in this area; number of times the ewe walked away from this area; number of times the ewe backed away from this area. Walking away and backing could occur before the test area was entered. Table 3.1 gives definitions for the behaviours recorded during the tests.

On the second day the test was repeated with the traffic cone placed in the centre of the test area. After each ewe the traffic cone was washed with water to remove any odours that may have altered its significance for subsequent ewes. In addition to the above measurements, the duration of time spent sniffing the cone and the number of aggressive behaviours (bites, kicks and head butts) directed at the traffic cone were also measured (defined in Table 3.1).

Table 3.1.
Definitions of ewe behaviours recorded during the novel object test.

Behaviour	Definition
Walk away	Ewe turns and walks away from the test area with her head facing away from the holding pen.
Backing	Ewe walks backwards away from the test area with her head still facing towards the holding pen.
Sniffs cone	Ewe contacts cone with end of muzzle, does not use teeth or top of head.
Bites cone	Ewe contacts the top of the cone with her teeth and grips it between them.
Kicks cone	Ewe briefly contacts cone with foot.
Head butts cone	Ewe briefly contacts cone with top of head, causes cone to move.

3.2.1.5. Statistical analysis.

The behaviour of the ewes when the novel object was present was compared with their behaviour in the control test. Their interactions with the traffic cone were also examined. Data were tested for normality and found to be skewed. Non-parametric statistical tests were therefore used for analysis. The ewe breeds were initially compared within their reproductive state group: pregnant or barren. Wilcoxon-signed ranks tests were used to compare the general behaviour of the ewes in the control test with their general behaviour in the test with the novel object. This analysis was made within each breed and allowed assessment of how the general behaviour of each breed was affected by the presence of the novel object. The interaction with the traffic cone was then compared between the two breeds. Mann-Whitney tests were used to compare the duration of time spent sniffing the traffic cone. The frequency of aggressive behaviour towards the cone was low therefore the number of ewes in each breed performing at least one aggressive behaviour toward the cone was compared using Fisher's Exact Probability test.

The effect of pregnancy on the behaviour of the two breeds was examined for behaviours in which the above analysis highlighted potential differences. Mann-Whitney tests were used to compare pregnant and barren ewes within the breeds. Fisher's Exact Probability tests were used to compare the number of ewes performing aggressive behaviours.

3.2.2. Experiment 2, maternal behaviour during the first 3 days post-partum.

The animals and husbandry methods for these observations have been described in Chapter 2.

3.2.2.1. Observations and measures of maternal behaviour.

During the lambing period ewes were kept under 24-hour surveillance. This was backed up by a continuous video record, using 8 cameras linked to a Panasonic 8-channel digital field switcher (WJ-FS20/B, Matsushita Communication Industrial, Japan). Ewes were observed for the first 30 minutes after the birth of a lamb, followed by five 10 minute observations every 30 minutes, finishing at 3 hours post-partum. For twin litters the observation schedule was restarted after the birth of the second lamb. Data on ewe vocalisation were collected live using a Psion Workabout handheld computer (Psion PLC, London, UK) and The Observer data collection software (Noldus et al. 2000). Data on maternal behaviour were collected from videotape, using The Observer software (Noldus et al. 2000). Table 3.2 gives the definitions of ewe and lamb behaviours recorded during the post-partum period.

Table 3.2.
Definitions of behaviours recorded during the first 3 hours post-partum.

Behaviour	Definition
Ewe	
Standing still	Ewe stands immobile without interacting with the lamb.
Grooming	Ewe licks or nibbles her lamb, includes chewing the remnants of foetal membranes during a grooming bout. A grooming bout ends when the ewe stops licking her lamb and is not chewing foetal membranes.
Circling [1.]	As the lamb investigates/contacts the udder or makes a suck attempt (interacts) the ewe walks around the lamb, usually finishing with the lamb at her head.
Backing [1.]	As the lamb interacts with the ewe she walks backwards away from the lamb.
Walking forward [1.]	As the lamb interacts, especially when attempting to reach the udder, the ewe walks forward, away from the lamb.
Butts	Ewe pushes the lamb with downwards or sideways movement of her head.
Retreats [2.]	Ewe walks backward away from the lamb when the lamb is not attempting to interact.
Leaves [2.]	Ewe walks away from her lamb, facing away from it, the lamb is not attempting to interact.
Low pitched Vocalisation	'mmm' bleat, low rumbly bleat made with the mouth closed.
High pitched Vocalisation	'baa' vocalisation, made with the mouth open.
Lamb	
Investigate dam [3.]	Lamb makes contact, with its head, with any part of the ewe's body except the udder region.
Contact udder [3.]	Lamb places its head underneath ewe in the udder region, in any position apart from inverse parallel (standing parallel to ewe but facing in opposite direction).
Suck attempt [3.]	Lamb places its head underneath the ewe in the udder region, in the inverse parallel position.

[1.] The performance by the ewe of any of these three behaviours, circling, backing and walking forward, usually resulted in the lamb losing contact with the dam or being removed from the udder region. These behaviours were considered to indicate that the ewe was not co-operating with the lamb's attempts to find the udder and suck. Co-operation with a suck attempt was indicated by the ewe standing still, grooming the lamb, or grooming the other lamb in twin litters. [2.] The behaviours 'retreat' and 'leave' were considered to indicate a withdrawal from the lamb. [3.] As the newborn lamb rarely goes straight to the udder and makes a suck attempt, three lamb behaviours were combined to represent the lamb's early attempts to find the udder and suck: investigate dam, contact udder and suck attempt.

Data on grooming behaviour, ewe vocalisations and negative behaviour (aggression and withdrawal from the lamb) were collected during the first 30 minute observation period only. Data on co-operation with sucking attempts were collected during all 6 observation periods.

A Maternal Behaviour Score (Dwyer and Lawrence 1998, after O'Connor et al. 1985) was assigned to the ewes during routine husbandry practices at 30 minutes post-partum (lamb's navels dipped in iodine, oral antibiotics administered, twin lambs marked for birth order) and approximately 24 hours post-partum (lambs weighed, tail-docked and ear tagged). The scoring criteria were as follows:

1. Ewe leaves lambs, shows no interest, and does not return when the handler leaves,
2. Ewe leaves lambs, returns when the handler leaves,
3. Ewe retreats to a distance of 5 or more metres, returns at once,
4. Ewe retreats to a distance of 1-5m, returns at once,
5. Ewe remains within 1m of lambs and the handler,
6. Ewe makes contact with lambs during handling.

If the birth interval was greater than 30 minutes in twin-bearing ewes the 30 minute score was assigned after the birth of the first lamb only. Both lambs were treated together during the second scoring at approximately 24 hours post-partum.

Over the first 3 days post-partum scan samples were made of lambed ewes at 2 hourly intervals. The ewe-lamb distance and the behaviours of the ewe and her lambs were recorded. During analysis, only ewe-lamb distance and interactions between the ewe and her lamb(s) were examined (defined in Table 3.3). These behaviours were chosen as they give information on the tendency for ewes to interact with their lambs. The ewe-lamb distance and the tendency to lie within 1m of each other may also indicate the strength of attraction between the ewe and her lambs.

Table 3.3.
Definitions of ewe and lamb behaviours recorded during scans.

Behaviour	Definition
Lies	Ewe or lamb lying down.
Stands	Ewe or lamb stands with all 4 feet on the ground.
Noses lamb	Ewe touches lamb with her muzzle.
Sucking interaction	Lamb has head underneath the ewe in the udder region, ewe is standing or lying still.

In twin litters the ewe could be lying with, or suckling, one or both of her lambs, by definition she could only nose one lamb at a time.

3.2.2.2. Statistical analysis.

In twin litters data were collected for each lamb and then averaged to give a single value for the ewe. Instances of negative behaviour were low therefore the number of ewes performing at least one of the behaviours in each category was recorded within each breed. Data on co-operation with initial sucking attempts were collected during all 6 observations following the birth of the full litter (single lamb or second twin) and then averaged to give a value for the full observation period. Two twin-bearing ewes (1 Blackface and 1 Suffolk) were excluded from this analysis as they had a birth interval of more than 2 hours. During the 2 hourly scans several ewes were omitted from the ewe-lamb distance and lying together data as they were penned for more than 24 hours during the first 3 days post-partum (see Chapter 2). One Suffolk ewe was omitted from all scans as she was contained in a foster crate. Table 3.4 gives the numbers of ewes used in the various analyses during the first 3 days post-partum.

Data were checked for normality and transformed as necessary. Data for latency to begin grooming were log (base 10) transformed and data for co-operation with initial sucking attempts were square root transformed. Data for low pitched vocalisations

Table 3.4.
Number of ewes included in the analyses during the first 3 days post-partum.

Analysis	Suffolk ewes	Blackface ewes
First 30 minutes: grooming behaviour, negative behaviour	16 (4,12)	14 (9,5)
Vocalisations	15 (4,11)	14 (9,5)
Co-operation with suck attempts	15 (4,11)	13 (9,4)
Maternal Behaviour Scores	16 (4,12)	14 (9,5)
3 day scans: ewe-lamb distance, lying together	11 (4,7)	14 (9,5)
Nosing lamb, sucking interactions	15 (4,11)	14 (9,5)

The single number shows the total number of ewes in each breed, the numbers in brackets show the number of single- and twin-bearing ewes.

were normally distributed but data for high pitched vocalisations were skewed and could not be transformed. Data from the 2 hourly scans were normally distributed but sucking interactions were rarely observed during the scans, therefore this behaviour was omitted from subsequent analysis. REML was used to analyse the differences in maternal behaviour between the two breeds. Sire breed, litter size and ewe breed were fitted as fixed effects in the model. Mann-Whitney tests were used to analyse the high pitched vocalisation and Maternal Behaviour Score data. Fisher's Exact Probability tests were used to analyse the performance of negative behaviour during the first 30 minutes post-partum.

3.2.3. Experiment 3, ability of ewes in the two breeds to recognise their lambs.

3.2.3.1 Animals.

At 3 days post-partum 31 ewes were tested for their ability to recognise their own lamb. One twin-bearing Suffolk ewe was not tested as she lambed 9 days after the other ewes. Consequently there were no suitable lambs available to act as alien lambs in her test. During the test the ewes were given a choice between their own and a similar alien lamb. Alien lambs were of the same breed as the own lamb and selected to resemble the ewe's own lamb as closely as possible in terms of age, size, coat colouring and facial markings. Twin-bearing ewes were given a choice between both twin lambs and 2 similar alien lambs, to prevent the ewe searching for the absent twin during the test. This was felt to be more likely to influence the behaviour of the ewe than any extra stimulus provided by 2 lambs, compared to the 1 lamb presented to the single-bearing ewes.

3.2.3.2. Test apparatus.

Figure 3.2 shows a diagram of the test apparatus. The test apparatus was constructed from sheep hurdles in an empty pen at the end of the lambing shed. The floor was covered in straw. Opaque plastic sheeting was used to make the sides of the test pen solid to prevent the ewe becoming distracted by events in the rest of the shed. The ewes had auditory and olfactory contact with the rest of the shed during the test. The

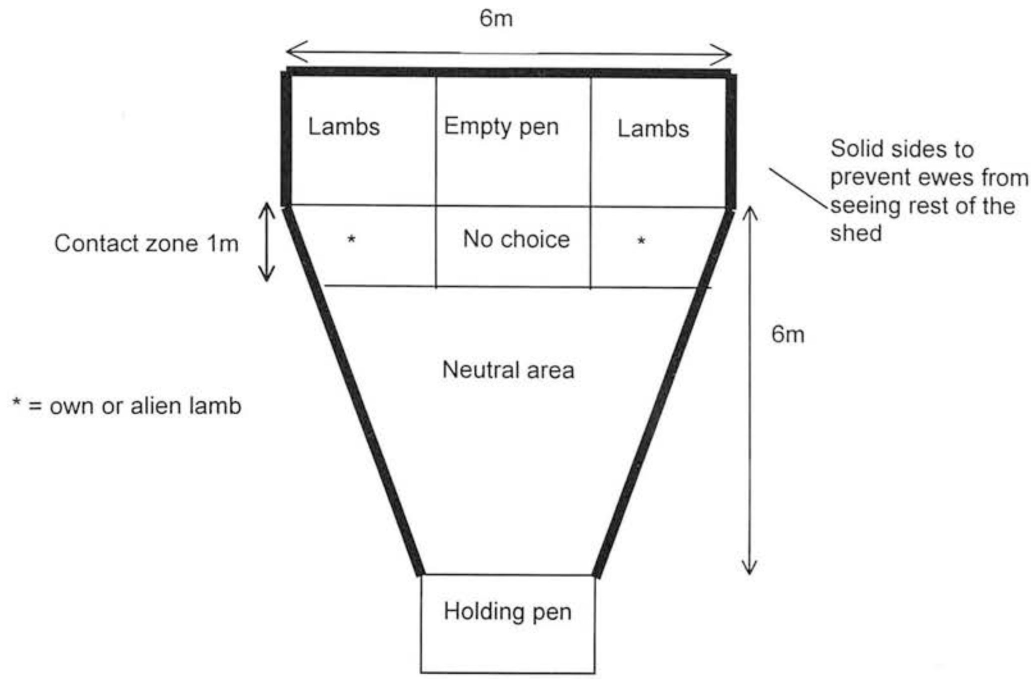

Figure 3.2. Diagram of the test apparatus used for the maternal choice test (not to scale).

fronts of the lamb pens were covered in wire mesh allowing visual, auditory, olfactory and some tactile contact between the ewes and lambs. Own lambs and alien lambs were placed in the 'lamb' pens in a pseudo-random fashion to ensure that presentation of the lambs was balanced for both groups and that results were not biased towards side preferences. The empty pen in between the lamb pens helped to determine which lamb the ewe was choosing to stand next to.

The floor of the test pen was divided into areas, marked on the straw using a white marker spray. The ewes did not appear to find this aversive as they showed no reaction toward it (sniffing or balking) and readily walked over the lines. The 1m deep area in front of the lamb pens (two lamb areas and the no choice area) was designated the 'contact zone'. Any ewes standing with their two front legs and at least half their body length in this zone were considered to be choosing to associate with the lambs. Standing in the individual area directly in front of a 'lamb' pen was considered to indicate a choice to associate with that specific lamb. A ewe standing in the area directly in front of the empty pen was considered to be choosing to associate with lambs but making no particular choice for one individual or the other. The holding pen was the only exit to the apparatus and also the only place in the test pen where the rest of the shed could be seen.

3.2.3.3. Testing.

Each ewe was tested once, for 5 minutes. During a test the ewe and her lamb(s) were moved from their lambing pen to the holding pen. The alien lamb was then removed from its dam and the own and alien lambs placed simultaneously in the lamb pens. The start of the test was delayed for 30 seconds to allow the ewe the opportunity to see and hear both lambs before she entered the pen. The gate between the holding pen and the rest of the apparatus was then opened and left open for the duration of the test. The following measures were recorded for each ewe: time taken to reach the contact zone, time taken to reach their own lamb, the first individual area in the contact zone entered and the first lamb approached. In addition, the location of the ewe was recorded every 10 seconds and the total number of ewe vocalisations tallied during the test. Once the test was completed the alien lamb was returned to its dam immediately and the ewe and her lambs reunited and led back to their lambing pen. Ewes and lambs were separated for the minimum period necessary to complete the test.

3.2.3.4. Statistical analysis.

All data were normally distributed with the exception of the time to reach own lamb which were log (base 10) transformed. REML was used to compare the ewes' behaviour during the test. Sire breed, litter size and ewe breed were fitted as fixed effects in the model. Paired T-tests were used to compare the time spent with own and alien lambs during the maternal choice test within the two breeds. Chi-squared tests were used to analyse the first contact zone entered and Fisher's Exact Probability tests to analyse the first lamb approached in the test.

3.2.4. Relationship between early maternal behaviour and behaviour in the maternal choice test.

The behaviour of the ewes during the maternal choice test was examined in relation to their behaviour immediately following parturition and over the first 3 days post-partum. This allowed more direct investigation of how early maternal behaviour relates to the ability of ewes to recognise their lambs. The relationship between early maternal behaviour and the behaviour of the ewe in the maternal choice test was examined using a factor analysis followed by orthogonal (Varimax) rotation of selected data from the first 3 days post-partum and the maternal choice test. The variables used in the factor analysis were: latency to groom lamb; duration of time spent grooming lamb; number of low pitched vocalisations; performance of negative behaviour; proportion of non-co-operation with sucking attempts; proportion of scans nosing lamb; proportion of scans lying within 1m of lamb; latency to reach own lamb in the maternal choice test; time spent with own lamb; time spent with alien lamb; and whether or not the ewe entered the holding pen during the test. These behaviours were chosen because they represent the provision of maternal care to the neonate (after Alexander 1988), the development of ewe-lamb bonding and the interactions of the ewe with her lamb, both during the post-partum period and in the maternal choice test. Several ewes of both breeds were omitted from the factor analysis as they had various items of data missing. In total 13 Suffolk and 13 Blackface ewes were included in this analysis.

3.3. Results.

3.3.1. Experiment 1, novel object test.

3.3.1.1. Breed comparisons in barren ewes.

3.3.1.1.1. General behaviour in the tests.

Table 3.5 shows the general behaviour of barren ewes during the control test and the test with the novel object present. All ewes entered the test area in front of the holding pen during both tests. In barren Suffolk ewes there was a non-significant tendency to reach the test area more quickly when the novel object was present (Wilcoxon W=0.0, N=5, p=0.059) but there was no significant difference in the time spent in that area (W=13.0, N=5, NS). There was no significant difference in the number of incidences of backing between the two tests (W=4.5, N=5, NS), but barren Suffolk ewes showed a tendency to perform more walking away during the test with the novel object, compared to the control test (W=15.0, N=5, p=0.059).

The presence of the novel object did not have a significant influence on the behaviour of the barren Blackface ewes. There were no significant differences in the

Table 3.5.
General behaviour of barren ewes during both novel object tests (control and novel object present).

Behaviour	Suffolk ewes	Blackface ewes
Latency to reach test area (s)		
Control	13.2 (10.6, 21.6)	49.1 (21.2, 251.1)
Novel object present	10.2 (9.6, 11.1)	11.4 (10.0, 31.1)
Duration in test area (s)		
Control	127.4 (73.7, 189.2)	80.6 (24.7, 140.9)
Novel object present	195.1 (88.6, 263.7)	127.3 (45.6, 225.6)
Walk away (no. during test)		
Control	1.0 (0.0, 3.5)	2.0 (1.0, 4.0)
Novel object present	5.0 (2.5, 5.5)	4.0 (2.0, 7.5)
Backing (no. during test)		
Control	6.0 (2.0, 7.0)	1.0 (0.0, 2.0)
Novel object present	3.0 (0.5, 6.0)	3.0 (0.5, 4.5)

Table shows medians (with Q1, Q3).

latency to reach the test area (W=4.0, N=5, NS), the time spent in that area (W=14.0, N=5, NS), and the number of incidences of walking away (W=6.0, N=3, NS) or backing (W=13.5, N=3, NS) between the two tests.

3.3.1.1.2. Interaction with the novel object.

The two breeds showed significant differences in their interaction with the traffic cone. Barren Suffolk ewes spent significantly longer sniffing the traffic cone compared to barren Blackface ewes (median duration (s) (with Q1, Q3): Suffolk=101.3 (15.8, 131.0), Blackface=5.4 (3.3, 11.7), Mann Whitney U=16.0, $p<0.05$). In addition, 4 Suffolk ewes performed at least one aggressive behaviour towards the traffic cone whereas none of the Blackface ewes showed any aggressive behaviours (Fisher's Exact Probability $p=0.023$).

3.3.1.2. Breed comparisons in pregnant ewes.

3.3.1.2.1. General behaviour during the tests.

Table 3.6 shows the general behaviour of pregnant ewes during the control test and the test with the novel object present. One Blackface ewe did not enter the test area in front of the holding pen in either test, all other ewes entered the area in both tests. The presence of the novel object did not have much influence on the behaviour of

Table 3.6.
General behaviour of pregnant ewes during both novel object tests (control and novel object present).

Behaviour	Suffolk ewes	Blackface ewes
Latency to reach test area (s)		
Control	13.6 (10.5, 16.2)	12.5 (10.5, 109.7)
Novel object present	10.8 (8.3, 13.9)	10.25 (9.1, 49.3)
Duration in test area (s)		
Control	282.5 (260.6, 285.6)	229.4 (62.0, 290.1)
Novel object present	229.0 (158.8, 289.4)	154.1 (76.4, 264.6)
Walk away (no. during test)		
Control	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)
Novel object present	1.0 (0.0, 3.8)	0.0 (0.0, 4.3)
Backing (no. during test)		
Control	0.0 (0.0, 2.0)	1.5 (0.0, 6.25)
Novel object present	1.5 (0.0, 5.0)	6.0 (0.0, 9.25)

Table shows medians (with Q1, Q3).

either breed. Both breeds were quicker to reach the test area when the novel object was present (Suffolk: W=6.0, N=9, p=0.058, Blackface: W=3.0, N=9, $p<0.05$) but there were no significant differences in the time spent in that area (Suffolk: W=16.0, N=10, NS, Blackface: W=14.0, N=9, NS), the number of incidences of walking away (Suffolk: W=11.5, N=5, NS, Blackface: W=14.0, N=5, NS) or backing (Suffolk: W=18.0, N=6, NS, Blackface: W=19.0, N=6, NS) between the two tests.

3.3.1.2.2. Interaction with the novel object.

There were also no differences between the two breeds in their interactions with the traffic cone. The duration of time spent sniffing the traffic cone did not differ between the two breeds (median duration (s) (with Q1, Q3): Suffolk=16.1 (7.0, 52.8), Blackface=12.6 (5.03, 24.37), U=93.0, NS). One Blackface ewe and 2 Suffolk ewes performed at least one aggressive behaviour towards the traffic cone but this was not significantly different between the two breeds.

3.3.1.3. Influence of pregnancy on interaction with the novel object.

From the above analysis it appears that pregnancy may have an effect on interaction with the traffic cone in Suffolk ewes but not Blackface ewes. In Suffolk ewes there was a tendency for barren ewes to spend longer sniffing the cone than pregnant ewes (median duration (s) (with Q1, Q3): barren=101.3 (15.8, 131.0), pregnant=16.1 (7.0, 52.8), U=55.0, p=0.076), but there were no differences between barren and pregnant Blackface ewes (median duration (s) (with Q1, Q3): barren=5.4 (3.3, 11.7), pregnant=12.6 (5.0, 24.4), U=31.0, NS). In the Suffolk ewes 4 barren (80%) and only 2 pregnant (20%) ewes performed at least one aggressive behaviour toward the traffic cone (p=0.047). Aggression towards the traffic cone was extremely rare in all Blackface ewes (1 bite from 1 pregnant ewe).

3.3.2. Experiment 2, maternal behaviour during the first 3 days post-partum.

Unless otherwise stated, litter size and sire breed had no significant effect on the maternal behaviour of the ewes.

3.3.2.1. Maternal behaviour during the first 3 hours post-partum.

3.3.2.1.1. Grooming behaviour.

Blackface ewes were quicker to begin grooming their lambs, compared to Suffolk ewes (mean latency to begin grooming (s) (with 95% confidence interval): Suffolk=132.43 (83.14-210.96), Blackface=44.26 (27.78-70.50), REML Wald=9.13, d.f.=1, $p<0.01$) and also groomed them for longer during the first 30 minutes post-partum (mean duration of grooming (s) (with s.e.m.): Suffolk=532.3 (78.1), Blackface=1110.0 (138.0), Wald=14.73, d.f.=1, $p<0.001$). Litter size had no significant effect on the latency to begin grooming but single-bearing ewes groomed their lambs for longer during the first 30 minutes post-partum than twin-bearing ewes (mean duration of grooming (s) (with s.e.m.): single=997.0 (175.0), twin=652.3 (80.1), Wald=6.04, d.f.=1, $p<0.05$).

3.3.2.1.2. Negative behaviour.

Blackface ewes were also less likely to show negative behaviour towards their newborn lambs, compared to Suffolk ewes (Table 3.7). There was a tendency for Blackface ewes to show less aggression towards their lambs than Suffolk ewes ($p=0.066$) and Blackface ewes showed significantly fewer retreats ($p=0.000$). However there was no significant difference in the number of ewes within each breed leaving their lamb during the first 30 minutes post-partum ($p=0.290$).

Table 3.7.
Negative behaviour during the first 30 minutes post-partum.

	Suffolk ewes	Blackface ewes
Aggression	4 (22.2%)	0
Retreat	10 (55.6%)	0
Leave	3 (16.7%)	1 (7%)

Table shows the number (and proportion) of ewes performing each of the negative behaviours.

3.3.2.1.3. Vocalisations.

The majority of ewes made low pitched vocalisations during the first 30 minutes following the birth of their lambs (100% of Suffolk ewes, 93% of Blackface ewes). There were no significant differences between the ewe breeds in the number of low pitched vocalisations at this time (mean number of vocalisations (with s.e.m): Suffolk=177.0 (34.0), Blackface= 91.7 (27.2), Wald=0.17, d.f.=1, NS).

Approximately half of the ewes in each group made high pitched vocalisations during the first 30 minutes post-partum (67% of Suffolk ewes, 50% of Blackface ewes). There was also no effect of ewe breed on the number of high pitched vocalisations made during this 30 minute period (median number of vocalisations (with Q1, Q3): Suffolk=1.0 (0.0, 24.0), Blackface=0.0 (0.0, 11.5), U=181.5, NS).

3.3.2.1.4. Co-operation with lamb suck attempts.

Blackface ewes showed more co-operation with early attempts to find the udder and suck, having a lower rate of circling, backing and walking forward over the top of lambs. Blackface ewes responded to 13.23% (95% confidence interval: 8.50-19.0) of lamb interactions with circling, backing or walking forward whereas Suffolk ewes responded negatively to 32.82% (95% confidence interval: 25.08-41.61) of lamb interactions (Wald=9.26, d.f.=1, $p<0.01$).

3.3.2.1.5. Maternal Behaviour Scores.

There were no significant differences in Maternal Behaviour Score between the ewe breeds at either 30 minutes (median score (with Q1, Q3): Suffolk=4.0 (4.0, 6.0), Blackface=4.5 (3.8, 5.0), U=195.5, NS) or 24 hours (median score (with Q1, Q3): Suffolk=4.0 (3.0, 4.0), Blackface=3.0 (3.0, 3.3), U=176.0, NS) post-partum.

3.3.2.2. First 3 days post-partum.

Table 3.8 shows the results from scans taken over the first 3 days post-partum. The two breeds did not differ in their ewe-lamb distance during this period (Wald=0.21, d.f.=1, NS). However single-bearing ewes were closer to their lambs than twin-bearing ewes during the first 3 days post-partum (mean distance (m) (with s.e.m.): single=0.0 (0.35), twin=0.76 (0.35), Wald=9.33, d.f.=1, $p<0.01$). There were no effects of ewe breed on the proportion of scans where ewes were observed to lie within 1m of their lamb (Wald=0.11, d.f.=1, NS) or to nose their lamb (Wald=1.89, d.f.=1, NS).

Table 3.8.
Ewe behaviour over the first 3 days post-partum.

	Ewe-lamb distance (m)	Nosing lamb (%)	Lying together (%)
Suffolk ewes	1.21 (0.15)	9.62 (2.28)	34.43 (5.09)
Blackface ewes	1.08 (0.13)	6.70 (1.56)	38.49 (3.13)

Table shows information collected in scan samples. Mean values are shown (with s.e.m.). Ewe-lamb distance is shown in metres. Data for nosing the lamb and lying together are given as the proportion of scans where the ewe was observed to perform these behaviours.

3.3.3. Experiment 3, ability of ewes in the two breeds to recognise their lambs.

Unless otherwise stated, litter size and sire breed did not have an effect on the behaviour of the ewes in the maternal choice test.

3.3.3.1. Latency to reach the contact zone and own lamb.

Blackface ewes showed a non-significant tendency to be quicker than Suffolk ewes to reach the contact zone (mean latency (s) (with s.e.m.): Suffolk=3.94 (0.29), Blackface=2.79 (0.37), Wald=3.58, d.f.=1, p=0.059) and their own lamb (mean latency (s) (with 95% confidence interval): Suffolk=8.16 (5.53-12.05), Blackface=3.63 (2.46-5.36),Wald=3.67, d.f.=1, p=0.055).

3.3.3.2. First contact zone entered.

Table 3.9 shows the first individual areas in the contact zone entered by the ewes. There was no significant difference in the likelihood of ewes in either breed to approach their own lamb first, compared to the other two areas within the contact zone (Chi-squared χ^2=0.92, d.f.=1, NS). Single-bearing ewes were more likely to approach their own lamb first, compared to twin-bearing ewes (χ^2=4.409, d.f.=1, p<0.05).

Table 3.9.
First individual contact zone entered by ewes.

	Own lamb	Alien lamb	No choice	Other (Alien & No choice)
Suffolk ewes	8	6	3	9
Blackface ewes	9	1	4	5
Twin ewes	7	6	5	11
Single ewes	10	1	2	3

Table shows the number of ewes in each group entering each of the individual contact zones. Due to the small numbers involved the number of ewes approaching the alien lamb or no choice area were combined in the category 'other' during analysis of the first contact zone entered.

3.3.3.3. First lamb approached.

82% of Suffolk ewes and 71% of Blackface ewes went directly to a lamb when first entering the contact zone (Table 3.9). When directly approaching a lamb, there was a non-significant tendency for Blackface ewes to be more likely to approach their own lamb first compared to Suffolk ewes (p=0.097). There was also a non-significant tendency for single-bearing ewes to be more likely to approach their own lamb first, compared to twin-bearing ewes (p=0.055).

3.3.3.4. Ewe vocalisations during the test.

There was no significant effect of ewe breed on the number of ewe vocalisations during the test. Suffolk ewes a mean of 50.8 (s.e.m.=3.96) vocalisations per test, Blackface ewes had a mean of 43.5 (s.e.m.=5.40) vocalisations per test, (Wald=1.99, d.f.=1, NS).

3.3.3.5. Time spent in different test areas.

Figure 3.3 shows the number of scans present in each of the areas within the test apparatus. There were no significant effects of ewe breed on the number of scans present in the neutral area (Wald=0.68, d.f.=1, NS), the contact zone (Wald=2.20, d.f.=1, NS) or the 'no choice' area within the contact zone (Wald=0.98, d.f.=1, NS). However Blackface ewes spent significantly more scans in the area in front of their

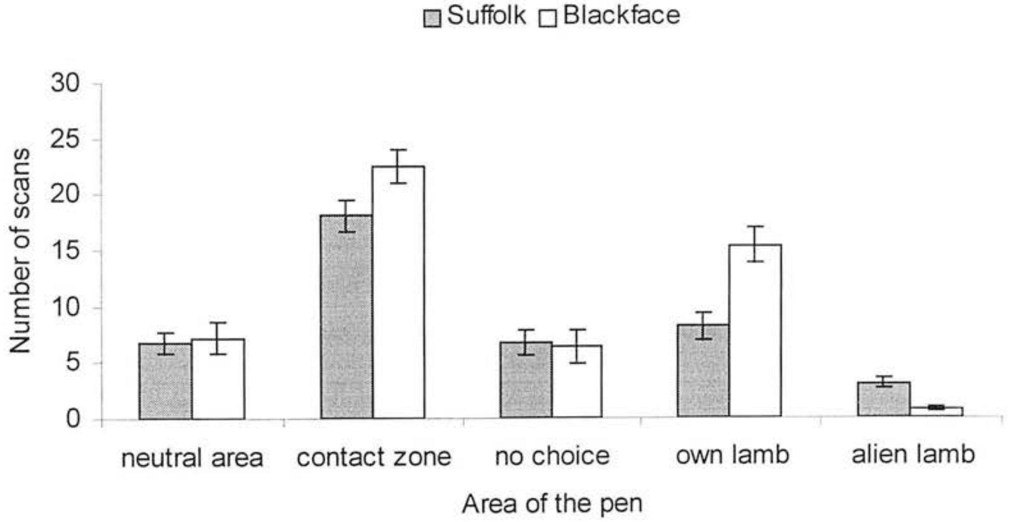

Figure 3.3. Number of scans spent in different areas of the test pen by the ewes in each group.

own lamb (Wald=14.64, d.f.=1, $p<0.001$) and significantly fewer scans in the area in front of the alien lamb (Wald=21.25, d.f.=1, $p<0.001$), compared to Suffolk ewes.

Both breeds spent significantly longer with their own lamb, compared to the alien lamb (Suffolk ewes, Paired T-test T=3.39, $p<0.01$, Blackface ewes, T=8.28, $p<0.001$). The time spent with own lamb was also calculated as a proportion of the time spent associating with any lamb. Blackface ewes spent a significantly greater proportion of this time with their own lamb, compared to Suffolk ewes (median proportion (%) (with Q1, Q3): Blackface=97.50 (91.48, 100.00), Suffolk=76.92 (50.00, 88.33), U=186.0, $p<0.001$).

71% of Suffolk ewes entered the holding pen during the test whereas only 21% of Blackface ewes entered this part of the apparatus. Suffolk ewes spent significantly more scans in the holding pen compared to Blackface ewes (median number of scans (with Q1, Q3): Suffolk=3.0 (0.0, 10.5), Blackface=0.0 (0.0, 0.25), U=150.0, $p<0.01$).

3.3.4. Relationship between early maternal behaviour and behaviour in the maternal choice test.

A factor analysis was used to examine the relationship between the ewes' initial maternal behaviour and their behaviour in the maternal choice test. PCA showed that 3 factors had eigen values above 1, accounting for 61.2% of the variation between the variables. The factor analysis was therefore run with 3 factors. The loadings of behaviours on the first 2 factors are shown in Figure 3.4.

Factor 1 accounted for 24% of the total variation. The duration of grooming the newborn lamb, the number of scans lying together and the time spent with own lamb in the maternal choice test all had strong negative loadings on this factor. The performance of negative behaviours during the first 30 minutes post-partum, the latency to reach own lamb in the maternal choice test, the time spent with the alien lamb and entry into the holding pen during the test all had strong positive loadings on

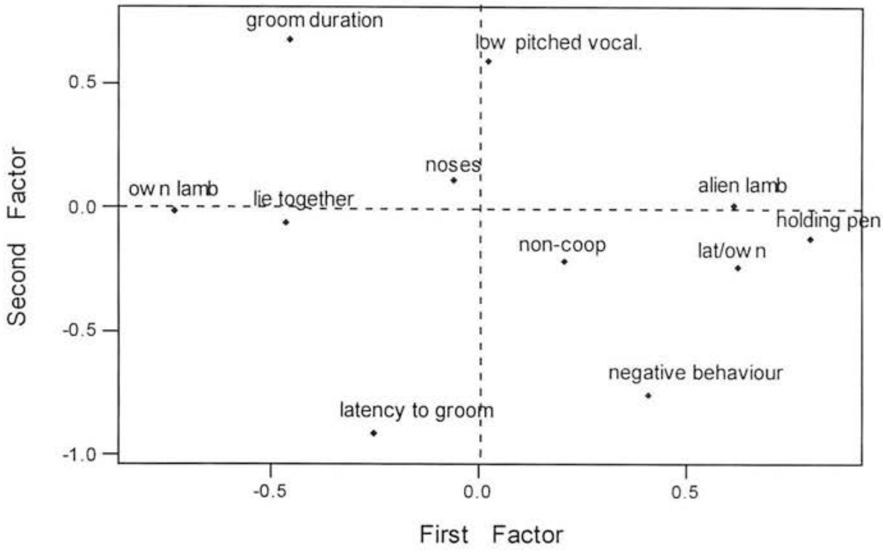

Figure 3.4. Loading plot for Factors 1 and 2 in the factor analysis of maternal behaviour over the first 3 days post-partum.

this factor. Factor 1 therefore appears to represent the ewe's attraction toward her own lamb.

Factor 2 accounted for 21% of the total variation. Grooming duration and low pitched vocalisation rate had strong positive loadings on Factor 2 whereas latency to groom and the performance of negative behaviours had strong negative loadings. This factor was therefore labelled 'initial maternal care'.

Factor 3 accounted for 16.2% of the total variation. Only 2 behaviours had strong positive loadings on this factor, nosing and non-co-operation with initial suck attempts, and there were no strong negative loadings. This factor was hard to interpret but also appeared to relate to the ewes' initial maternal behaviour. As it was not possible to give an accurate interpretation of Factor 3, only the first 2 factors were used to describe the relationship between initial maternal behaviour and behaviour in the maternal choice test.

3.3.4.1. Expression of maternal behaviour within the two breeds of ewe.

Figure 3.5 shows the score plots on the first 2 factors for individual ewes. The majority of Blackface ewes were tightly bunched together indicating that there was not much variation in maternal behaviour in this breed. Their behaviour was mostly characterised by behaviours indicating positive interaction and interest in their lamb, both immediately after birth (Factor 2) and for the whole 3 days (Factor 1). One Blackface ewe was separated from the other ewes. This ewe did not interact with her newborn lamb during the first 30 minutes but did not reject it and over the next 3 days her maternal behaviour resembled that of other Blackface ewes. This probably explains her separation from the other Blackface ewes, as she showed a similar negative loading on Factor 1 (attraction of ewe towards her lamb over the first 3 days) but a very different loading on Factor 2 (initial maternal care).

In contrast to the tight bunching of the Blackface ewes, the Suffolk ewes were very spread out, indicating much more variation in their maternal behaviour. They showed more variation in their general attraction for their lamb (Factor 1) than in their initial maternal behaviour (Factor 2). In comparison to the Blackface ewes their behaviour

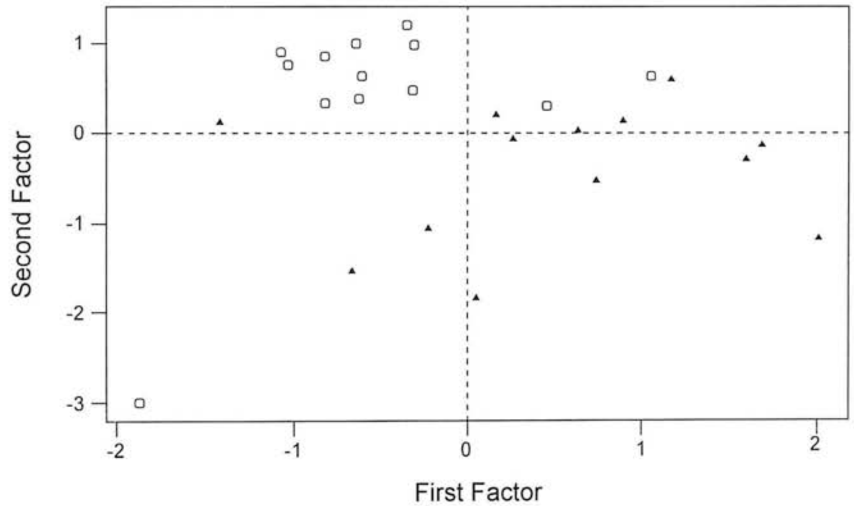

Figure 3.5. Score plot for the individual ewes in the factor analysis of maternal behaviour over the first 3 days post-partum. Blackface ewes are represented by open squares, Suffolk ewes by solid triangles.

was characterised by less interaction with their lamb over the first 3 days post-partum and less positive behaviour during the initial post-partum period.

3.4. Discussion.

3.4.1. Experiment 1, novel object test.

3.4.1.1. Interpretation of the ewes' behaviour in the novel object test.

The behaviours of animals during tests designed to elicit an emotional reaction are notoriously hard to interpret as they are invariably unique to the design of the apparatus and experiment (Romeyer and Bouissou 1992). The use of a control test in the current experiment helped give a clearer idea of how the presence of the novel object affected the behaviour of the ewes. Fear can be expressed via active avoidance (flight, hiding, escape), immobility or through active defence (attack, threat) (Boissy 1995). As close proximity with the cone is voluntary, avoidance is likely to be the behaviour most indicative of fear in this test. Avoidance of the cone would be expected to delay the ewes' entry into the area in front of the holding pen and to reduce the time spent in this area when the cone was present. This was not the case for any of the ewes. Backing or walking away from the cone may also indicate avoidance as the ewe is distancing herself from the aversive stimulus. In addition, backing may imply a greater fear than walking away as the ewe is maintaining visual contact with the cone. This would be necessary to enable reaction to any sudden movement the stimulus may make. The incidence of backing was not increased by the presence of the cone but barren Suffolk ewes had a tendency to walk away from the area in front of the holding pen more frequently when the cone was present.

Aggression toward the cone may also indicate fear as the ewe could be considered to be attacking a stimulus she finds threatening (Boissy 1995). Reproductive state affected the way the Suffolk ewes, but not Blackface ewes, interacted with the cone. Barren Suffolk ewes appeared to spend longer sniffing the cone than pregnant Suffolk ewes and were more likely to behave aggressively toward it. Exposure to a novel object is also known to elicit aggression in mice (Misslin and Ropartz 1981) and pigs (Wood-Gush et al. 1990). It therefore appears that the traffic cone does invoke a fear response in barren Suffolk ewes. However, the fact that these ewes did not approach the cone cautiously (latency to reach area in front of holding pen was reduced when the novel object was present) and also spent a third of the test time sniffing the cone, implies that the fear response was only mild.

3.4.1.2. Influence of pregnancy on the fear reaction of ewes.

Pregnancy appeared to reduce the fear response of Suffolk ewes toward a novel object as pregnant Suffolk ewes showed less walking away and less aggression in response to the traffic cone, compared to barren Suffolk ewes. Vierin and Bouissou (2001) also found that pregnancy reduced the fear reaction of Ile-de-France ewes toward a novel object combined with a surprise effect. They suggested a reduction in fear response during pregnancy was beneficial for the young as prenatal stress is known to have negative effects on the physiology and behaviour of the young (reviewed by Braastad 1998). A decrease in reactivity to fear-eliciting stimuli could therefore be a mechanism to ensure optimal development of the offspring (Vierin and Bouissou 2001). An alternative explanation is that pregnant ewes have a reduced fear response to aid certain changes in behaviour during pregnancy. Pregnancy places a greater nutritional requirement on the ewe and she may have to forage further away from the flock than normal to meet this requirement. Ewes also seek isolation for parturition (Vince et al. 1985), and a reduction in the fear response of pregnant ewes would aid both of these behaviours.

Neither pregnant nor barren Blackface ewes appeared to find the novel object aversive and it is therefore not possible to use the results of this experiment to assess the effect of pregnancy on their fear reaction. None of the Blackface ewes avoided the cone or showed much interaction with it, sniffing it for less than 15 seconds, and only one pregnant ewe showed aggression towards it (a single bite).

3.4.1.3. Influence of previous experience on the fear reaction of ewes.

It is interesting that barren Suffolk ewes showed some fear toward the cone whereas barren Blackface ewes did not. This may be due to the different environments these two breeds are accustomed to. Blackface sheep are traditionally a hill breed, living in a very heterogeneous environment. Conversely Suffolk ewes are farmed in lowland areas and maintained in comparatively homogeneous fields. The Suffolk ewes in this study came from a lowland farm whereas the Blackface ewes were raised in a hill environment. Greater exposure to variety in the heterogeneous environment may make the Blackface ewes less reactive to a mild stressor, such as an inanimate traffic cone, compared to the Suffolk ewes raised in a more homogeneous environment. Pigs raised in bare or barren environments have been found to pay more attention toward novel objects than pigs raised in enriched environments (Stolba and Wood-Gush 1980, Wood-Gush et al. 1990). This could be due to animals from the barren environment having fewer relevant experiences to call upon, and thus being slower to learn about, and classify the novel object (Wood-Gush et al. 1990). Whilst the homogeneous field environment of the Suffolk ewes cannot be described as barren, it is likely to be less complex than the hill environment of the Blackface ewes and to provide fewer opportunities to experience novelty.

3.4.1.4. The role of a fear of novelty in breed differences in initial maternal behaviour.

The aim of this experiment was to compare the reactivity of the two breeds to novelty. Although this was somewhat confounded by the apparent low aversiveness of the novel object chosen, especially for Blackface ewes, the results do suggest that pregnant ewes in the two breeds do not differ in their reactivity towards this type of novelty. Therefore, the breed differences in maternal behaviour observed in primiparous ewes may not be due to differences in their fear of novelty. In addition, pregnancy also appears to reduce the reaction of Suffolk ewes to novelty and may consequently make them less likely to find the novelty of a newborn lamb aversive. However, a newborn lamb would be expected to provide much more potential for aversion than an inanimate traffic cone. It would not only present unfamiliar visual, olfactory and auditory cues but is also likely to attempt to interact with the ewe. This could be aversive as some ewes have been shown to groom their recumbent newborn lamb but butt it aggressively when it moved (Lynch et al. 1992). Consequently, it is not possible to definitively conclude that the differences in maternal behaviour between the two breeds are not caused by differences in their reactivity to novelty and further investigation is needed.

There are two approaches which could be used to investigate this further. Firstly, the test could be repeated using a novel object designed to reproduce the cues presented by a newborn lamb, for example unfamiliar smell and random movements. However the difficulty of discerning which cues are most important, or aversive, to the ewe and problems associated with reliably reproducing some of the cues, especially the lamb's attempts to interact with the ewe, will make this a complicated process. Alternatively, and potentially more successfully, primiparous ewes could be familiarised with newborn lambs before their own parturition. Their behaviour towards the neonate could then be compared to that of ewes with no prior experience of newborn lambs. This was not possible in the current study as it would have interfered with the main focus of the project as a whole.

3.4.2. Experiment 2, maternal behaviour during the first 3 days post-partum.

3.4.2.1. Initial post-partum period.

The ewes in this study showed similar behavioural differences to those seen in previous studies (Dwyer and Lawrence 1998, Dwyer et al. 1998, Dwyer and Lawrence 1999a). Blackface ewes were quicker to begin grooming their lambs than Suffolk ewes and groomed them for more of the first 30 minutes post-partum. They were less likely to perform negative behaviours than Suffolk ewes and showed more co-operation with their lambs' attempts to suck. One result that differed from the findings of Dwyer et al. (1998) was that the two breeds did not differ in their performance of low pitched bleating during the post-partum period. It is not clear why this is so but the finding may have been due to the frequent bad weather, gales and thunder storms, during the current study. The noise of wind and rain made it difficult to be sure of hearing all of these quiet vocalisations.

3.4.2.2. Maternal Behaviour Score.

Maternal Behaviour Scores did not differ between the two breeds at either 30 minutes or 24 hours post-partum. This agrees with the previous findings of scores assigned at 30 minutes post-partum (Dwyer and Lawrence 1998). As discussed by Dwyer and Lawrence (1998) this test may not have been equivalent in the two breeds as motivation to avoid the handler may have been stronger in hill-bred Blackface ewes. Additionally, as lambing proceeded, the number of ewes present in the pens changed as lambed ewes were moved into different pens (Chapter 2). As a consequence the 30 minute post-partum score may have had different significance for ewes tested later in the lambing period, compared to those tested early on, as they had fewer flock mates. The opposite of this is true for the 24 hour post-partum score, ewes tested early on in lambing would have had fewer flock mates than ewes tested later in lambing. Sheep are gregarious animals and social isolation is known to induce stress (Romeyer and Bouissou 1992), therefore a low number, or absence, of flock mates during tests may have increased the stressfulness of the test situation. This may have altered the behaviour of these ewes, for example making them more reactive towards the handler. As a result of these confounding factors the Maternal Behaviour Score is not a reliable indicator of maternal behaviour in this experimental situation.

3.4.2.3. Scan samples over the first 3 days post-partum.

As with the findings of Dwyer and Lawrence (1999a) there were no breed differences in ewe-lamb distance over the first 3 days post-partum. Litter size did have an effect, with single-bearing ewes being closer to their lambs than twin-bearing ewes. As ewe-lamb distance was averaged for twin-bearing ewes this could be caused by the twin lambs being apart, resulting in the ewe being unable to be close to both of them. Unfortunately the distance between littermates was not recorded during the scans. No breed differences were found in the frequency of ewes nosing their lambs. Dwyer and Lawrence (1999a) found that Blackface ewes nosed their lambs more frequently than Suffolk ewes.

In summary, only two results, low pitched vocalisation frequency and frequency of nosing, were not in agreement with previous findings. Therefore it is safe to conclude that the ewes in this study show the same differences in maternal behaviour as those in previous studies (Dwyer and Lawrence 1998, Dwyer et al. 1998, Dwyer and Lawrence 1999a) and can be used in the investigation of breed differences in maternal behaviour throughout lactation.

3.4.3. Experiment 3, ability of ewes in the two breeds to recognise their lambs.

3.4.3.1. Recognition of own lamb.

Both breeds seemed able to recognise their own lamb at 3 days post-partum as they both spent longer with their own lamb compared to the alien lamb. This indicates a preference for their own lamb and consequently the ability to recognise it. Differences in the early maternal behaviour of the two breeds therefore do not affect the ability of ewes to recognise their lambs at 3 days post-partum.

Initial recognition of the lamb appeared to be made at a distance as the majority of ewes went straight to a lamb and reached the contact zone in less than 10 seconds. Blackface ewes may have been slightly better at distance recognition as there was a tendency for them to be quicker to reach their own lamb than the Suffolk ewes. However this could also have been due to these ewes having a greater maternal motivation than Suffolk ewes, this is discussed below. There was also a slight tendency for Blackface ewes to be more likely to approach their own lamb first, compared to Suffolk ewes, but this is too indefinite to conclude that Blackface ewes are better able to recognise their lambs from a distance.

Single-bearing ewes also showed a non-significant tendency to approach their own lamb first, compared to twin-bearing ewes. As twin-bearing ewes have two, rather than one, lambs to recognise they may be slower at learning to distinguish their lambs than single-bearing ewes.

3.4.3.2. Breed differences in maternal motivation.

Several aspects of ewe behaviour during the test suggest that Blackface ewes have a stronger bond with their lambs and are more maternally motivated than Suffolk ewes. This difference appears to be linked exclusively to the motivation to be with their own lamb, rather than with lambs in general, as both breeds were equally attracted to all lambs and spent a similar number of scans in the contact zone. As discussed above, the tendency for Blackface ewes to reach their lambs faster than Suffolk ewes may be an indicator of stronger maternal motivation. Blackface ewes also spent longer with their own lambs and less time with the alien lambs compared to Suffolk ewes. This may indicate a greater maternal motivation in the Blackface ewes compared to the Suffolk ewes, although this could also be linked to their ability to recognise their lambs. Whilst both breeds are ultimately able to recognise their lambs, the speed at which they achieve recognition may be different. The longer time spent with their own lamb in Blackface ewes could be due to a faster ability to recognise their lamb, compared to Suffolk ewes. The Suffolk ewes may need to spend longer assessing all the lambs before identifying their own.

The lower incidence of Blackface ewes entering the holding pen, compared to Suffolk ewes, provides further support for their stronger maternal motivation. This was the area of the test pen furthest away from the lambs and entry into this area meant that ewes had to leave their lambs (in an unfamiliar environment) at a distance of more than 6m. The holding pen was also the only part of the test apparatus where the rest of the shed could be seen and the only exit to the apparatus. Presence in this area may therefore have indicated an attempt to return to the home pen and flock mates.

Although it is not possible to definitively separate maternal motivation and recognition ability, the combination of a lower likelihood of leaving their lamb to enter the holding pen and a stronger preference for their own lamb suggests that Blackface do have a stronger maternal motivation than Suffolk ewes, regardless of their ability to recognise their lambs. Consequently it appears that Blackface ewes have a stronger ewe-lamb bond than Suffolk ewes at 3 days post-partum.

3.4.4. Relationship between early maternal behaviour and behaviour in the maternal choice test.

From the above analysis and discussion it can be seen that differences in initial maternal behaviour between the two breeds appear to be linked to the strength of the ewe-lamb bond at 3 days post-partum. Blackface ewes show more affiliative behaviour towards their newborn lambs and also have a stronger bond with them, compared to Suffolk ewes. Further investigation using factor analysis showed that the largest source of variation between the ewes (Factor 1) was the attraction of the ewe towards her own lamb(s) at each stage of the study. Immediately after birth this was reflected in the grooming of the neonate and tolerance of the lamb's attempts to interact with its dam, find the udder and suck. Seeking proximity to own lamb was important during the first 3 days post-partum (when both animals were lying down) and during the maternal choice test. Avoidance of the alien lamb and an absence of leaving the lamb to enter the holding pen also indicated attraction to own lamb during the test.

The second largest source of variation (Factor 2) was due to the reaction of the ewe towards her newborn lamb and her initial maternal behaviour. Grooming duration and low pitched vocalisation rate were linked, opposite the latency to begin grooming and the performance of negative behaviour during the first 30 minutes post-partum. Both factors, jointly accounting for 45% of the variation between ewes, represent the ewe's willingness to interact with her lambs. The behavioural differences seen between the two breeds throughout the first 3 days post-partum may therefore be mainly due to differences in the ewes' attraction towards their own lambs and their willingness to interact with them.

3.4.4.1. Variation in the expression of maternal behaviour within the two breeds.
The majority of Blackface ewes showed little variation in their maternal behaviour, which was characterised by a willingness to interact with their lambs throughout the first 3 days post-partum. In contrast Suffolk ewes showed much more variation in their behaviour and were more likely to be associated with behaviours which indicated a lack of interest in their lambs, for example entering the holding pen during the maternal choice test. The two breeds therefore appear to differ in the strength of their maternal motivation throughout the first 3 days. Lower maternal motivation in Suffolk ewes may account for their lower performance of affliative behaviours during the initial post-partum period compared to Blackface ewes. The reason for this, and the differences in behavioural variation in the two breeds, is discussed further in Chapter 6.

3.5. Summary.

It was not possible to definitively assess the role of a fear of novelty in breed differences in initial behaviour as the novel object did not appear to be aversive to many of the ewes. However pregnancy did appear to reduce the fear response of Suffolk, but not Blackface, ewes. The two breeds showed very similar differences in their early maternal behaviour to those reported in previous studies (e.g. Dwyer and Lawrence 1998, Dwyer et al. 1998, Dwyer and Lawrence 1999a). Blackface ewes showed more affiliative behaviour towards their newborn lambs and less negative behaviour compared to Suffolk ewes. There was no difference in the ability of ewes in the two breeds to recognise their lambs at 3 days post-partum but Blackface ewes showed a stronger motivation to be with their own lamb during the maternal choice test than Suffolk ewes. A factor analysis showed that the willingness of the ewe to associate with her lamb during the maternal choice test was related to her willingness to interact with it throughout the first 3 days post-partum. Blackface ewes did not show much variation in their early maternal behaviour, which was characterised by a strong motivation to interact with their lambs. Suffolk ewes showed more variation in their maternal behaviour and were more likely to show behaviours indicating low interest in their lamb. Suffolk ewes therefore appear to be less maternally motivated than Blackface ewes during the initial post-partum period.

Chapter 4: General maternal behaviour during later lactation.

4.1. Introduction.

As discussed in Chapter 1, the comparison of maternal behaviour in Suffolk and Blackface ewes has previously concentrated on the initial post-partum period. In sheep the mother-offspring bond can remain strong for up to 100 days after birth and natural weaning is not complete until lambs are about 6 months old (Arnold et al. 1979). Maternal care therefore extends beyond the immediate post-partum period and the aim of this part of the study is to investigate whether Suffolk and Blackface ewes continue to show differences in their maternal behaviour during later lactation.

4.1.1. Features of ewe behaviour to be examined.

In order to assess maternal behaviour during later lactation it is important to identify which aspects of ewe behaviour should be examined. As maternal care functions to ensure survival of the young (Alexander 1988), behaviour that is beneficial for lamb survival may be considered a logical basis for such an investigation. In a review of ungulate maternal care, Alexander (1988) compiled a list of dam behaviours thought to be beneficial for the survival of offspring (as discussed in Chapter 1). However the behaviours identified in this review (Alexander 1988) are confined to the post-partum period and do not relate to later maternal care. Nonetheless, by applying the same principle of survival value to later behaviour it is possible to identify which ewe behaviours are likely to have a survival benefit for young at this time.

Various features of ovine behavioural ecology would be expected to influence which behaviours are beneficial for offspring survival. Sheep are mobile, social animals, living in flocks that travel as they graze (Lynch et al. 1992), the lambs employing a following strategy (Lent 1974). Due to the flock situation and the mobility of the young, it is essential that the dam develops a bond with her lamb and maintains contact in order to ensure exclusive suckling of her young. Indiscriminate suckling will reduce the milk supply for the ewe's own lamb and may decrease its chances of survival. It is also important that the ewe does not lose track of her young as she grazes (Alexander 1988). This would not only expose it to the risk of predation but also the chance of becoming separated from the flock and left behind.

4.1.2. Maintenance of proximity between ewes and lambs.

Maintenance of proximity in a dynamic flock situation requires mutual awareness by both the ewe and lamb and is achieved through following or approaching each other as the ewe-lamb distance increases. Mutual awareness may be indicated by behavioural synchrony between ewes and their lambs and this may also facilitate the maintenance of proximity. The motility of the flock also means that the behaviour of the ewe whilst the lamb is recumbent is important in the prevention of separation.

4.1.3. Communication between ewes and lambs.

Communication between ewes and their lambs would be expected to play a significant role in the maintenance of contact between the animals. The most obvious form of communication is vocalisation, however Lawrence (1984) has also identified the use of a head-up posture in communication between ewes and their lambs. During a head-up posture the ewe stands rigidly and raises her head vertically to her body. This acts as a signal to her lamb that it may approach and/or suck. As the lamb ages the ewe becomes less willing to move towards her young and responsibility for maintaining contact passes over to the lamb (Hinch et al. 1987). The ewe maintains control of the dynamics of the relationship using the head-up posture to influence the movements of her lamb (Lawrence 1984).

4.1.4. Sucking interactions in later lactation.

As discussed in Chapter 1 the lamb is dependent on the dam for nutrition during the first few weeks post-partum (Ewbank 1967). Her co-operation with sucking interactions is therefore essential for lamb survival during this period. As the lamb ages it becomes less dependent on the ewe for nutrition, reflected by less frequent suckling and the development of grazing (Morgan and Arnold 1974). This would be expected to reduce the importance of maternal care and the dam's reaction to suck attempts. However, the older lamb may still benefit from a close association with its dam, gaining information about socialisation and diet preferences from her (Hinch et al. 1987). Suckling and nosing by the ewe are known to have a role in the maintenance of association between ewes and their lambs, especially as the lambs become older and less dependent on the ewe for nutrition (Shillito and Hoyland 1971, Arnold et al. 1979). Nosing primarily occurs during sucking interactions and involves the ewe briefly touching the rear end of her lamb with her muzzle. It is the only form of physical interaction, other than suckling, between the ewe and lamb beyond the immediate post-partum period (Bareham 1976, Alexander 1988). The behaviour of the ewe during her lamb's sucking attempts is therefore important for lamb survival throughout lactation, not just when her lamb is dependent on her for nutrition.

4.1.5. Maternal behaviours examined in this study.

Three main aspects of ovine behaviour appear to have an influence on young survival during later lactation: the spatial relationship a ewe has with her lamb, the communication between them and the nature of their sucking interactions. These three areas were therefore chosen as the basis for the assessment of continued differences in maternal behaviour between Blackface and Suffolk ewes. The maternal care of Blackface ewes during the immediate post-partum period is characterised by more affiliative behaviour towards their lambs and more interest in them, compared to Suffolk ewes (Chapter 3). It was therefore hypothesised that Blackface ewes would continue to have a closer relationship with their lambs than Suffolk ewes. Blackface ewes were expected to have a shorter ewe-lamb distance, to show more behavioural synchrony with their lambs and to be more likely to have their own lamb as nearest neighbour, compared to Suffolk ewes. As communication, either vocal or postural, indicates awareness of the lamb, and an intention to maintain contact, it was hypothesised that Blackface ewes would show more communication with their lambs, compared to Suffolk ewes. It was also hypothesised that Blackface ewes would show more positive behaviour towards their lambs during sucking interactions, compared to Suffolk ewes, and that they would also nose their lambs more frequently than Suffolk ewes.

4.2. Methods.

4.2.1. Animals.

The animals used in these observations have been described in Chapter 2. Dependent on the weather and health of the animals, ewes and their lambs left the lambing shed and entered the study field at approximately 3 days post-partum. The average age for leaving the shed was 5 days post-partum. One single-bearing Blackface ewe died at 5 weeks post-partum. Data from this ewe were excluded from analysis from this point onward.

4.2.2. Observation of maternal behaviour.

The maternal behaviour of Suffolk and Blackface ewes was observed for the first 10 weeks post-partum. Data collection began the day after the animals had been moved from the lambing shed to the study field. The average lamb age for starting data collection was 6 days old. Two methods of data collection were used: scan samples of the whole flock and continuous focal observations on individual ewes and their lambs. More data were collected during the first 4 weeks post-partum as this has previously been identified as a period of great change in the ewe-lamb relationship (e.g. Morgan and Arnold 1974, Hinch et al. 1987). Figure 4.1 shows a timeline diagram of data collection during the study. Data were collected between 08.00 and 20.00 each day. Focal observations on individual animals were made in a pseudo-random manner to ensure that, over time, data collection for each ewe was equally spaced within the daily collection period. The timing of scans was also balanced to ensure that equal numbers of scans were made throughout the day. When two scans or observations were made on the same day, the first was made in the morning

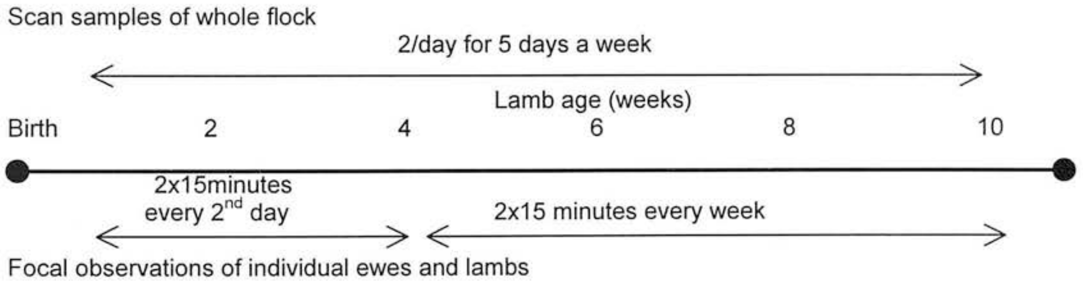

Figure 4.1. Timeline of data collection between birth and 10 weeks post-partum.

(between 08.00 and 12.59) and the second in the afternoon (between 13.00 and 20.00).

4.2.2.1. Scan samples of the flock.

During a scan the following data were collected for each animal: posture, behaviour and nearest neighbour. The ewe-lamb distance was also recorded between each lamb and its dam. Table 4.1 contains an ethogram of the behaviours recorded during the scans and the classifications of nearest neighbours. This information was used to investigate ewe-lamb distances, nearest neighbours, behavioural synchrony between ewes and their lambs and the distance between ewes and their lambs whilst the lambs were recumbent. When assessing behavioural synchrony between ewes and their lambs it was necessary to set a distance limit over which animals were considered to be too far apart to be aware of each other and actively choosing the same activity. A limit of 10m was used as this distance has previously been used to determine association between ewes and their lambs (Arnold and Grassia 1985).

4.2.2.2. Focal observations.

Focal observations were made on individual ewe-lamb groups using a Psion Workabout handheld computer (Psion PLC, London, UK) and The Observer data

Table 4.1.
Definitions of behaviours and ewe's nearest neighbour recorded during scan samples.

Behaviour	Definition
Posture	
Stand	Animal on feet.
Lie	Animal lying down.
Behaviour	
Inactive	Animal standing or lying immobile.
Walk	Animal walking.
Graze	Animal standing or walking slowly with head down, includes biting, chewing, pulling grass and searching for food patches.
Ruminate	Animal lying or standing with head horizontal to the body, making chewing movements with mouth.
Suck	Lamb standing with head underneath the ewe in the udder region or the ewe allowing her lamb to do this.
Play	Lamb running in an apparently purposeless manner, stotting or jumping on a stationary object with peers.
Nearest Neighbour	
Own lamb	Ewe's own lamb, twin id recorded if multiple litter.
Other lamb	Lamb which isn't own, breed recorded.
Other ewe	Breed recorded.

collection software (Noldus et al. 2000). During an observation all the behaviours of both the ewe and her lamb(s) were recorded. Where the ewe and lamb were too far apart to be observed simultaneously, the ewe was observed and the lamb recorded as out of sight. An ethogram for the focal observations is shown in Table 4.2. Information from these observations was used to examine the behaviour of ewes during sucking interactions and communication between ewes and their lambs.

Table 4.2.
Definitions of ewe and lamb behaviours recorded during focal observations.

Behaviour	Definition
Ewe	
Stand (s)	Standing inactive or ruminating, head is horizontal to the body.
Graze (s)	Standing or walking slowly with head down, includes biting, chewing, pulling grass and searching for food patches.
Walk (s)	Walking with head raised, no definite movement towards the lamb.
Lie (s)	Lying inactive or ruminating.
Approach lamb (s)	Walks directly towards the lamb, may be a full (to within 1m) or partial approach.
Refuse suck attempt (e)	As the lamb attempts to reach the udder by placing its head underneath the ewe she moves her hind leg between the lamb and her udder or steps over the lamb, preventing the lamb from making contact with the udder.
Terminate suck attempt (e)	After a successful suck the ewe causes the lamb to remove its head from the udder region by moving her hind leg between the lamb and her udder, or by stepping over the lamb.
Nose lamb (e)	Ewe touches the rear of the lamb with the end of her muzzle.
Head-up (s)	Standing with head raised above the body, neck is vertical.
High pitched vocalisation (e)	'Baa' vocalisation, made with mouth open.
Low pitched vocalisation (e)	'Mmm' bleat, low, rumbly bleat made with mouth closed.
Lamb	
Stand (s)	All behaviours performed whilst on feet, including ruminating, grazing and playing.
Lie (s)	Lying inactive or ruminating.
Walk (s)	Walking with head raised, no definite movement towards dam.
Follow dam (s)	Lamb follows dam closely (less than 5m apart) as she walks.
Approach dam (s)	Walking directly towards dam, may be full (to within 1m) or partial.
Successful suck attempt (s)	Lamb places head under ewe's body and makes contact with the udder for at least 5 seconds.
Unsuccessful suck attempt (s)	Lamb attempts to place head under ewe's body in the udder region but is prevented from making contact with the udder by the ewe moving her leg or stepping over the lamb.
Terminate suck attempt (e)	Lamb terminates successful suck attempt by removing head from the udder region.
Vocalisation (e)	Lamb bleats.

Behaviours recorded as states are indicated by an 's', behaviours recorded as events are indicated by an 'e'.

4.2.3. Statistical analysis.

Data on maternal behaviour were averaged over 2 week blocks for the purposes of analysis. This was to ensure that sufficient examples of infrequent behaviours such as sucking interactions and communication would be included in the analysis of individual time periods. By averaging across a 2 week block the chances of collecting information on several instances of an infrequent behaviour were greatly increased and the accuracy of the data improved. All other data were analysed in the same way to allow comparisons and the identification of patterns or relationships between behaviours. Where ewes had twins an average was taken of the data collected for each lamb. Due to the time spent in the lambing shed after parturition the time period 'week 2' relates primarily to the second week post-partum, rather than the first 2 weeks. All other time periods relate to the full 2 weeks.

Data on lamb approaches to their dam were not analysed, due to difficulty in interpreting this behaviour. Lambs were very active and it was not always possible to confidently distinguish an intentional approach to the dam from general activity taking place in close proximity to her. Ewes were more sedentary and approaches to their lambs were easier to identify.

Data were checked for normality and transformed as necessary. Data for high pitched vocalisations, head-up postures, full approaches by the ewe and nosing behaviour were square root transformed. Data for low pitched vocalisations and ewe termination of sucking interactions could not be transformed, all other data were normally distributed. REML was used to analyse the maternal behaviour of the ewes. Sire breed, litter size and ewe breed were fitted as fixed effects in the model. Mann Whitney tests were used to analyse data on low pitched vocalisations and ewe terminations of sucking bouts. Paired T-tests were used for within breed comparisons between the ewe-lamb distance whilst the lamb was lying and during all other behaviour. The probability of a ewe randomly having her lamb as her nearest neighbour was calculated by dividing the number of lambs in her litter (1 or 2) by the number of individuals in the flock (minus 1). This was compared to the proportion of scans in which the ewe had her own lamb as nearest neighbour.

4.3. Results.

Unless otherwise stated, sire breed and litter size did not have an effect on the behaviour of the ewes.

4.3.1. The spatial relationship between the ewes and lambs.

4.3.1.1. Ewe-lamb distance.

Figure 4.2 shows the changes in ewe-lamb distance over the study period. Distance increased in both breeds up to 6 weeks post-partum and then remained relatively constant until the end of the study. Blackface ewes were closer to their lambs than Suffolk ewes at weeks 2 (REML Wald=4.46, d.f.=1, $p<0.05$), 6 (Wald=6.48, d.f.=1, $p<0.05$), 8 (Wald=34.02, d.f.=1, $p<0.001$) and 10 (Wald=4.27, d.f.=1, $p<0.05$) but not at week 4 (Wald =1.16, d.f.=1, NS). Sire breed and litter size also had an effect on ewe-lamb distance at week 8. Ewes with lambs sired by a Blackface sire were significantly closer to their lambs at this time than ewes with lambs sired by a Suffolk sire (mean distance (m), (with s.e.m.): Suffolk sire=28.83 (4.60), Blackface sire=20.36 (2.46), Wald=9.62, d.f.=1, $p<0.01$). Ewes with single lambs were significantly closer to their lambs than ewes with twin lambs (mean distance (m), (with s.e.m.): single=14.6 (2.46), twin=30.69 (3.45), Wald=29.58, d.f.=1, $p<0.001$).

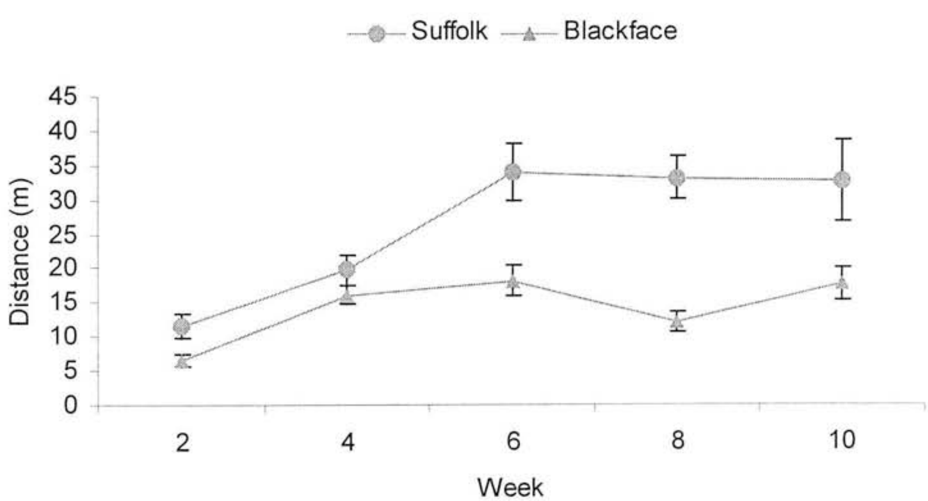

Figure 4.2. Mean distance (m) between Suffolk and Blackface ewes and their lambs over the 10 week study period (with s.e.m.).

4.3.1.2. Full approaches to the lamb by the ewe.

The majority of Blackface ewes were observed to make full approaches to their lambs for most of the 10 week study period (13, 14, 9, 9 and 7 ewes in weeks 2, 4, 6, 8, and 10 respectively). After 4 weeks post-partum less than half of Suffolk ewes were observed to make a full approach to their lamb (14, 15, 3, 6 and 5 ewes in weeks 2, 4, 6, 8 and 10 respectively). Breed comparisons were made during the first 4 weeks post-partum. There was no significant difference in the number of full approaches made by the ewes at week 2 (mean number per observation (with 95% confidence interval): Suffolk=0.40 (0.27-0.54), Blackface=0.29 (0.19-0.42), Wald=0.47, d.f.=1, NS) but Blackface ewes made significantly more full approaches to their lambs than Suffolk ewes at week 4 (mean number per observation (with 95% confidence interval): Suffolk=0.13 (0.08-0.18), Blackface=0.29 (0.22-0.36), Wald=4.78, d.f.=1, $p<0.05$).

Litter size also had an effect on ewe approaches to their lamb. Single-bearing ewes made more full approaches to the lambs than twin-bearing ewes at week 2 (mean number per observation (with 95% confidence interval): single=0.64 (0.48, 0.82), twin=0.14 (0.07, 0.23), Wald=17.66, d.f.=1, $p<0.001$) and week 4 (mean number per observation (with 95% confidence interval): single=0.25 (0.18, 0.32), twin=0.13 (0.10, 0.21), Wald=7.21, d.f.=1, $p<0.01$).

4.3.1.3. The lamb as the ewe's nearest neighbour.

During the study period ewes had their own lamb as nearest neighbour in an average of 49.65% of scans. As the chance of a ewe randomly having her own lamb as a nearest neighbour was 1.22% for single-bearing ewes and 1.23% for twin-bearing ewes, ewes appeared to be actively associating with their own lambs. Figure 4.3 shows the data collected on the proportion of scans in which ewes had their own lamb as nearest neighbour. There were no breed differences in the likelihood of a ewe having her own lamb as nearest neighbour at weeks 2 (Wald=2.15, d.f.=1, NS), 4 (Wald=0.70, d.f.=1, NS), 6 (Wald=1.03, d.f.=1, NS) and 10 (Wald=1.92, d.f.=1, NS). However at week 8 Blackface ewes were more likely to have their own lamb as nearest neighbour than Suffolk ewes (Wald=4.09, d.f.=1, $p<0.05$).

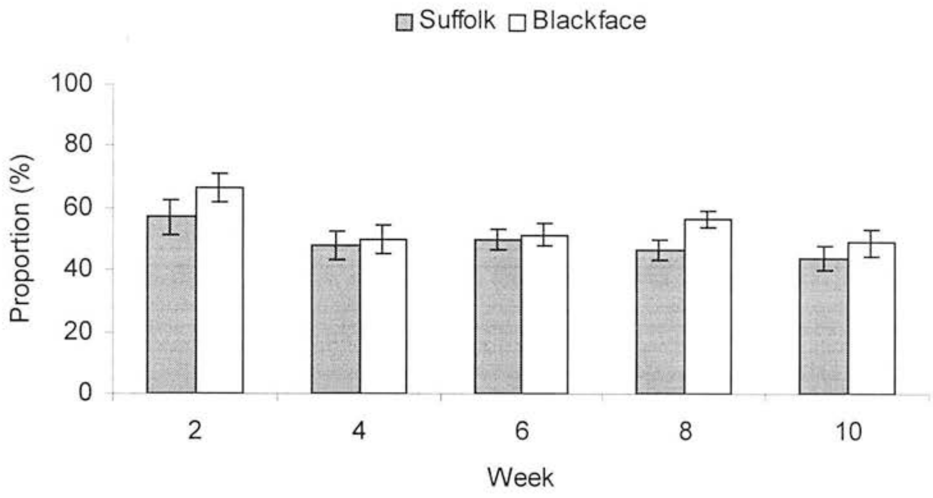

Figure 4.3. Mean proportion of scans in which Suffolk and Blackface ewes had their own lamb as nearest neighbour, over the 10 week study period (with s.e.m.).

4.3.1.4. Behavioural synchrony between ewes and lambs.

Over the entire study period all ewes showed behavioural synchrony with their lambs in an average of 43.7% of scans. Figure 4.4 shows the changes in behavioural synchrony between ewes and their lambs over the study period. There were no breed differences in the level of behavioural synchrony between a ewe and her lamb(s) at any of the time points during the study: week 2 (Wald=0.59, d.f.=1, NS), week 4 (Wald=0.12, d.f.=1, NS), week 6 (Wald=0.00, d.f.=1, NS), week 8 (Wald=0.80,

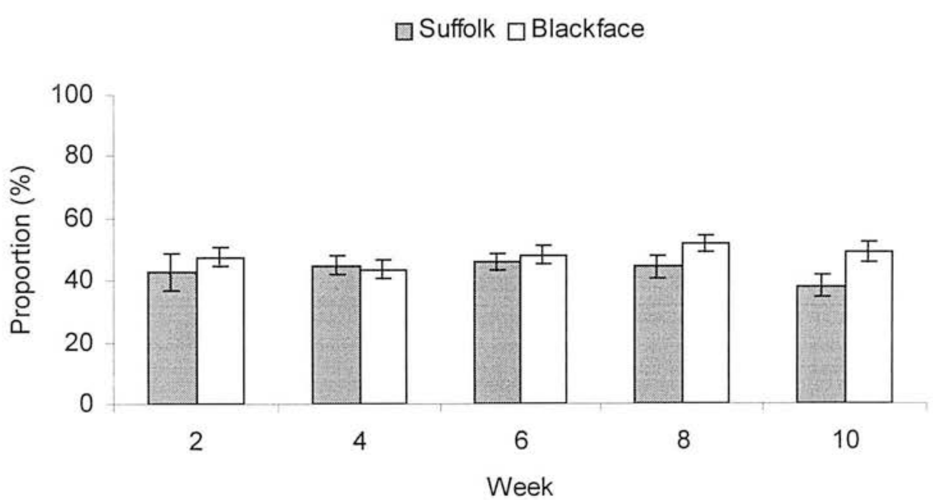

Figure 4.4. Mean proportion of scans in which Suffolk and Blackface ewes showed behavioural synchrony with their lambs, over the 10 week study period (with s.e.m.).

d.f.=1, NS) and week 10 (Wald=2.11, d.f.=1, NS).

4.3.1.5. Ewe-lamb distance whilst the lamb is recumbent.

Both breeds of ewe were closer to their recumbent lamb, compared to all other behaviours (Figures 4.5a & 4.5b), at weeks 6 (Suffolk: Paired T-test T=4.44, $p<0.001$, Blackface: T=3.13, $p<0.01$), 8 (Suffolk: T=5.47, $p<0.001$, Blackface: T=2.23, $p<0.05$), and 10 (Suffolk: T=1.86, p=0.08, Blackface: T=2.50, $p<0.05$) but not weeks 2 (Suffolk: T=-0.19, NS, Blackface: T=1.54, NS) or 4 (Suffolk: T=1.76, NS, Blackface: T=0.99, NS).

Comparing between the two breeds, Blackface ewes were closer to their recumbent lambs than Suffolk ewes at week 8 (Wald=7.44, d.f.=1, $p<0.01$) but not at weeks 2 (Wald=2.43, d.f.=1, NS), 4 (Wald=0.18, d.f.=1, NS), 6 (Wald=2.34, d.f.=1, NS) and 10 (Wald=0.71, d.f.=1, NS). Single-bearing ewes were closer to their lambs than twin-bearing ewes at weeks 2 (mean distance (m), (with s.e.m.): single=5.15 (1.19), twin=9.87 (1.54), Wald=4.24, d.f.=1, $p<0.05$), 8 (mean distance (m), (with s.e.m.): single=10.16 (2.34), twin=20.64 (2.80), Wald=8.73, d.f.=1, $p<0.01$) and 10 (mean distance (m), (with s.e.m.): single=9.88 (1.84), twin=25.2 (4.23), Wald=6.26, d.f.=1, $p<0.05$).

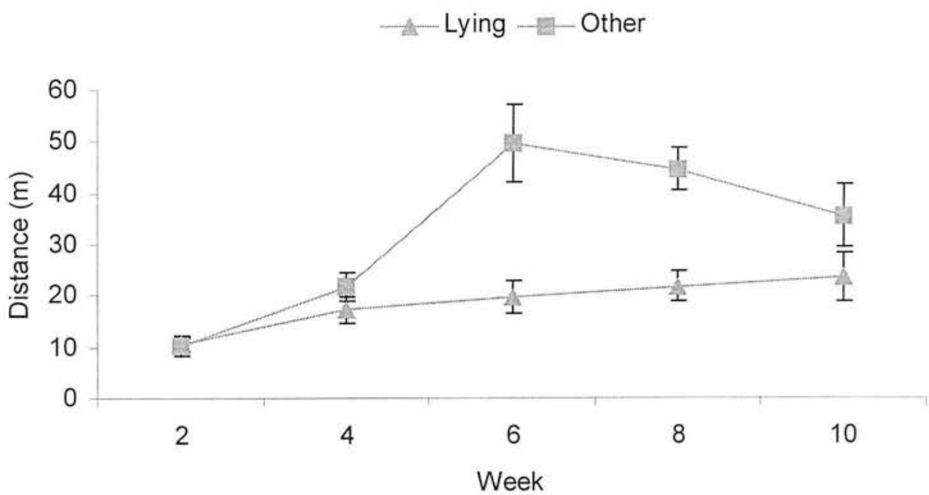

Figure 4.5a. Mean distance between Suffolk ewes and their lambs whilst the lamb is lying and during all other behaviours, over the 10 week study period, (with s.e.m.).

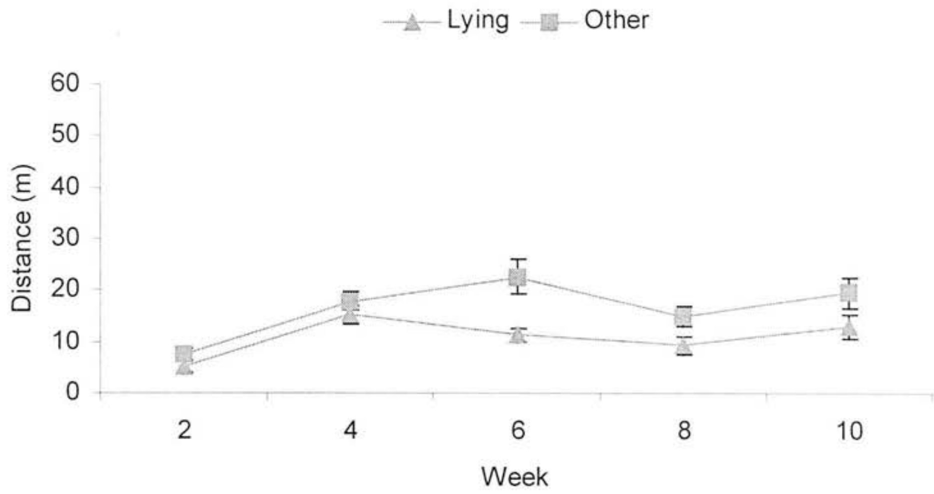

Figure 4.5b. Mean distance between Blackface ewes and their lambs whilst the lamb is lying and during all other behaviours, over the 10 week study period (with s.e.m.).

4.3.2. Communication between the ewes and lambs.

4.3.2.1. Low pitched vocalisations.

Figure 4.6 shows the median number of low pitched vocalisations expressed per observation. These vocalisations were expressed very infrequently after week 4 and the two breeds were only compared during the first two time periods. There were no differences in the frequency of low pitched vocalisations during either week 2 (Mann Whitney U=314.5, NS) or week 4 (U=332.5, NS).

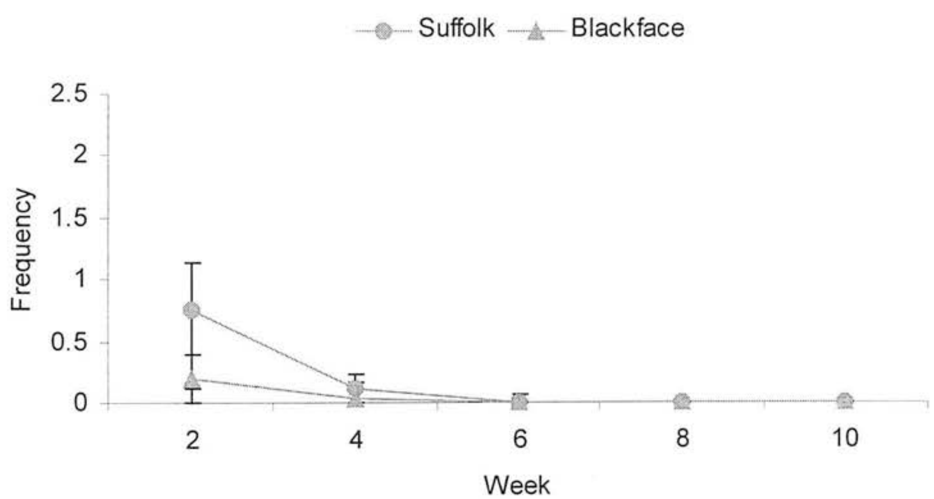

Figure 4.6. Median number of low pitched vocalisations made by Suffolk and Blackface ewes during an observation, over the 10 week study period (with Q1, Q3).

4.3.2.2. High pitched vocalisations.

Figure 4.7 shows the mean number of high pitched vocalisations expressed per observation. These vocalisations also decreased in frequency as the study progressed. There were no differences in the frequency of high pitched vocalisations during any of the time periods: week 2 (Wald=0.06, d.f.=1, NS), week 4 (Wald=0.14, d.f.=1, NS), week 6 (Wald=0.05, d.f.=1, NS), week 8 (Wald=1.41, d.f.=1, NS) and week 10 (Wald=2.26, d.f.=1, NS).

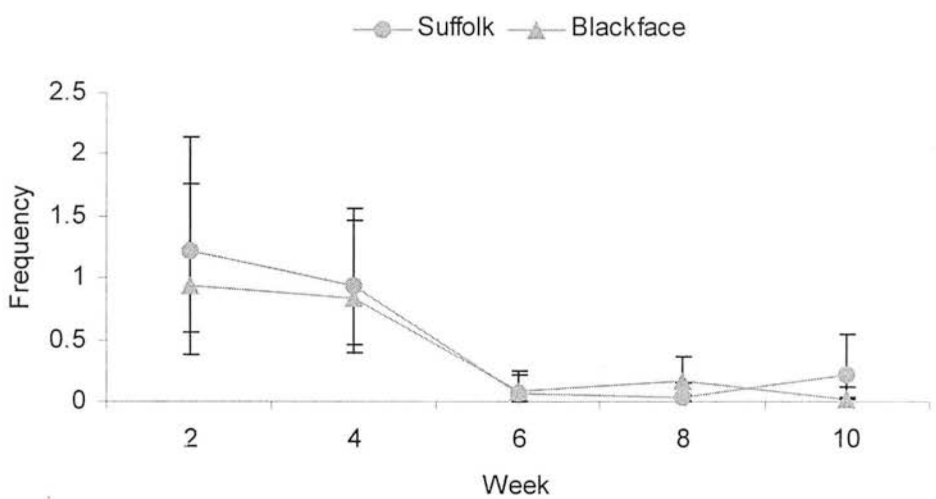

Figure 4.7. Mean number of high pitched vocalisations made by Suffolk and Blackface ewes during an observation, over the 10 week study period (with 95% confidence interval).

4.3.2.3. Head-up postures.

Figure 4.8 shows the changes in the performance of the head-up posture over the study period. In Blackface ewes performance of the head-up posture showed a pronounced peak at 4 weeks post-partum, followed by a steady but slow decline between weeks 6 and 10. Suffolk ewes did not show a peak in their performance of the head-up posture and showed a fairly steady but low performance during the first 6 weeks post-partum. This also declined between weeks 6 and 10. Over time Blackface ewes performed a higher frequency of head-up postures than Suffolk ewes at weeks 4 (Wald=9.13, d.f.=1, $p<0.01$), 6 (Wald=3.85, d.f.=1, $p<0.05$), 8 (Wald=10.04, d.f.=1, $p<0.01$) and 10 (Wald=4.13, d.f.=1, $p<0.05$) but there was no difference between the two breeds at week 2 (Wald=0.28, d.f.=1, NS).

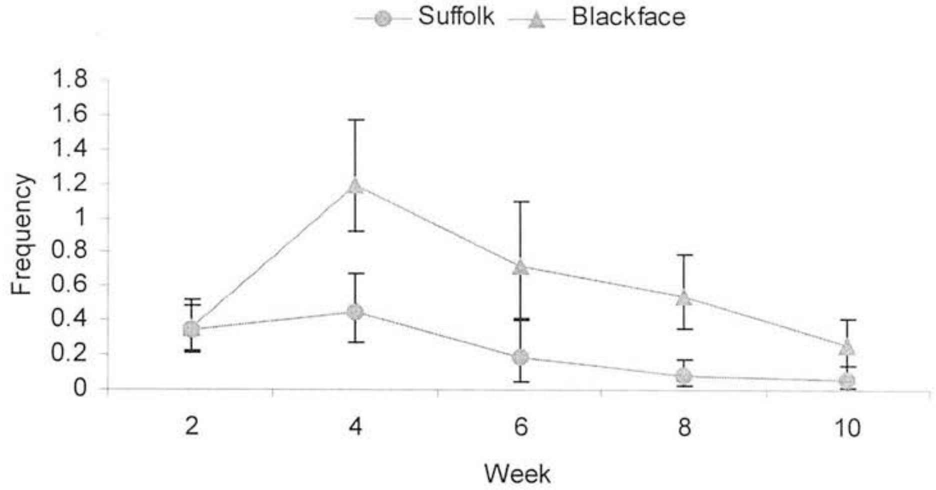

Figure 4.8. Mean number of head-up postures made by Suffolk and Blackface ewes during an observation, over the 10 week study period (with 95% confidence interval).

4.3.3. Sucking interactions in later lactation.

4.3.3.1. Proportion of suck attempts which were successful.

Figure 4.9 shows the proportion of successful suck attempts made by lambs throughout the study period. A successful suck attempt was one in which the lamb made contact with the udder for at least 5 seconds. Lambs with a Blackface dam had a higher proportion of successful sucking attempts at weeks 2 ($U=207.5$, $p<0.01$),

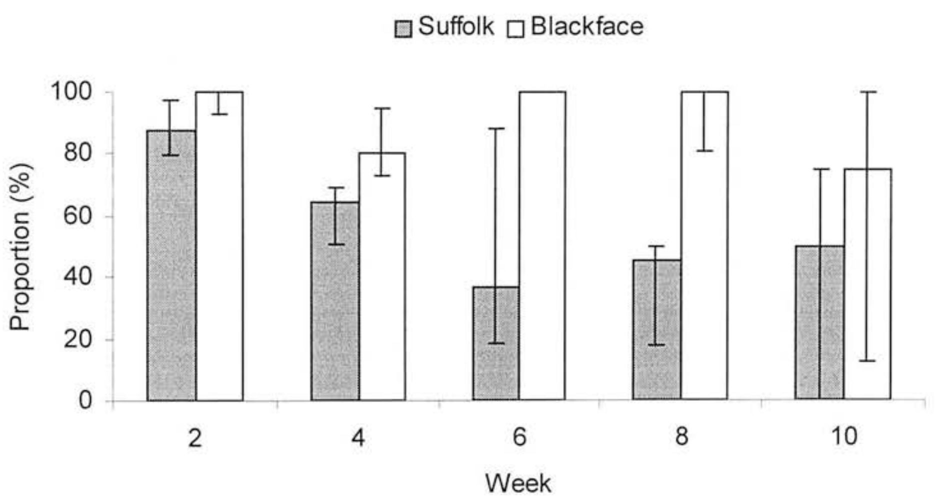

Figure 4.9. Median proportion of lamb suck attempts accepted by Suffolk ewes and Blackface ewes, over the 10 week study period (with Q1, Q3).

4 (U=215.0, p<0.01), 6 (U=142.0, p<0.01) and 8 (U=156.5, p<0.01), compared to lambs with a Suffolk dam. There were no breed differences in the proportion of successful sucks at week 10 (U=100.0, NS).

4.3.3.2. Frequency and length of sucking bouts.

Figure 4.10 shows the number of sucking bouts per observation. Lambs with a Blackface dam had a lower frequency of sucking bouts than lambs with a Suffolk dam at week 2 (Wald=4.37, d.f.=1, p<0.05) but not at weeks 4 (Wald=1.75, d.f.=1, NS), 6 (Wald=0.07, d.f.=1, NS), 8 (Wald=1.39, d.f.=1, NS) and 10 (Wald=1.24, d.f.=1, NS).

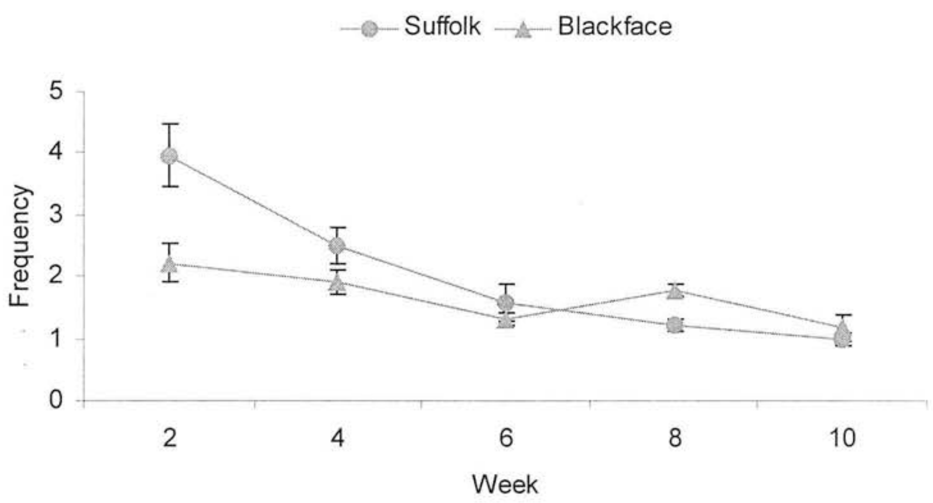

Figure 4.10. Mean number of sucking bouts made by the lambs of Suffolk and Blackface ewes during an observation, over the 10 week study period (with s.e.m.).

Lambs with a Blackface dam also had a longer bout length at week 2 (Wald=5.69, d.f.=1, p<0.05) but not at weeks 4 (Wald=0.05, d.f.=1, NS), 6 (Wald=0.51, d.f.=1, NS), 8 (Wald=0.72, d.f.=1, NS) and 10 (Wald=0.11, d.f.=1, NS), compared to lambs with a Suffolk dam (Figure 4.11). Single lambs had a longer bout duration than twin lambs at weeks 2 (mean duration (s) (with s.e.m.): single=31.43 (3.62), twin=20.38 (2.69), Wald=6.03, d.f.=1, p<0.05) and 10 (mean duration (s) (with s.e.m.): single=8.21 (1.73), twin=4.41 (1.15) Wald=4.86, d.f.=1, p<0.05).

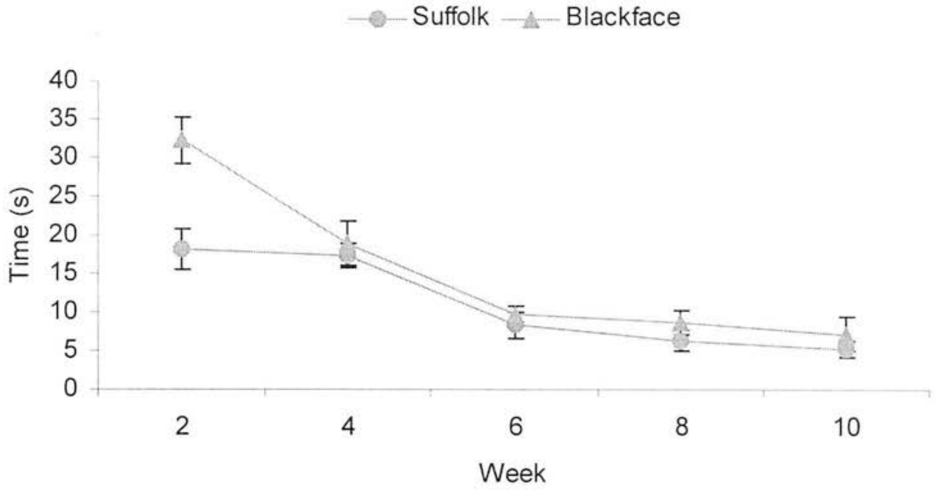

Figure 4.11. Mean duration of a sucking bout made by the lambs of Suffolk and Blackface ewes, over the 10 week study period (with s.e.m.).

4.3.3.3. Termination of sucking bouts by the ewe.

The proportion of successful sucking bouts terminated by the ewe was also compared between the two breeds (Figure 4.12). During the first 2 weeks post-partum ewes of both breeds terminated around half of all sucking bouts. The frequency of ewe terminations then increased and by 6 weeks post-partum ewes of both breeds terminated almost 100% of sucking bouts. This continued until the end of the study. There were no breed differences in the proportion of ewe terminations at any time

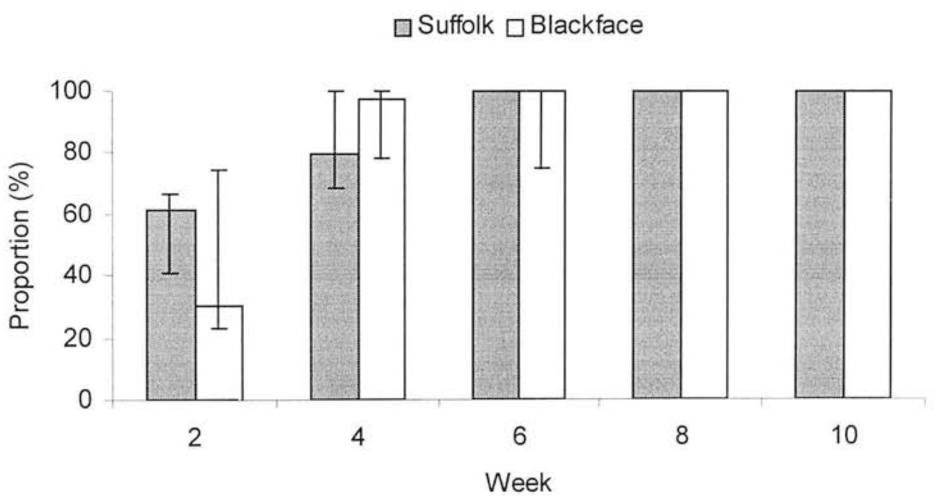

Figure 4.12. Median proportion of successful sucks terminated by Suffolk and Blackface ewes, over the 10 week study period (with Q1, Q3).

period: week 2 (U=287.0, NS), week 4 (U=262.0, NS), week 6 (U=158.0, NS), week 8 (U=165.5, NS) and week 10 (all ewes terminated all successful suck attempts).

4.3.3.4. Nosing by the ewe.

Figure 4.13 shows the number of instances of nosing per observation. Nosing was very infrequent after 4 weeks post-partum and the two breeds were only compared during the first 4 weeks. Blackface ewes nosed their lamb(s) more frequently at week 2 compared to Suffolk ewes (Wald=5.74, d.f.=1, $p<0.05$) but not at week 4 (Wald=0.71, d.f.=1, NS). Single-bearing ewes also nosed their lambs more frequently at weeks 2 (mean number of noses per observation (with 95% confidence interval): single=1.46 (1.08-1.91), twin=0.51 (0.29-0.79), Wald=16.25, d.f.=1, $p<0.001$) and 4 (mean number of noses per observation (with 95% confidence interval): single=1.03 (0.83-1.26), twin=0.59 (0.44-0.77), Wald=7.59, d.f.=1, $p<0.01$).

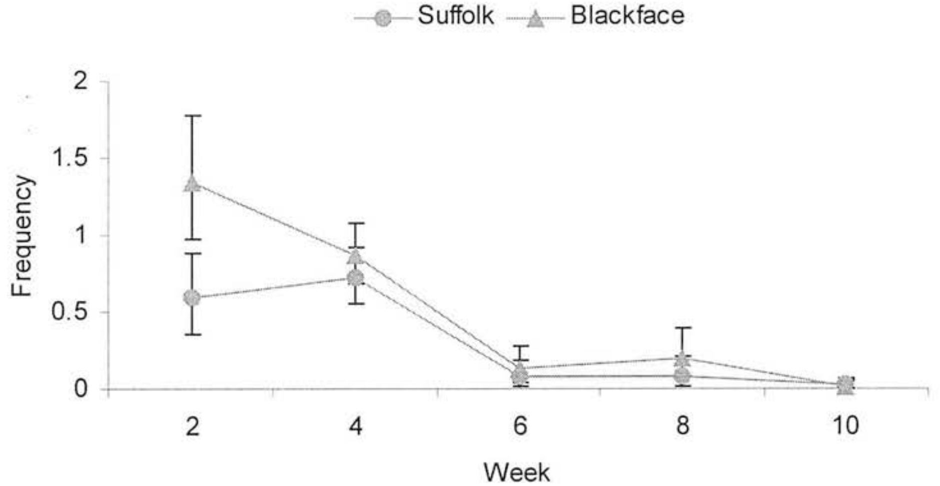

Figure 4.13. Mean number of times Suffolk and Blackface ewes nose their lamb during an observation, over the 10 week study period (with 95% confidence interval).

4.4. Discussion.

As hypothesised, Suffolk and Blackface ewes continue to show differences in their maternal care beyond the post-partum period. Ewes of the two breeds show differences in some aspects of their spatial relationship with their young, their communication with them and their behaviour during sucking interactions.

4.4.1. The spatial relationship between the ewes and lambs.

On the whole the two breeds did not differ in the proportion of scans in which the ewes had their own lamb as nearest neighbour, or in which they showed behavioural synchrony with their lambs. The exception to this was week 8, when Blackface ewes were more likely to have their own lamb as a nearest neighbour than Suffolk ewes. However Blackface ewes were significantly closer to their lambs than Suffolk ewes for the majority of the study period, the exception being week 4. They also made more approaches to their lamb at week 4 than Suffolk ewes, and appeared to continue making them for longer than Suffolk ewes.

4.4.1.1. Reasons for the breed differences in ewe-lamb distance.

Although ewe-lamb proximity is maintained by both the ewe and her lamb, the manner by which it is maintained changes over time and the lamb assumes increasing responsibility as it becomes older (e.g. Arnold and Grassia 1985, Hinch et al. 1987, Hinch et al. 1990). Consequently, although the behaviour of the ewe is likely to have a key role at all times, the means by which it influences ewe-lamb distance will alter with increasing lamb age and the corresponding changes in the dynamics of the ewe-lamb relationship.

During the first few weeks post-partum ewes take an active role in the maintenance of proximity and will approach their lambs when separated from them (e.g. Arnold and Grassia 1985, Hinch et al. 1987). The shorter ewe-lamb distance in Blackface ewes at week 2 could therefore be directly due to ewe behaviour, with Blackface ewes approaching their lambs more frequently than Suffolk ewes. However the two breeds did not show a significant difference in the frequency of approaches to their lambs at this time, and this cannot be the explanation for the shorter ewe-lamb distance in Blackface ewes. During the first week post-partum ewes are known to show greater maternal protection towards their lambs and are likely to stay close to them in order to do this (Morgan and Arnold 1974). In addition, lying is a common behaviour in lambs during the first 2 weeks post-partum and behaviours which may result in the lamb moving away from its dam, for example grazing and playing, do not occur frequently at this time (Morgan and Arnold 1974). It is therefore possible that Blackface ewes simply stayed closer to their lambs than Suffolk ewes during the first 2 weeks post-partum. The maternal choice test in Chapter 3 showed that Blackface ewes have a closer association with their lambs at 3 days post-partum compared to Suffolk ewes. Their closer ewe-lamb distance at 2 weeks post-partum could therefore result from a continuation of the Blackface ewes' greater motivation to stay close to their lambs.

Beyond the first month post-partum responsibility for directly maintaining ewe-lamb proximity passes to the lamb and ewes show less inclination to approach their lambs (e.g. Hinch et al. 1987, Hinch et al. 1990). Consequently, although Blackface ewes appeared to continue making approaches for longer than Suffolk ewes, the closer ewe-lamb distance between Blackface ewes and their lambs after 4 weeks is unlikely to be solely due to these ewes approaching their lambs more frequently than Suffolk ewes. It must therefore also be due to the motivation of the lambs to seek proximity with their dams. This would be expected to be related to the relationship the lambs have with them, which is in turn influenced by the ewes' behaviour towards their lambs, both at the present time and earlier in the relationship.

4.4.1.1.1. Influence of the ewe's earlier behaviour.

Previous studies have shown that the ewe's response to initial sucking interactions affects the development of attachment between a lamb and its dam (Nowak 1996). The two breeds of ewe in this study are known to show differences in their maternal behaviour during the post-partum period, including their response to initial sucking attempts (Dwyer and Lawrence 1998, Chapter 3). The greater co-operation with suck attempts shown by Blackface ewes, compared to Suffolk ewes, may therefore result in lambs with a Blackface dam establishing a stronger attraction towards their dam than lambs with a Suffolk dam, and consequently being more motivated to seek proximity to her during later lactation. This is investigated further in Chapter 8.

4.4.1.1.2. Influence of the ewe's present behaviour.

As the lamb ages, the ewe's behaviour during sucking interactions continues to be important in maintaining the ewe-lamb relationship (Shillito and Hoyland 1971, Arnold et al. 1979). As a result, her reaction to suck attempts in later lactation is likely to influence her lamb's attraction towards her and, consequently, their ewe-lamb distance. Ewes are also known to control the movements of their older lambs with postural (Lawrence 1984) and vocal communication (Shillito-Walser et al. 1984). Therefore, although the ewe does not contribute to the maintenance of proximity by actively approaching her lamb after 4 weeks post-partum, her behaviour continues to influence the spatial relationship she has with her lamb. Breed differences in the ewes' response to sucking interactions and their communication with their lambs may therefore account for the shorter ewe-lamb distance in Blackface ewes, compared to Suffolk ewes. The roles of communication and sucking interactions in the maintenance of ewe-lamb proximity are discussed in the relevant sections below.

4.4.1.2. Behaviour of ewes whilst lambs were recumbent.

Between 6 and 10 weeks post-partum all ewes maintained a shorter ewe-lamb distance whilst their lamb was recumbent, compared to all other behaviours. As discussed above the responsibility for maintaining proximity gradually passes from the ewe to the lamb at around 1 month of age. It is therefore interesting that the differentiation between ewe-lamb distance for recumbent lambs and ewe-lamb distance for all other behaviours does not occur until the lambs are 5-6 weeks old and have assumed responsibility for the maintenance of proximity. It would appear that, although the ewes are no longer helping to maintain proximity with their lambs when both are active, they are still maintaining proximity when their lambs are recumbent. However, it is also possible that the shorter ewe-lamb distance is a function of the whole flock coming together to rest. Further work is needed to clarify whether this is a flock reaction or if ewes are independently seeking proximity to their recumbent lambs.

There were no breed differences in the ewe-lamb distance when lambs were recumbent with the exception of week 8 when Blackface ewes were closer to their recumbent lambs, compared to Suffolk ewes. The behaviour of the ewes when their lambs were recumbent is therefore unlikely to be contributing to the differences in general ewe-lamb proximity.

4.4.2. Communication between the ewes and lambs.

4.4.2.1. Ewe vocalisations.

Vocalisation by the ewes in this study was infrequent, especially as their lambs became older. Low pitched vocalisations were particularly infrequent. These vocalisations are almost exclusively associated with newborn lambs (Shillito 1972) and beyond the immediate post-partum period the low pitched rumble is thought to be associated with early recognition between ewes and their lambs (Shillito and Hoyland 1971). Consequently it is unsurprising that these vocalisations were most frequently heard during the first few weeks post-partum. Given their association with newborn lambs and early recognition it is unlikely that low pitched vocalisations are used directly in the maintenance of proximity between ewes and their older lambs. This likelihood is further reduced by their low amplitude and the consequent improbability of them being heard across long distances. However, low pitched vocalisations may have an indirect contribution to the maintenance of proximity by facilitating early recognition between ewes and their lambs.

In contrast, high pitched vocalisations are known to be used in communication with older lambs, principally when the ewes and lambs are separated (e.g. Shillito 1972, Shillito-Walser et al. 1984). Consequently they have the potential for a role in the maintenance of ewe-lamb proximity. However vocalisations are not just audible to lambs and are likely to attract predators (Hansen et al. 2001). It would therefore be expected that ewes would vocalise infrequently, as reflected in the present study, and use an alternative form of communication with their lambs for the frequent interaction needed for the maintenance of proximity. Vocalisation may only be used when the ewes and lambs are separated by a large distance and/or for a long time period, and consequently out of sight of each other.

High-pitched vocalisations were expressed most frequently during the first 4 weeks of this study, after which there was a marked decrease, and for the remaining weeks they were very infrequent in both breeds. Ewe-lamb separation is likely to be most frequent during the first few weeks post-partum, compared to later lactation, as ewes and lambs learn to recognise each other at a distance and establish their spatial relationship. This may account for the higher frequency of high-pitched vocalisations during the first few weeks post-partum.

In conclusion, it appears unlikely that vocalisation is used in the regular maintenance of ewe-lamb proximity. The lack of breed differences in the expression of ewe vocalisations also makes it unlikely that this ewe behaviour accounts for the differences in ewe-lamb distance between the two breeds.

4.4.2.2. Postural communication.

Postural communication is silent and therefore less likely to attract predators than vocalisations, giving it greater potential for a role in the regular maintenance of ewe-lamb proximity. The head-up posture has also previously been shown to act as a signal for lambs to approach their dam (Lawrence 1984). Blackface ewes had a higher rate of performance of the head up posture than Suffolk ewes from week 4 onwards, the time when the dynamics of the ewe-lamb relationship begin to change and the lamb assumes responsibility for maintaining proximity. Blackface ewes therefore appear to be using this method of maintaining contact at a higher rate than Suffolk ewes and this may be a reason for their shorter ewe-lamb distance compared to Suffolk ewes. The use of the head-up posture by the two breeds of ewe, and the response of their lambs, is further explored in Chapter 5.

4.4.2.3. Learning the association between the head-up posture and approach to the dam.

The performance of the head-up posture in Blackface ewes increased to a peak at 4 weeks post-partum and then declined in frequency. This peak occurs at the time when the ewe stops seeking her young (Hinch et al. 1987, Hinch et al. 1990) and the lamb must learn to maintain contact with its dam. The high performance rate of the head-up posture at this time may therefore facilitate the association of the head-up posture with approach to the dam in lambs with a Blackface dam. The peak in the performance of the head-up posture also coincided with a dip in the proportion of successful suck attempts in the Blackface ewes. Blackface ewes accepted almost 100% of their lambs' suck attempts at weeks 2, 6, and 8 but only around 80% at week 4. Several studies (Ewbank 1967, Lawrence 1984, O'Connor 1990) have reported that ewes occasionally signal for their lamb to approach, through a vocalisation or, more frequently, a postural change, but then refuse sucking and walk off with the lamb following. It is possible that similar behaviour in the Blackface ewes helped their lambs learn to associate the head-up posture with approach to the dam. The lower proportion of successful sucks may have resulted from ewes signalling for their lambs to approach, refusing a suck by walking away and then, after the lamb had followed, allowing a suck. The sucking interaction would have acted as a reward for approaching and following the ewe, facilitating the lambs learning to do so in response to the head-up posture. This is also investigated further in Chapter 5.

4.4.3. Sucking interactions in later lactation.

4.4.3.1. Sucking interactions during the first few weeks post-partum.

During the first few weeks post-partum ewes are expected to allow their lambs to suck ad libitum and not restrict access to the udder (Ewbank 1967). At this time lambs with a Suffolk dam had a higher frequency of successful sucking attempts per observation, compared to lambs with a Blackface dam. However, the duration of these sucking bouts was shorter and, overall, Suffolk ewes were less likely than Blackface ewes to accept a suck attempt. This suggests that Suffolk ewes were showing less co-operation with their lamb's suck attempts, compared to Blackface ewes. Suffolk ewes also showed less co-operation with their lambs' initial suck attempts than Blackface ewes (Chapter 3) and this may reflect a continuation of the Suffolk ewes' lower co-operation with sucking interactions.

The ewe's co-operation with suck attempts is likely to have consequences for the attachment between the lamb and ewe, a higher rate of co-operation leading to a stronger attachment. The greater co-operation by the Blackface ewes may therefore contribute to their shorter ewe-lamb distance by facilitating a stronger dam attachment by their lambs. In contrast, the lower co-operation seen in the Suffolk ewes is likely to lead to a comparatively lower attraction toward the dam and, consequently, a greater ewe-lamb distance.

4.4.3.2. Sucking interactions after ewes begin to control their lambs' sucking behaviour.

After the first few weeks post-partum ewes begin to control the duration and frequency of sucking interactions, restricting access to the udder (Trivers 1985). This is reflected by the fact that almost all sucking interactions were terminated by the ewe from week 5 onwards. The two breeds did not differ in the proportion of terminations made by the ewe but Blackface ewes were more likely than Suffolk ewes to accept suck attempts up to week 8. Their lambs consequently had a higher rate of successful sucks than lambs with a Suffolk dam. As the ewes were not allowing ad libitum sucking after the first few weeks post-partum this must be due to differences in the ewes' method of control, rather than their co-operation with suck attempts.

4.4.3.3. Control of sucking interactions.

Lambs with a Blackface dam were allowed to suck almost every time they approached their dam and made an attempt. This suggests that the Blackface ewes were using 'distance control' of sucking, signalling when their lambs could approach and make a suck attempt. The use of distance control by Blackface ewes is supported by their frequent performance of the head-up posture.

Suffolk ewes do not appear to use distance control of suck attempts. Not only do they have a lower performance of the head-up posture compared to Blackface ewes, their higher proportion of unsuccessful suck attempts suggests that Suffolk ewes control when their lambs may suck once they have approached and made an attempt. Their lambs must therefore approach randomly and make a suck attempt, rather than being signalled to do so by their dam. Only once the lamb has begun an attempt will the ewe indicate whether or not it may proceed to a successful suck. These apparent differences in the control of sucking interactions by the two breeds are investigated further in Chapter 5.

4.4.3.4. Influence of control methods on the lamb's attachment to its dam.

As discussed above, sucking interactions are thought to reinforce the ewe-lamb bond (Arnold et al. 1979), and the response of the ewe to a suck attempt is important for lamb attachment to the dam during later lactation. By using a signal to control when their lambs may approach and suck Blackface ewes are more likely to have consistent, positive responses to their lambs' attempts to suck. In contrast, the control of sucking interactions once their lambs have made an attempt will result in Suffolk ewes having inconsistent responses, sometimes allowing them to suck, sometimes preventing them. A consistent positive response is more likely to encourage the lamb to be attracted to its dam and maintain a close proximity. This may be another reason for the Blackface ewes' closer proximity to their lambs, compared to Suffolk ewes, once the responsibility for maintenance of proximity passes to the lamb.

4.4.4. Breed differences in maternal motivation and strength of the ewe-lamb bond in later lactation.

The behaviour of the ewes in the maternal choice test (Chapter 3) suggested that Blackface ewes have a greater maternal motivation and stronger ewe-lamb bond than Suffolk ewes at 3 days post-partum. Several of the results from the observation of maternal behaviour during later lactation suggest that this continues as the lambs become older. For the majority of the study period Blackface ewes were closer to their lambs than Suffolk ewes. Ewe-lamb distance has previously been used as an indicator of the strength of the ewe-lamb bond (Morgan and Arnold 1974). Therefore the closer ewe-lamb distance in Blackface ewes suggests a stronger bond with their lambs compared to that between Suffolk ewes and their lambs.

As discussed earlier, the closer ewe-lamb distance observed in Blackface ewes during the first 2 weeks, whilst ewes share the responsibility for maintaining proximity with their lambs (e.g. Arnold and Grassia 1985, Hinch et al. 1987), may also indicate the continuation of a greater maternal motivation and desire to associate with their lambs, compared to Suffolk ewes. In addition, their greater co-operation with suck attempts and higher rate of nosing at this time are likely to be further indicators of a stronger maternal motivation, compared to Suffolk ewes. Nosing is thought to be used by ewes to identify and maintain contact with their lambs during the first few days post-partum (Shillito and Hoyland 1971).

Beyond the first few weeks post-partum the ewe does not take a very active role in the ewe-lamb relationship and it is hard to interpret her behaviour in terms of maternal motivation. However, the fact that the majority of Blackface ewes, but only a few Suffolk ewes, were observed to continue making approaches to their lambs until the end of the study may also indicate a greater maternal motivation in Blackface ewes. In addition, the head-up posture appears to be one of the main methods by which the ewe maintains contact with her lamb during later lactation (Lawrence 1984, O'Connor 1990). Consequently, the higher expression of this posture in Blackface ewes could indicate that they are more maternally motivated than Suffolk ewes at this time, as it suggests a greater desire to maintain contact with their lambs.

4.4.5. Comparisons of the spatial relationship between ewes and lambs at different ages.

The spatial relationship of ewes and lambs in these two breeds has previously been studied between 2 and 4 months post-partum (Dwyer and Lawrence 1999b, 2000a), allowing comparisons of ewe and lamb behaviour at different ages. In the later studies Blackface ewes were found to be consistently closer to their lambs than Suffolk ewes, more likely to have their own lamb as nearest neighbour (Dwyer and

Lawrence 1999b) and to show more behavioural synchrony with their lambs compared to Suffolk ewes (Dwyer and Lawrence 2000a). The ewes in the present study showed similar results for ewe-lamb distance but did not show consistent differences in their likelihood of having their lamb as nearest neighbour or the behavioural synchrony shown with their lambs. These different results could be due to the ages of the animals studied or differences in experimental set-up between the two studies.

4.4.5.1. The influence of age on the ewe-lamb spatial relationship.

The present study was conducted during a time of great change in the both the ewe-lamb relationship and the behaviour of the lambs. During the first few weeks post-partum lambs switch from being fully dependent on their dam for nutrition to using grazing as their main source of food (e.g. Morgan and Arnold 1974). They also change from having very close contact with their dams during the first week (Arnold and Grassia 1985) to associating and playing with peers at around 2 weeks old (Morgan and Arnold 1974). They then begin to graze and return to a close proximity with their dams over the following months (Arnold and Grassia 1985). In contrast, grazing is the main activity for the ewe throughout lactation (Morgan and Arnold 1974). Consequently the different activities of younger lambs, compared to their dams, decreases the likelihood of them showing behavioural synchrony with their dams and their association with peers during the first few weeks decreases their chances of being nearest neighbours. Conversely, the tendency for older lambs to graze by their mother's side (Arnold and Grassia 1985) increases their chances of showing behavioural synchrony and being nearest neighbours. Breed differences in behavioural synchrony and the tendency to be nearest neighbours may therefore only be present when the ewes and lambs show similar behaviour.

4.4.5.2. The influence of environment on the ewe-lamb spatial relationship.

The two studies were also conducted in different environments, the present study in a relatively small (6.4 ha) field, the other studies in larger fields (9 ha and 21.4 ha). This could have affected the behaviour of the animals and the tendency for ewes and lambs to be nearest neighbours. In their natural environment hill breeds have a small subgroup size, compared to those of lowland breeds (Arnold and Pahl 1967, Shillito-Walser and Hague 1981). A small subgroup would increase the chance of a ewe having her own lamb as nearest neighbour, compared to a ewe in a large group. Dwyer and Lawrence (1999b) found that Blackface ewes altered their grouping behaviour according to the features of their environment. In a large (21.4 ha) geographically heterogeneous field the Blackface ewe subgroup size was similar to those found on an established home range, whereas in a small (9 ha) geographically homogeneous field they made closer associations with other ewes and aggregated into larger subgroups. Given the comparatively small size of the current study field (6.4 ha) it is possible that Blackface ewes aggregated into large subgroups, decreasing the likelihood of their own lamb being their nearest neighbour, even when they were in close proximity. This also highlights the problem of using 'nearest neighbour' as a measure of ewe-lamb association as only one individual can be recorded as an animal's nearest neighbour. Ewe-lamb proximity does not have this confounding factor and is therefore a more accurate measure of ewe-lamb association.

4.5. Summary.

Breed differences in maternal behaviour persisted throughout lactation. Blackface ewes were closer to their lambs than Suffolk ewes for the majority of the study period and also showed more postural communication with them and a more positive response to sucking interactions. Ewe-lamb distance has previously been used as an indicator of the strength of the ewe-lamb bond (Morgan and Arnold 1974) and the closer ewe-lamb distance in the Blackface ewes therefore suggests a stronger ewe-lamb bond during later lactation, compared to the Suffolk ewes. This is a continuation of observations in Chapter 3 which suggested a stronger maternal motivation in Blackface ewes, compared to Suffolk ewes. Other differences in maternal behaviour, such as higher rate of nosing at week 2, more co-operation with suck attempts during the first few weeks and more frequent postural communication with their lambs, also suggest that Blackface ewes continue to be more maternally motivated than Suffolk ewes.

Beyond 4 weeks post-partum the lamb assumes responsibility for the maintenance of proximity and the ewe influences ewe-lamb distance indirectly, via her behaviour toward her lamb and the nature of the relationship she has with it. Therefore, although Blackface ewes appeared to continue making approaches to their lambs for longer than Suffolk ewes, the differences in maternal behaviour between the two breeds are likely to be the main reason for the shorter ewe-lamb distance in Blackface ewes. The more frequent performance of the head-up posture and the higher proportion of suck attempts accepted by Blackface ewes, compared to Suffolk ewes, may alter the attraction of their lambs towards them, and their motivation to seek proximity with them. This is investigated further in Chapter 5.

Chapter 5: Interactions between ewes and their lambs during later lactation.

5.1.Introduction.

The aim of this chapter is to expand ideas developed in Chapter 4 and to explore in detail the use of postural communication by Suffolk and Blackface ewes and their behaviour during sucking interactions with their lambs.

5.1.1. The use of postural communication.

In Chapter 4 Blackface ewes were found to be significantly closer to their lambs than Suffolk ewes, and also performed the head-up posture at a higher rate. As the head-up posture has previously been identified in the maintenance of ewe-lamb proximity (Lawrence 1984, O'Connor 1990), it was proposed that Blackface ewes were using the head-up posture to maintain contact with their lambs, resulting in their shorter ewe-lamb distance compared to Suffolk ewes. The low rate of postural communication in Suffolk ewes suggests that they are either not using the head-up posture, or are using it inefficiently compared to the Blackface ewes. By looking at the response of lambs to the performance of the head-up posture by their dam, it is possible to examine whether or not postural communication plays a role in the maintenance of proximity between ewes and their lambs. A tendency for the lambs to approach their dam after a head-up posture would be expected to result in ewes and lambs being closer together over time, if the posture is performed frequently. It was hypothesised that, compared to Suffolk ewes, Blackface ewes received a higher response rate from their lambs when they performed the head-up posture and that these responses were more likely to result in the lambs moving closer to their dam.

5.1.2. The behaviour of the ewes during sucking interactions.

The head-up posture has also previously been linked with sucking interactions between ewes and their lambs (Lawrence 1984, O'Connor 1990). In Chapter 4 Blackface ewes were found to have a higher proportion of successful suck attempts compared to Suffolk ewes. It was proposed that Blackface ewes may employ a different control strategy to Suffolk ewes, using certain behaviours or signals to indicate that their lambs may approach and make a suck attempt, thereby controlling the sucking interactions from a distance. Conversely Suffolk ewes may not use signals but control whether or not their lambs can suck once they have approached and made an attempt. The higher frequency of head-up postures in Blackface ewes raises the possibility that they could be using this signal for the distance control of sucking.

To investigate this further, the behaviour of ewes before and during sucking attempts was examined. In particular, the tendency for successful suck attempts to follow specific ewe behaviours would be expected to indicate that ewes are using a signal to control when their lambs may approach and suck. It was hypothesised that Blackface ewes would perform specific behaviours that were consistently associated with successful sucks but Suffolk ewes would not show such associations.

Other features of sucking interactions, such as the behaviour of the lamb after a refusal, can also provide important information about the ewes' control of their lambs' sucking behaviour. Inconsistent control of sucking by the ewe may result in a refusal being followed by multiple attempts over a short period of time. As Blackface ewes appear to be more consistent in their control of sucking interactions it was hypothesised that Blackface ewes would receive fewer chains of multiple attempts than Suffolk ewes and that these chains would be shorter than those seen in Suffolk ewes.

5.2. Methods.

Data were taken from the focal observations described in Chapter 4, therefore animals, husbandry practices and data collection methods are as detailed in Chapters 2 and 4.

5.2.1. Lamb response to head-up postures.

The response of lambs to the performance of a head-up posture by their dam was determined by analysing the behaviour of lambs following a head-up posture. Definitions of the head-up posture and lamb behaviours are given in Table 5.1. The proportion of head-up postures followed by each of the 8 possible responses was recorded for each ewe. Twin lambs were recorded as separate animals. A lamb was considered to have responded to a head-up posture if it changed its behaviour within 25 seconds of the ewe first performing the head-up posture. This time period was calculated in a previous study using log survivorship curves of time intervals between changes in ewe behaviour and the lamb approaching its dam (O'Connor 1990). Log survivorship curves describe the probability of an event occurring relative to the time elapsed since the last event (Lehner 1979, cited in O'Connor

Table 5.1.
Definitions of the head-up posture and lamb responses to the head-up posture.

Behaviour	Definition
Head-up posture	Ewe stands rigidly with her head held above her body, her neck is vertical.
Lamb responses	
None	Lamb does not show any change in its behaviour following a head-up response.
Approach and successful suck	Lamb walks or runs directly towards its dam and places its head underneath her in the udder region, makes contact with the udder.
Approach and unsuccessful suck	Lamb walks or runs directly towards its dam and tries to place its head underneath her in the udder region but is prevented from making contact with the udder by the ewe.
Approach only	Lamb walks or runs directly towards its dam and stands within 1m of her, the lamb does not attempt to suck.
Partial approach	Lamb walks or runs directly towards its dam but stops before it is within 1m of her.
Already following	Lamb is already following, within 5m, behind the walking ewe when she stops and makes a head-up posture.
Already sucking	Lamb has its head underneath the ewe in the udder region and is making contact with the udder.
Other	Lamb shows a change in its behaviour (e.g. stands from lying, stops walking) but does not approach the ewe.

1990). This analysis identified 25 seconds as the time period indicative of an immediate response by the lamb to a change in its dam's behaviour.

5.2.2. Statistical analysis.

Data were checked for normality and transformed as necessary. Data for all lamb responses involving a full approach were log (base 10) transformed. Data for lamb response over the whole observation period were normally distributed. ANOVA was used to analyse changes in lamb response to the head-up posture over time. REML was used to analyse the data at individual time points. Sire breed, litter size and ewe breed were fitted as fixed effects in the model.

5.2.3. Ewe and lamb behaviour in association with sucking interactions.

This analysis focused on the behaviour of the ewe immediately before a suck attempt and the reaction of the ewe towards the suck attempt i.e. did she accept or refuse it? The behaviour of the ewe was examined for 25 seconds before a suck attempt (as above). Any communication between the ewe and her lambs concerning willingness to allow sucks must take the form of a signal, i.e. a change in her behaviour. The analysis therefore consisted of two stages, firstly determining if suck attempts were associated with a change in the behaviour of the ewe and secondly, whether any specific ewe behaviours were involved. During the first part of the analysis ewe behaviour was classified as either 'no change' if she made no change in her behaviour for the 25 seconds before a suck attempt or 'change' if she did change her behaviour during this time. Subsequently the changes in ewe behaviour were defined (Table 5.2.) and examined separately to determine if any individual behaviours showed significant associations with lamb suck attempts. Suck attempts were divided into successful and unsuccessful interactions to allow assessment of the ewes' response to them. Successful sucks, where the ewe accepted the suck attempt, were defined as the lamb being able to place its head underneath the ewe in the udder region for at least 5 seconds. Unsuccessful sucks, or ewe refusals, were defined as the ewe preventing the lamb placing its head in the udder region by moving her leg between the lamb and her udder or by walking forward as the lamb attempted to reach the udder. The ewes often performed more than one behavioural change in the

Table 5.2.
Definitions of the individual changes in ewe behaviour observed before a suck attempt.

Behaviour	Definition
Head-up posture	Ewe stands rigidly with head held vertically to her body.
Lying to standing	Ewe rises from lying on the ground to standing on all 4 feet, head held horizontally.
Grazing to standing	Ewe raises her head from grazing (standing or walking slowly with the head down, in close contact with the grass) to standing with head held horizontally.
Approach	Ewe walks directly towards the lamb to within 1m of it and stands with head held horizontally.
Vocalisation	Ewe makes a high pitched bleat (mouth open) or low pitched rumble (mouth closed).
Nose lamb	Ewe touches the rear of her lamb with her muzzle.
Walking to Standing	Ewe stops walking and stands with her head held horizontally, lambs were following ewe as she walked.
Nurse other lamb	Only in twin-bearing ewes. Ewe is already accepting a suck from one twin when the other twin makes a suck attempt.

25 seconds before a suck attempt (e.g. vocalisation or nose lamb). As it was impossible to determine whether the lamb was responding to one or both of the behaviours, both were recorded.

The style of sucking interactions was also examined by looking at whether attempts occurred individually or within a chain. To do this the number of individual attempts was compared with the number of chains of attempts. Suck attempts were considered to be within a chain if there was an interval of less than 30 seconds between consecutive attempts. Chains of successful attempts comprised only successful sucks, chains of unsuccessful attempts comprised mostly unsuccessful attempts but could also contain successful attempts in addition to unsuccessful attempts.

5.2.4. Statistical Analysis.

Data were highly skewed and it was not possible to transform the data. Mann-Whitney tests were used to analyse the behaviour of ewes around sucking interactions. In addition to ewe breed, Mann-Whitney tests were also used to look at the effects of sire breed and litter size. Kruskall-Wallis tests were used to analyse temporal changes in the proportion of sucking attempts occurring individually.

5.3. Results.

Unless otherwise stated, litter size and sire breed did not have an effect on ewe and lamb behaviour.

5.3.1. Lamb response to head-up postures.

5.3.1.1. Most common lamb responses.

Lamb response was assessed over the whole observation period. The two most common lamb responses were the same for both breeds: none, approach and successful suck. However Blackface ewes received fewer none responses (REML Wald=13.9, d.f.=1, p<0.001) and more approaches to suck (Wald=13.7, d.f.=1, p<0.001) from their lambs, compared to Suffolk ewes (Figure 5.1).

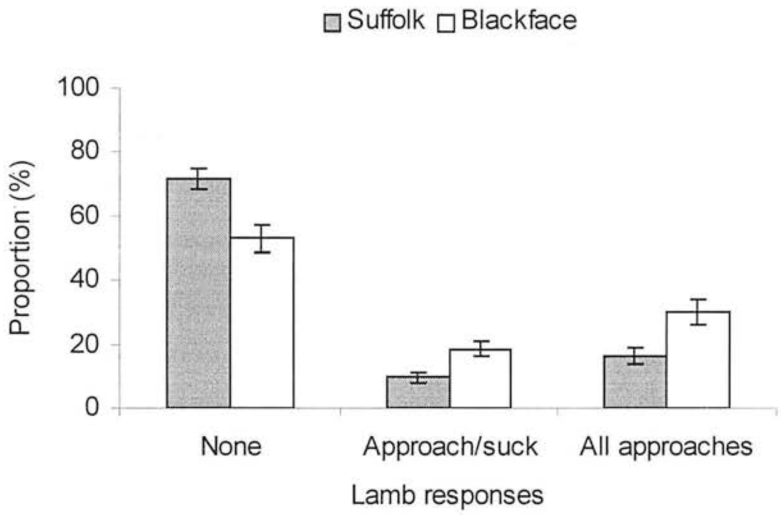

Figure 5.1. Mean proportion of lamb responses to head-postures made by Suffolk and Blackface ewes over the whole observation period (with s.e.m.). The figure shows the two most common responses (none, approach and suck) for both breeds and the 3 responses involving full approaches grouped together.

5.3.1.2. Responses involving a full approach to the ewe.

All types of response involving a full approach (i.e. approach and successful suck, approach and unsuccessful suck, approach only) were analysed together. Blackface ewes received more approaches from their lambs compared to Suffolk ewes (Wald=10.3, d.f.=1, p<0.001) (Figure 5.1).

Ewes with lambs sired by a Blackface sire were more likely to receive a full approach than ewes with lambs sired by a Suffolk sire (mean proportion of all full approaches (%) (with 95% confidence interval): Blackface sire=29.94 (25.54-34.34), Suffolk sire=15.76 (11.36-20.16), Wald=7.9, d.f.=1, p<0.01).

5.3.1.3. Changes in lamb response to the head-up posture over time.

Table 5.3 shows the four most common lamb responses following a head-up posture for the two breeds at each time point. Over time the most common lamb response was 'none' for both breeds. However the likelihood of a 'none' response remained relatively constant for Blackface ewes (ANOVA F=0.46, d.f.=4, NS) but increased over time in Suffolk ewes (F=4.04, d.f.=4, p<0.01). From week 8 Suffolk ewes received almost exclusively none responses from their lambs. Blackface ewes received fewer 'none' responses than Suffolk ewes at weeks 4 (Wald=5.82, d.f.=1, p<0.05), 8 (Wald=4.25, d.f.=1, p<0.05) and 10 (Wald=3.17, d.f.=1, p=0.075) but not at weeks 2 (Wald=0.06, d.f.=1, NS) or 6 (Wald=0.34, d.f.=1, NS).

The second most common lamb response at each time period tended to include an approach to the ewe. During the first 6 weeks post-partum 'approach and successful suck' was the second most frequent lamb response in both breeds. Blackface ewes received a higher proportion of approaches with successful sucks than Suffolk ewes at week 4 (Wald=5.82, d.f.=1, p<0.05) but not at weeks 2 (Wald=0.42, d.f.=1, NS) or 6 (Wald=0.89, d.f.=1, NS).

The two ewe breeds also showed differences in the proportion of lamb responses resulting in a full approach to the dam (i.e. approach and successful suck, approach and unsuccessful suck, approach only) (Figure 5.2). Suffolk ewes received approaches from their lambs for the first 6 weeks only whereas Blackface ewes received approaches until the end of the study. In the weeks when both breeds of ewe received approaches, Blackface ewes received a higher lamb response rate than Suffolk ewes at week 4 (Wald=4.62, d.f.=1, p<0.05) but not at weeks 2 (Wald=2.31, d.f.=1, NS) or 6 (Wald=0.16, d.f.=1, NS).

Table 5.3.
The four most common lamb responses to a head-up posture at each time point.

Time	No. ewes	Behaviour 1	Behaviour 2	Behaviour 3	Behaviour 4
Week 2					
Suffolk	15	None 70.97% (6.81)	Approach/suck 16.56% (7.14)	Already follow 5.93% (3.36)	Already suck 2.64% (2.23)
Blackface	14	None 67.70% (6.90)	Approach only 14.72% (7.14)	Approach/suck 9.26% (2.65)	Other 4.0% (2.47)
Week 4					
Suffolk	17	None 65.58% (6.28)	Approach/suck 9.68% (2.47)	Already follow 7.11% (3.89)	Already suck 7.07% (5.87)
Blackface	14	None 50.49% (5.77)	Approach/suck 21.44% (3.33)	Already follow 11.13% (3.56)	Approach only 7.53% (2.00)
Week 6					
Suffolk	13	None 49.7% (11.4)	Approach/suck 13.46% (8.31)	Approach only 13.46% (7.81)	Approach/unsucc 7.82% (4.37)
Blackface	9	None 59.4% (11.7)	Approach/suck 17.3% (8.40)	Other 11.44% (4.13)	Approach only 6.02% (4.34)
Week 8					
Suffolk	5	None 95.0% (5.00)	Already follow 5.0% (5.00)		
Blackface	7	None 58.8% (16.50)	Approach/suck 34.4% (17.50)	Already follow 3.40% (2.43)	Approach only 2.38% (2.38)
Week 10					
Suffolk	7	None 100.0% (0.00)			
Blackface	9	None 57.40% (16.00)	Other 20.40% (12.40)	Already follow 12.96% (8.69)	Approach only 5.56% (5.56)

Table shows the mean proportions of the four most common lamb responses following a head-up posture by the ewe (with s.e.m.). Head-up postures were not observed in all ewes during each time period, the number of ewes observed performing head-up postures in each 2 week period is given in column two.

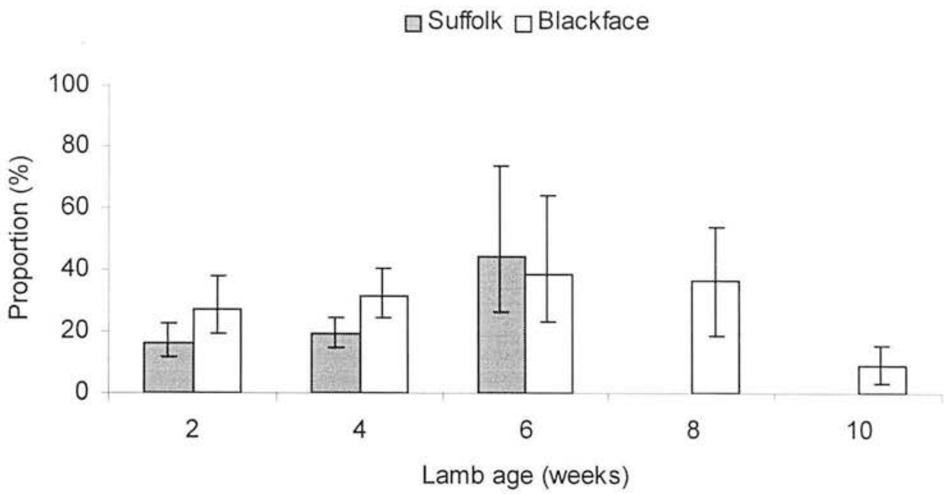

Figure 5.2. Mean proportion of lamb responses involving a full approach received by Suffolk ewes and Blackface ewes with increasing lamb age (with 95% confidence interval). A full approach included: approach and successful suck, approach and unsuccessful suck, approach only.

Ewes with lambs sired by a Blackface sire were more likely to receive approaches from their lamb at week 2 than ewes with lambs sired by a Suffolk sire (mean proportion of responses involving a full approach (%) (with 95% confidence interval): Blackface sire=36.73 (24.41-47.25), Suffolk sire=19.23 (14.95-24.74), Wald=13.5, d.f.=1, $p<0.001$).

5.3.2. Ewe and lamb behaviour in association with sucking interactions.

5.3.2.1. Ewe behaviour before suck attempts.

For both breeds the majority of suck attempts (both successful and unsuccessful) were made after a change in ewe behaviour (Figure 5.3). Blackface ewes were more likely than Suffolk ewes to receive a suck attempt after a change in their behaviour at week 4 (Mann Whitney U=308.5, $p<0.01$) but not at weeks 2 (U=259.0, NS), 6 (U=175.5, NS), 8 (U=144.5, NS), or 10 (U=79.0, NS).

5.3.2.2. Ewe response to suck attempts made after a change in their behaviour.

Figure 5.4 shows the response of ewes to suck attempts made after a change in their behaviour. Throughout the study Blackface ewes almost exclusively allowed their lambs to make a successful suck following a change in their behaviour.

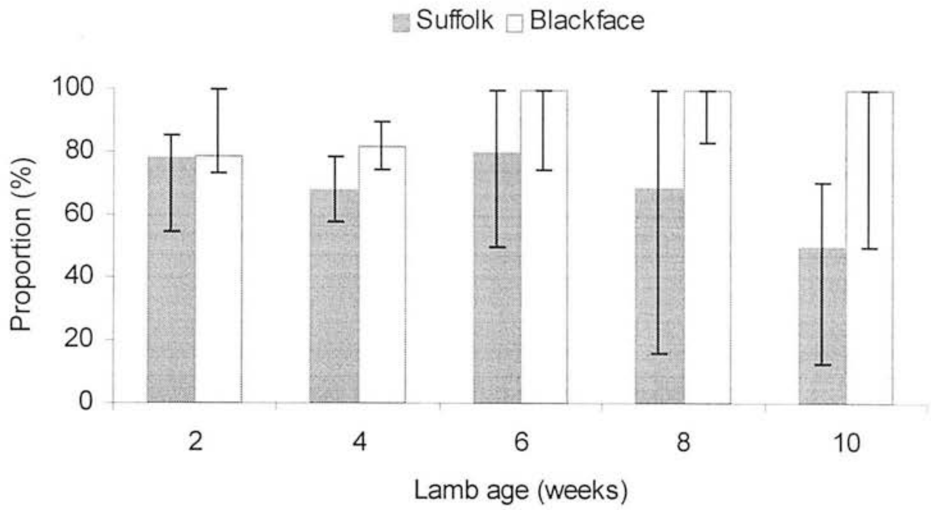

Figure 5.3. Median proportion of suck attempts (successful and unsuccessful) made after a change in ewe behaviour (with Q1, Q3).

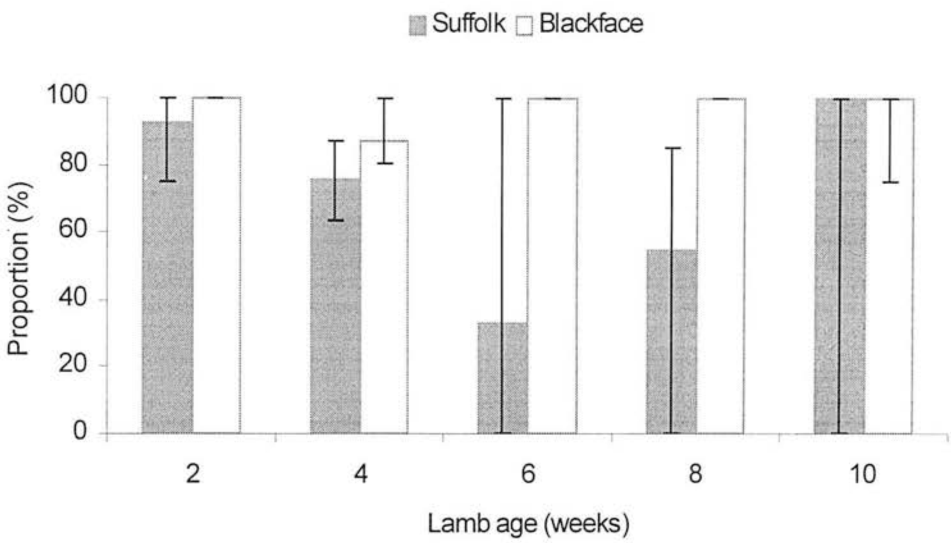

Figure 5.4. Median proportion of suck attempts made after a change in ewe behaviour resulting in a successful sucking interaction (with Q1 and Q3). All Blackface ewes accepted 100% of suck attempts made after a change in ewe behaviour at weeks 6 and 8.

In contrast, Suffolk ewes were more restrictive towards their lambs. During the first 4 weeks they allowed a high proportion of successful sucks following a change in behaviour but this rapidly decreased at week 6. There was some increase towards the end of the study but they still showed a high level of restriction towards their lambs.

When the two breeds were compared, Blackface ewes were more likely than Suffolk ewes to allow successful sucks after a change in behaviour at weeks 2 (U=282.0, $p<0.01$), 4 (U=289.5, $p<0.05$), 6 (all Blackface ewes had same value of 100.0%, Suffolk median=33.33%, Q1=0.0, Q3=100.0) and 8 (all Blackface ewes had same value of 100.0%, Suffolk median=55.0%, Q1=0.0, Q3=85.0) but not at week 10 (U=50.0, NS).

5.3.2.3. Suck attempts made with no change in ewe behaviour.

As would be expected from Figure 5.3, the number of suck attempts made with no previous change in ewe behaviour was low in both breeds of ewe. However the two breeds did show differences in the number of ewes receiving this type of suck attempt (Figure 5.5). The majority of Suffolk ewes continued to receive some suck attempts with no previous change in behaviour throughout the study but after week 4 very few Blackface ewes received such attempts.

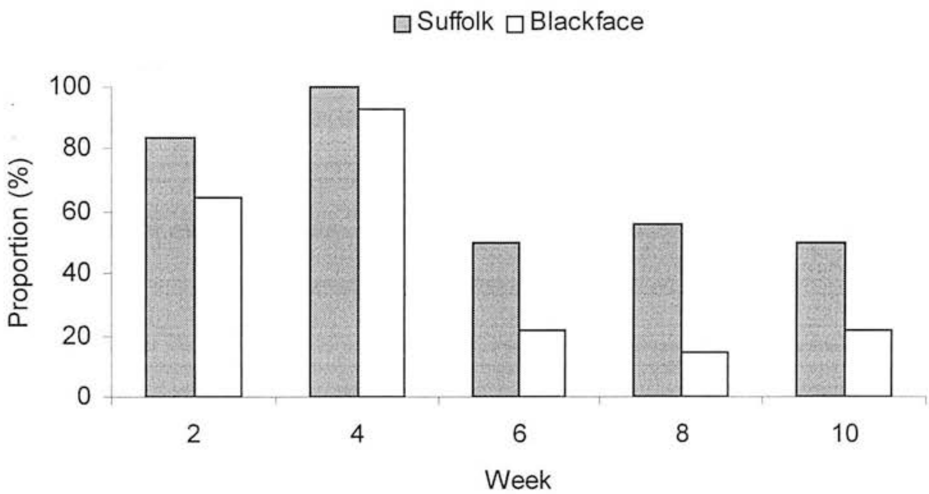

Figure 5.5. Proportion of ewes receiving a suck attempt with no previous change in behaviour.

5.3.2.4. Ewe response to suck attempts made with no change in their behaviour.

Due to the low numbers of Blackface ewes receiving this type of suck attempt statistical comparisons were only possible during the first 4 weeks. There was a tendency for Blackface ewes to accept more suck attempts than Suffolk ewes at week 2 (median proportion (%) (with Q1, Q3): Suffolk=87.50 (58.33, 100.0), Blackface=100.0 (100.0, 100.0), U=142.0, p=0.071). There were no breed differences in the response of ewes at week 4 (median proportion (%) (with Q1, Q3): Suffolk=43.75 (6.25, 75.69), Blackface=20.0 (0.0, 80.0), U=189.5, NS).

During the first 2 weeks post-partum ewes with single litters were more likely to accept suck attempts compared to ewes with twin litters (all singles 100%, twin median=83.75%, Q1=61.61, Q3=100.0).

5.3.2.5. Specific changes in ewe behaviour before successful sucking interactions.

The changes in ewe behaviour before successful sucks are given in Table 5.4. Blackface ewes showed an increase in their performance of the head-up posture before a successful suck to week 6 and continued to perform the head-up posture until the end of the study. Suffolk ewes showed frequent performance of the head-up posture during the first 4 weeks only and did not perform it at all beyond 6 weeks post-partum. Rising from lying to standing before a successful suck attempt was infrequent in Blackface ewes throughout the study and occurred frequently in Suffolk ewes during the first 4 weeks only. Raising the head from grazing to standing increased in frequency with lamb age in both breeds. The ewe approaching her lamb before a successful suck was common in both breeds during the first 4 weeks post-partum and appeared to increase in frequency in Blackface ewes at week 8. Vocalisation before a successful suck attempt was fairly frequent in Suffolk ewes throughout the study but only seen with any frequency in Blackface ewes during the first 4 weeks and at week 8. Nosing was most frequent in both breeds during the first 4 weeks post-partum. Changing from walking to standing was frequent during the first 4 weeks only in Suffolk ewes. It remained at a low frequency throughout the study in Blackface ewes, with the exception of week 4. Nursing the other twin was very rare in both breeds.

5.3.2.5.1. Breed differences in the specific changes in ewe behaviour, first 4 weeks post-partum.

At weeks 2 and 4 Blackface ewes were more likely to nose their lambs before a suck than Suffolk ewes (week 2: U=272.5, $p<0.05$, week 4: U=290.0, $p<0.05$). At week 4 Blackface ewes were more likely than Suffolk ewes to perform the head-up posture

Table 5.4.
Changes in ewe behaviour before successful suck attempts.

		Change in ewe behaviour							
Lamb age (weeks)	Ewe Breed	Head-up	Lying to standing	Grazing to standing	Approach lamb	Vocalise	Nose lamb	Walking to standing	Nurse other lamb-twins
2	Suffolk	6.25	11.81	0.00	22.22	33.33	10.42	12.50	0.00
	(16)	(0.00, 29.17)	(0.00, 23.75)	(0.00, 15.63)	(0.00, 40.48)	(14.38, 57.59)	(0.00, 33.33)	(0.00, 33.33)	(0.00, 0.00)
	Blackface	8.33	0.00	0.00	33.33	33.33	43.75	0.00	0.00
	(14)	(0.00, 35.00)	(0.00, 37.27)	(0.00, 17.50)	(7.50, 50.00)	(0.00, 50.00)	(13.64, 66.67)	(0.00, 9.09)	(0.00, 0.00)
4	Suffolk	15.48	29.17	0.00	20.00	21.11	0.00	16.96	0.00
	(18)	(0.00, 28.57)	(4.69, 51.39)	(0.00, 13.54)	(0.00, 28.79)	(0.00, 34.37)	(0.00, 1.79)	(0.00, 36.65)	(0.00, 7.63)
	Blackface	38.00	7.56	2.94	19.05	33.33	9.19	27.78	0.00
	(14)	(20.24, 59.74)	(0.00, 15.71)	(0.00, 16.27)	(8.33, 42.50)	(0.00, 34.00)	(0.00, 36.11)	(8.82, 43.25)	(0.00, 0.00)
6	Suffolk	0.00	0.00	16.67	0.00	16.67	-	0.00	-
	(8)	(0.00, 25.00)	(0.00, 0.00)	(0.00, 91.7)	(0.00, 0.00)	(0.00, 87.50)		(0.00, 25.00)	
	Blackface	50.00	0.00	0.00	0.00	0.00	-	0.00	-
	(11)	(0.00, 100.00)	(0.00, 0.00)	(0.00, 25.00)	(0.00, 0.00)	(0.00, 0.00)		(0.00, 0.00)	
8	Suffolk	-	0.00	16.67	0.00	0.00	-	0.00	-
	(10)		(0.00, 75.00)	(0.00, 100.00)	(0.00, 25.00)	(0.00, 12.50)		(0.00, 0.00)	
	Blackface	12.50	0.00	12.50	37.50	25.00	0.00	0.00	-
	(8)	(0.00, 50.00)	(0.00, 18.75)	(0.00, 43.70)	(0.00, 87.50)	(0.00, 31.20)	(0.00, 0.00)	(0.00, 18.75)	
10	Suffolk	-	0.00	0.00	0.00	0.00	-	0.00	0.00
	(5)		(0.00, 45.00)	(0.00, 80.00)	(0.00, 75.00)	(0.00, 100.00)		(0.00, 41.70)	(0.00, 10.00)
	Blackface	0.00	-	100.00	0.00	0.00	-	0.00	-
	(5)	(0.00, 25.00)		(25.00, 100.00)	(0.00, 25.00)	(0.00, 25.00)		(0.00, 25.00)	

Table shows median proportions of successful suck attempts preceded by each type of change in ewe behaviour (with Q1, Q3). Not all ewes were observed to make a change in behaviour before receiving a suck attempt during each time period. The number of ewes observed showing a change in behaviour before a receiving a suck attempt from their lamb in each 2 week period is shown in brackets after the ewe breed. '–' indicates that none of the suck attempts were preceded by that particular change in behaviour.

before a successful suck (U=299.0, p<0.01) and Suffolk ewes were more likely to rise from lying to standing than Blackface ewes (U=170.0, p<0.05).

At weeks 2 and 4 single-bearing ewes were more likely to nose their lambs before a suck attempt than twin-bearing ewes (median proportion (%) (with Q1, Q3): week 2: single=58.33, (20.83, 66.67), twin=4.17, (0.0, 33.33), U=257.0, p<0,01, week 4: single=20.0, (0.0, 38.89), twin=0.0, (0.0, 4.0), U=276.5, p<0.01). At week 2 only, twin-bearing ewes were more likely to change from walking to standing before a successful suck attempt, compared to single-bearing ewes (median proportion (%) (with Q1, Q3): single=0.0, (0.0, 0.0), twin=16.25, (0.0, 37.27), U=140.5, p<0.05).

5.3.2.5.2. Breed differences in behavioural changes between weeks 6 and 10.

At week 6 Blackface ewes showed a tendency to perform more head-ups than Suffolk ewes (U=132.0, p=0.058). At week 8 Blackface ewes were the only ewes to perform the head-up posture before a successful suck and one Blackface ewe was the only ewe to nose her lamb before a successful suck. At week 10 one Blackface ewe was the only ewe to perform a head-up posture and two Suffolk ewes were the only ewes to rise from lying to standing before a successful suck.

5.3.2.6. Specific changes in ewe behaviour before unsuccessful sucking interactions.

Changes in ewe behaviour preceding an unsuccessful sucking interaction were very rare in Blackface ewes with the exception of week 4. In contrast a number of Suffolk ewes showed changes in their behaviour before unsuccessful sucks up to week 8. The changes in ewe behaviour for both breeds are shown Table 5.5.

At week 4 the head-up posture and changing from walking to standing were the most frequent changes in ewe behaviour before an unsuccessful suck in Blackface ewes.

For Suffolk ewes performance of the head-up posture before an unsuccessful suck attempt was very rare throughout the study. Rising from lying to standing was low in the first 4 weeks but then increased in frequency from 6 weeks post-partum.

Table 5.5.
Changes in ewe behaviour before unsuccessful suck attempts.

		Change in ewe behaviour							
Lamb age (weeks)	Ewe Breed	Head-up	Lying to standing	Grazing to standing	Approach lamb	Vocalise	Nose lamb	Walking to standing	Nurse other lamb-twins
2	Suffolk (10)	0.00 (0.00, 0.00)	0.00 (0.00, 16.67)	0.00 (0.00, 0.00)	0.00 (0.00, 100.00)	0.00 (0.00, 27.70)	-	0.00 (0.00, 100.00)	0.00 (0.00, 0.00)
	Blackface (1)	66.67	-	-	33.33	-	-	-	-
4	Suffolk (14)	0.00 (0.00, 25.00)	0.00 (0.00, 15.15)	9.09 (0.00, 37.50)	0.00 (0.00, 0.00)	0.00 (0.00, 5.00)	0.00 (0.00, 0.00)	41.67 (23.75, 56.67)	0.00 (0.00, 0.00)
	Blackface (10)	50.00 (0.00, 100.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	-	-	55.00 (0.00, 100.00)	-
6	Suffolk (7)	0.00 (0.00, 0.00)	60.00 (0.00, 100.00)	0.00 (0.00, 50.00)	0.00 (0.00, 0.00)	-	-	0.00 (0.00, 75.00)	0.00 (0.00, 0.00)
	Blackface (0)	-	-	-	-	-	-	-	-
8	Suffolk (9)	0.00 (0.00, 0.00)	0.00 (0.00, 100.00)	0.00 (0.00, 100.00)	0.00 (0.00, 0.00)	-	-	0.00 (0.00, 41.67)	0.00 (0.00, 0.00)
	Blackface (0)	-	-	-	-	-	-	-	-
10	Suffolk (3)	-	100 (50.00, 100.00)	-	-	-	-	0.00 (0.00, 50.00)	-
	Blackface (1)	-	-	50.00	-	-	-	50.00	-

Table shows median proportions of unsuccessful suck attempts preceded by each type of change in ewe behaviour (with Q1, Q3). Not all of the ewes were observed to make a change in behaviour before receiving a suck attempt in each time period. The number of ewes showing a change in behaviour before a receiving a suck attempt from their lamb during each 2 week period is shown in brackets after the ewe breed. '–' indicates that none of the suck attempts were preceded by that particular change in behaviour.

Raising the head from grazing to standing also increased in frequency with lamb age. Approaching the lamb occurred with some frequency in the first 2 weeks post-partum but was rare throughout the rest of the study. Changing from walking to standing was quite common throughout the study.

5.3.2.6.1. Breed differences in the specific changes in ewe behaviour before unsuccessful sucking interactions.

Comparisons were only possible at week 4 due to the low number of Blackface ewes showing changes in behaviour before an unsuccessful suck attempt at other times. Suffolk ewes were the only ewes to vocalise and nose their lambs before an unsuccessful suck at week 4.

5.3.2.7. Style of sucking interaction.

5.3.2.7.1. Proportion of individual successful suck attempts.

Figure 5.6 shows the proportion of individual successful suck attempts. From week 4 the majority of successful sucks were made as single attempts in both breeds of ewe and statistical comparison was only possible during the first 4 weeks. The proportion of individual successful suck attempts did not differ between the two breeds at weeks 2 (U=239.0, NS) or 4 (U=245.5, NS).

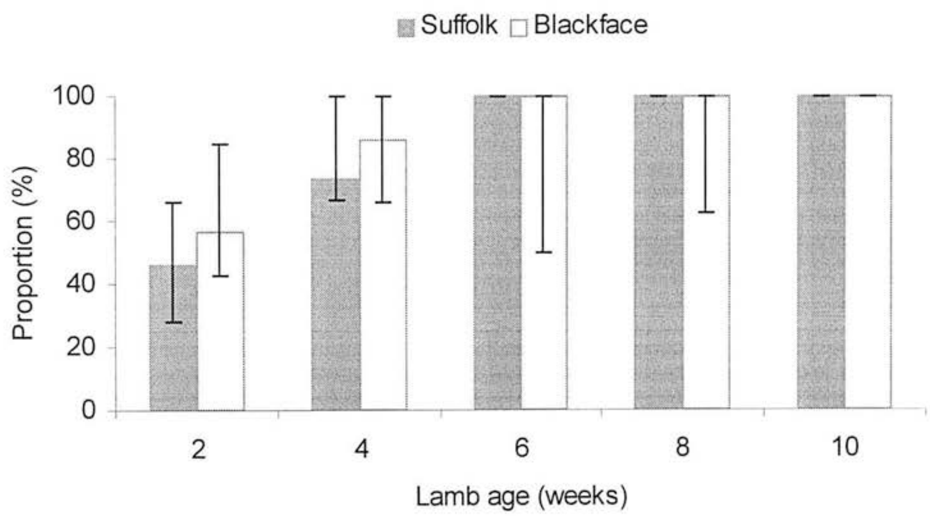

Figure 5.6. Median proportion of successful sucking interactions made as single attempts (with Q1, Q3).

5.3.2.7.2. Proportion of individual unsuccessful suck attempts.

Unsuccessful suck attempts were rare in Blackface ewes, with the exception of week 4, but fairly common in Suffolk ewes (Figure 5.7).

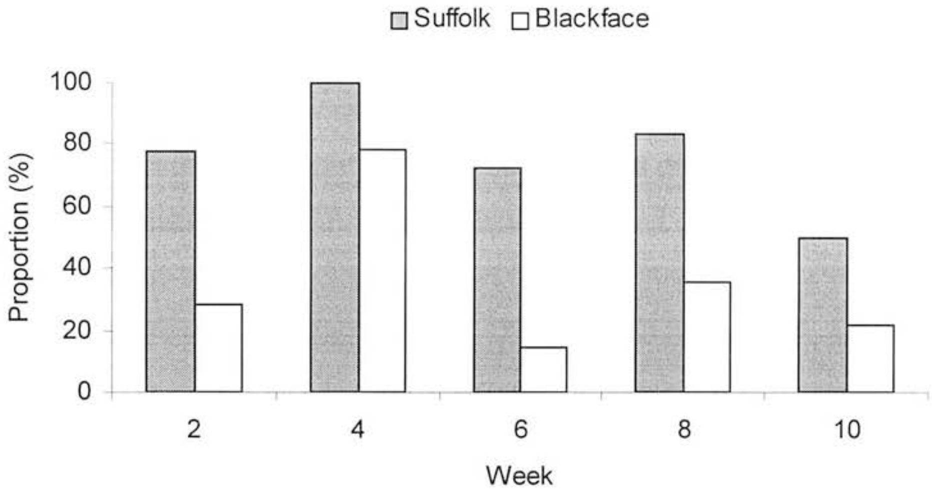

Figure 5.7. Proportion of ewes receiving unsuccessful suck attempts.

Suffolk ewes showed a steady increase in the proportion of individual unsuccessful suck attempts as their lambs increased in age (Kruskal Wallis H=13.12, d.f.=4, $p<0.05$) (Figure 5.8). Due to the low number of Blackface ewes receiving unsuccessful suck attempts statistical comparisons were only possible at week 4. At

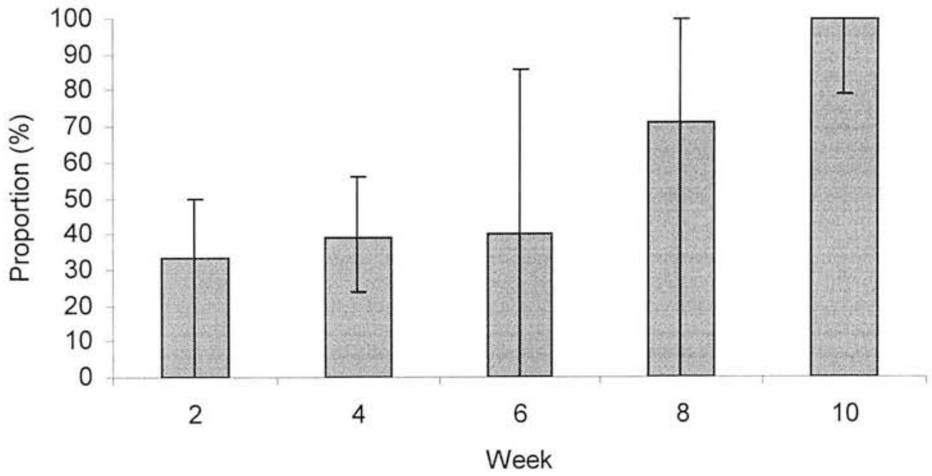

Figure 5.8. Median proportion of unsuccessful suck attempts involving a single attempt in Suffolk ewes (with Q1, Q3).

this time Blackface ewes received a higher proportion of single unsuccessful suck attempts than Suffolk ewes (U=221.0, $p<0.05$).

5.3.2.7.3. Length of chains of successful suck attempts.

From week 6 successful suck attempts were primarily made as single attempts in both breeds (ewes receiving chains of successful sucks: 12, 9, 3, 2, 0 Blackface ewes in weeks 2, 4, 6, 8 and 10 respectively, 14, 12, 0, 2, 1 Suffolk ewes in weeks 2, 4, 6, 8 and 10 respectively). Therefore breed comparisons of the average length of chains of successful sucks were only possible in weeks 2 and 4. At week 2 Blackface ewes received shorter chains of successful sucking bouts than Suffolk ewes (median number of attempts per chain (with Q1, Q3): Suffolk=3.31, (2.75, 3.92), Blackface=2.0, (2.0, 2.88), U=104.5, $p<0.01$). There was no difference in chain length at week 4 (median number of attempts per chain (with Q1, Q3): Suffolk=2.37, (2.0, 3.83), Blackface=2.33, (2.0, 2.75), U=93.0, NS).

5.3.2.7.4. Length of chains of unsuccessful suck attempts.

Chains of unsuccessful suck attempts were fairly common in Suffolk ewes up to week 8 (13, 17, 6, 6 and 4 ewes in weeks 2, 4, 6, 8 and 10 respectively) but rare in Blackface ewes (3, 8, 2, 4 and 0 ewes in weeks 2, 4, 6, 8 and 10 respectively). The length of chains of unsuccessful suck attempts received by Suffolk ewes (Figure 5.9)

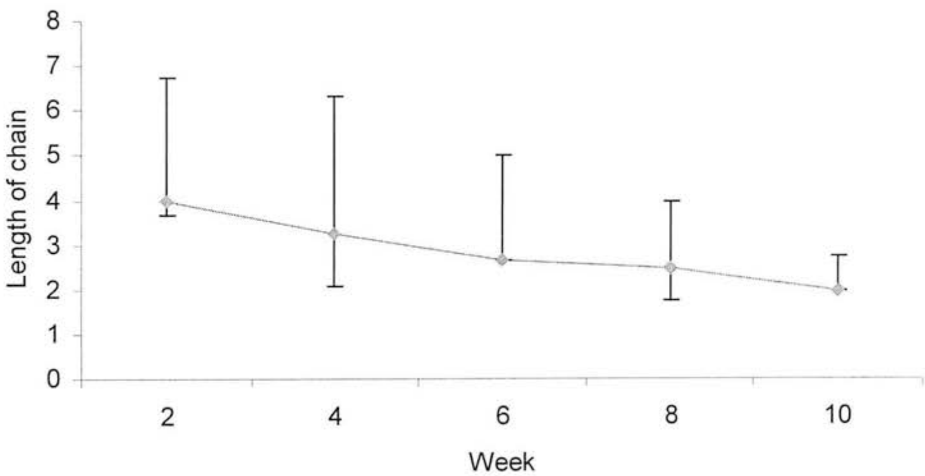

Figure 5.9. Median length of chains of unsuccessful suck attempts received by Suffolk ewes (with Q1, Q3).

had a tendency to get shorter as their lambs aged (H=8.34, d.f.=4, p=0.08).

A statistical comparison between the breeds was only possible at week 4. At this time there were no effects of ewe breed (median number of attempts per chain (with Q1, Q3): Suffolk=3.25, (2.55, 6.32), Blackface=3.42, (2.25, 4.03), U=94.0, NS).

5.4. Discussion.

5.4.1. Lamb response to head-up postures.

Ewes from the two breeds received different responses from their lambs following their performance of a head-up posture. Over the whole observation period Blackface ewes received more approaches and fewer 'none' responses compared to Suffolk ewes. Blackface ewes also continued to receive approaches throughout lactation and the likelihood of their lambs making no response to a head-up posture remained constant with increasing lamb age. In contrast, Suffolk ewes received approaches from their lambs for the first 6 weeks only and the likelihood of receiving a 'none' response increased with lamb age, resulting in almost exclusively 'none' responses from week 8 onwards.

5.4.1.1. The role of the head-up posture in maintaining ewe-lamb proximity.

When the two breeds were compared, Blackface ewes received a higher number of lamb approaches in response to a head-up posture from week 4 onwards. Although there were no breeds differences in approach rate at week 6, the greater performance of the head-up posture by Blackface ewes would have resulted in the actual number of approaches from their lambs being higher than those made by lambs with a Suffolk dam. The higher approach rate, combined with a higher performance of the head-up posture by the ewe (Chapter 4), is likely to result in the lambs of Blackface ewes moving closer to their dams more frequently than lambs with a Suffolk dam. This would be expected to result in a shorter ewe-lamb distance in the Blackface ewes, compared to Suffolk ewes and as proposed in Chapter 4, suggests that postural communication is involved in the breed differences in ewe-lamb distance.

5.4.1.2. The use of postural communication by ewes in the two breeds.

The temporal changes in lamb response, combined with the differences in expression rate of the head-up posture (Chapter 4), suggest that Blackface ewes, but not Suffolk ewes, use postural communication with their lambs. Lambs with a Blackface dam continued to change their behaviour in response to the head-up posture throughout lactation. During the first 8 weeks their response most frequently involved an approach to the ewe but at week 10, 'other' was the second most frequent lamb response received by Blackface ewes. During an 'other' response lambs changed their behaviour, for example stood from lying, vocalised or stopped walking. This suggests that they were still paying attention to their dams' behaviour and responding to the head-up posture. Therefore, although this response does not involve the lamb moving closer to its dam it still represents an active response from the lamb. In contrast, the likelihood of lambs with a Suffolk dam performing a 'none' response increased as they aged and after week 6 they showed very little response to the performance of a head-up posture by their dam. Combined with the low performance of the head-up posture by Suffolk ewes (Chapter 4), this suggests that postural signals are either not used in the communication between Suffolk ewes and their lambs, or they are used inefficiently.

5.4.2. Ewe and lamb behaviour in association with sucking interactions.

5.4.2.1. The use of signals in the control of sucking interactions.

The previous discussion on postural communication suggests that Blackface ewes, but not Suffolk ewes, were using postural signals in their communication with their lambs. Observations on the behaviour of ewes and lambs around sucking interactions suggest that this may also be the case in the ewes' control of their lambs' sucking behaviour. In both breeds the majority of suck attempts (successful and unsuccessful) were made after a change in ewe behaviour, indicating that lambs were using changes in their dams' behaviour as a cue to make an attempt. However, the response of the ewe to these suck attempts differed between the two breeds and Blackface ewes were more likely than Suffolk ewes to accept a suck attempt if it was made after a change in behaviour. Blackface ewes almost exclusively accepted suck attempts made after a change in behaviour but Suffolk ewes were inconsistent in their response, being almost as likely to refuse, as accept such attempts. This may indicate that Blackface ewes, but not Suffolk ewes, intended a change in their behaviour to act as a signal for their lamb to make a suck attempt.

In addition, Blackface ewes were very unlikely to receive a suck attempt with no previous change in their behaviour after week 4, whereas the majority of Suffolk ewes continued to receive such attempts throughout lactation. Ewes begin to control their lambs' sucking behaviour at approximately 4 weeks of age (Ewbank 1967, Trivers 1985). Therefore the cessation of suck attempts with no previous change in ewe behaviour after this time may indicate that Blackface ewes taught their lambs to associate a signal with the invitation to suck. Conversely, the continued attempts from lambs with a Suffolk dam may represent a failure to teach their lambs, or the lack of signal use in the control of suck attempts by Suffolk ewes.

5.4.2.2. Specific behaviours associated with suck attempts in the two breeds.

In order to investigate this further it is useful to look at the specific ewe behaviours occurring before lamb suck attempts. From 4 weeks post-partum the head-up posture was the behaviour most commonly associated with suck attempts in Blackface ewes and they were more likely than Suffolk ewes to perform a head-up posture before a successful suck attempt. Blackface ewes therefore appear to use the head-up posture as the main signal inviting their lambs to make a suck attempt. Suffolk ewes did not show a consistent performance of any specific behaviour before a suck attempt and therefore do not appear to use postural signals in the control of their lambs' sucking interactions.

5.4.2.3. Significance of a change in ewe behaviour for lambs with a Suffolk dam.

Although suck attempts by lambs with a Suffolk dam were not associated with a specific behavioural change, the majority of their attempts did occur after a change in ewe behaviour, indicating that this must have some significance for the lamb. Throughout the study the proportion of suck attempts accepted by Suffolk ewes was higher if the attempt was made following a change in their behaviour. Consequently the significance of a change in dam behaviour may be the opportunity to suck with less chance of refusal, rather than a signal indicating willingness to allow sucking.

5.4.2.4. The nature of suckling relationships in the two breeds.

As proposed in Chapter 4 the two breeds of ewe appear to have different methods of controlling their lambs' suck attempts and, as a consequence, the nature of their suckling relationship with their lambs is very different. Blackface ewes have a clear suckling relationship and, as described above, use behavioural signals to indicate when their lambs may make a suck attempt. The rare occurrence of unsuccessful suck attempts with Blackface ewes gives further indication of the use of signals and the success of Blackface ewes in teaching their lambs to respond to them. An unsuccessful suck attempt occurs when the ewe prevents her lamb from sucking as it tries to make contact with the udder. A low frequency of unsuccessful attempts must consequently indicate that the ewe is controlling her lamb's sucking from a distance, before it has tried to make contact with the udder. Blackface ewes are therefore proactive in their suckling relationship with their lambs and use distance control in the regulation of sucking interactions.

In contrast, Suffolk ewes have a much more confused suckling relationship with their lambs. Their response to suck attempts was inconsistent, both in relation to general changes in behaviour and also specific behavioural changes. Suffolk ewes therefore do not appear to control their lambs' suck attempts from a distance, using a signal, but, as proposed in Chapter 4, exert control once the lamb has approached and tried to make contact with udder. Suffolk ewes are therefore more reactive than Blackface ewes in the control of their lambs' sucking behaviour and appear to regulate sucking interactions in response to their lambs' attempts.

5.4.2.5. Style of sucking interactions.

Chains of successful sucks were only frequent during the first few weeks post-partum, when ewes are expected to allow ad libitum sucking by their lambs (Ewbank 1967). At this time Blackface ewes received shorter chains of successful sucks than Suffolk ewes and this, coupled with the longer duration of these sucks (Chapter 4), could be further indication of the greater co-operation with sucking in Blackface ewes, as discussed in Chapter 4. The majority of successful sucks after week 4 were made as individual attempts in both breeds. This suggests that both breeds were allowing their lambs to satisfy their hunger during a successful suck.

The breed differences in the ewes' response to suck attempts and their control of sucking interactions may be expected to result in differences in the sucking behaviour of their lambs. The inconsistent response of Suffolk ewes to suck attempts would be expected to result in their lambs being more persistent in their attempts to suck after a refusal, making longer and more frequent chains of unsuccessful attempts compared to the lambs of Blackface ewes. In agreement with this, Suffolk ewes received unsuccessful suck attempts throughout lactation whereas Blackface ewes only received such attempts at a high frequency at week 4. At this time Blackface ewes received a higher proportion of single unsuccessful suck attempts, compared to Suffolk ewes, and this may result from their more consistent response to their lambs' suck attempts.

Although Suffolk ewes received chains of unsuccessful sucks throughout the study there was a steady increase in the proportion of individual unsuccessful suck attempts. In addition, the length of any chains of unsuccessful attempts also became progressively shorter with increasing lamb age. This suggests that over time the lambs of Suffolk ewes learnt that a refusal meant they could not suck. However it is also possible that Suffolk ewes may have become more definite in their refusals as their lambs aged or their lambs may have became less persistent as they began to graze and milk became less nutritionally important. Further work is needed to investigate this matter.

5.4.2.6. Ewe behaviour associated with early sucking interactions.

The previous discussion has focused on sucking interactions after 4 weeks post-partum, once ewes begin to control their lambs' sucking behaviour. However observations of the ewes' behaviour before early sucking interactions also showed some interesting results. Ewes are known to allow ad libitum sucking during the first few weeks post-partum (Ewbank 1967, Trivers 1985) but the results of this study suggest that ewes may also actively encourage their lambs to suck at this time. In both breeds of ewe, nosing, vocalising, and approaching the lamb were changes in behaviour almost exclusively associated with the first 4 weeks post-partum. Nosing the lamb was very rarely seen beyond the first month and the frequency of approaches and vocalisation also decreased markedly after week 4. These behaviours occurred most frequently before successful sucks and this, combined with their interactive nature, especially nosing and approaching the lamb, suggest active encouragement of sucking by the ewe. Nosing has previously been identified in the encouragement of initial sucking behaviour in the neonate (Sharafeldin and Kandeel 1971) and this may reflect a continuation of such encouragement during the first few weeks of life. As the ewe is known to only be physically active in the maintenance of ewe-lamb distance for the first 4 weeks post-partum (Hinch et al. 1987, Hinch et al. 1990) it is appropriate that the putative encouragement of sucking should also only be seen at this time. Further work is needed to clarify whether or not ewes are using these behaviours to encourage their lambs to suck during the first month post-partum.

There were no breed differences in the likelihood of a ewe vocalising or making an approach before a suck attempt during the first 4 weeks but Blackface ewes were more likely than Suffolk ewes to nose their lamb at this time. This may be further indication of Blackface ewes having greater maternal motivation than Suffolk ewes, as discussed in Chapters 3 and 4. However single-bearing ewes were also more likely than twin-bearing ewes to nose their lambs at this time. The analysis does not allow the separation of ewe breed and litter effects and it is therefore not possible to definitively conclude that ewe breed was responsible for Blackface ewes performing more nosing than Suffolk ewes.

5.4.3. Learning the association between the head-up posture and approach to the dam.

In Chapter 4 Blackface ewes were found to have a peak in their performance of the head-up posture at week 4 and it was proposed that this might facilitate their lambs' learning to approach their dam in response to postural communication. Several observations from this chapter give further support to this proposal and suggest that the opportunity to suck may act as a positive reinforcer of the lambs' response to the head-up posture.

At week 4 Blackface ewes were more likely than Suffolk ewes to receive an approach and successful suck in response to a head-up posture. Although the approach is made by the lamb, the outcome of the approach, the chance to suck or not, is under the ewe's control and the higher incidence of an 'approach and suck' response is due to maternal behaviour. In addition, week 4 was the only time period when unsuccessful sucks were seen in any frequency in the Blackface ewes. At this time, changes in Blackface ewe behaviour before an unsuccessful suck most frequently involved a head-up posture or a change from walking, with lambs following, to standing. Successful sucks were also associated with these changes in behaviour at week 4, and this was the only time period when the behavioural change 'walking to standing' was strongly associated with successful sucks. Ewes are known to occasionally signal for their lamb to approach, through a vocalisation or postural change, refuse sucking and then walk off with the lamb following. This has been proposed as an attempt by the ewe to maintain close contact with her lambs (Ewbank 1967). The combination of frequent unsuccessful sucks and the association of both types of suck attempt with postural communication and the ewes leading their lambs may indicate a similar strategy in the Blackface ewes at this time. The behaviour of the Blackface ewes, especially giving their lambs the opportunity to suck after following the dam, has the potential to facilitate their lambs' learning to associate the head-up posture with approach to their dam. Further investigation is needed to clarify whether or not this is intentional teaching by the Blackface ewes.

5.4.4. Benefits of the use of the head-up posture in ewe-lamb communication.

The previous discussion shows the implications of differences in the use of the head-up posture for ewe-lamb proximity, sucking interactions and ewe-lamb attachment. These may not be the only areas which benefit from the use of postural communication between ewes and their lambs as the head-up posture is very similar to the postural communication seen in adult sheep.

The most common postures in sheep are grazing, resting and walking. Two postures, which differ from the normal postures seen in sheep, have been identified by Geist (1971) as having communicative value. During normal walking a sheep's head is held quite low with its nose pointing to the ground. By raising its head and holding it rigidly whilst walking with tense steps, it draws the attention of others (Geist 1971). In addition a sheep suddenly freezing and staring in one direction alerts others to do the same. Geist (1971) identified these two behaviours as the 'alarm posture' and the 'attention posture'. The attention posture is very similar to the head-up posture. The use of the head-up posture in communication between ewes and their lambs may therefore also provide a way of teaching lambs about adult postural communication. An early familiarity with the head-up posture would be expected to make it easier for lambs to learn to respond to similar postures in other animals as they become older. In addition, by combining the posture with a positive outcome, such as the opportunity to suck, response to the posture will be strongly reinforced in the lamb.

The natural reaction of alarmed sheep is to form a tight group and to move away from the perceived danger in this group (Geist 1971, Hansen et al. 2001). The association of the head-up posture with approach to other sheep may help to reinforce flocking behaviour in the Blackface-raised lambs. In agreement with this, heavy breeds, including the Suffolk, have been found to have looser flocking behaviour in response to a predator than lighter breeds (Hansen et al. 2001). However the reasons for this were not investigated and further work is needed to examine the role of postural communication in these differences in antipredatory behaviour.

5.4.5. Long-term benefits of postural communication between ewes and lambs.

In conclusion, the more frequent use of the head-up posture by the Blackface ewes, compared to Suffolk ewes, and the positive associations linked to it, may have various benefits for their lambs, other than just closer ewe-lamb proximity, increasing their lambs' chances of survival both before and after weaning.

5.5. Summary and conclusions.

Blackface and Suffolk ewes were found to differ in their use of postural communication with their lambs and in their control of sucking interactions. Postural communication between Blackface ewes and their lambs was strongly associated with approaches by their lambs and it is therefore apparent that Blackface ewes use the head-up posture in their maintenance of ewe-lamb proximity. Suffolk ewes did not appear to use the head-up posture in their maintenance of ewe-lamb proximity. These differences in the use of postural communication are likely to contribute to the closer ewe-lamb distance seen in Blackface ewes, compared to Suffolk ewes, as lambs with a Blackface dam would have been moving closer to their dams more frequently than lambs with a Suffolk dam.

The head-up posture was also strongly associated with successful sucking interactions between Blackface ewes and their lambs throughout lactation, suggesting that this behaviour is used as a signal in the distance control of sucking interactions by Blackface ewes. Unsuccessful suck attempts were very rare in Blackface ewes, providing further evidence of their distance control of sucking interactions. The use of distance control of sucking interactions indicates that Blackface ewes are proactive in their sucking interactions with their lambs and are therefore likely to have a positive suckling relationship with them. In contrast, Suffolk ewes had a high number of unsuccessful suck attempts and showed no consistent associations between specific ewe behaviours and suck attempts. This indicates that Suffolk ewes do not control lamb suck attempts from a distance but regulate them once the lamb has attempted to contact the udder. This is likely to result in a very confused suckling relationship between Suffolk ewes and their lambs, as lambs would not be able to predict when their suck attempt would be accepted.

During the first month post-partum all ewes showed behaviours indicative of active encouragement of sucking by their lambs. These behaviours, nosing, vocalising, and approaching the lamb, were almost exclusively associated with the first 4 weeks and most frequently occurred before successful sucks. Further work is needed to clarify whether or not ewes actively encourage their lambs to suck at this time.

The use of the head-up posture in communication between Blackface ewes and their lambs, in particular its association with approaching the ewe, may also have advantages for their lambs in later life. This is due to it being similar to the postural communication used by adult sheep in reaction to perceived danger.

In conclusion, the results of this chapter point to Blackface ewes having more interaction with their lambs during later lactation, compared to Suffolk ewes. This adds to the data collected on breed differences in maternal care in previous studies (e.g. Dwyer and Lawrence 1998, Dwyer et al. 1998, Dwyer and Lawrence 1999a) and previous chapters in the current study. Together, these indicate that Blackface ewes show more affiliative behaviour towards their lambs from birth onwards, compared to Suffolk ewes, suggesting that the two breeds have different maternal styles. This is investigated further in the next chapter.

Chapter 6: Maternal style in the two breeds and consistency in the maternal behaviour of individual ewes.

6.1. Introduction.

The previous three chapters have shown that the two breeds of ewe exhibit differences in their maternal care throughout lactation. Blackface ewes have a closer relationship with their lambs than Suffolk ewes, showing more affiliative behaviour to the neonate, and positively interacting and communicating with their lambs beyond the post-partum period. In contrast Suffolk ewes are less inclined than Blackface ewes to interact with their lambs during the initial post-partum period and show infrequent communication with them as they become older. The expression of maternal behaviour therefore not only differs between the two breeds but the differences appear to be consistent throughout lactation, suggesting that Suffolk and Blackface ewes have different maternal styles.

6.1.1. Investigating the maternal style of Suffolk and Blackface ewes.

Analysis in previous chapters has concentrated on the individual maternal behaviours expressed at various stages of lactation. In order to investigate maternal style the behaviour of the ewes throughout the lactation period must be considered as a whole. By using a factor analysis, relationships between the various behaviours can be identified and then used to examine variation in the long-term expression of maternal behaviour between the two breeds.

Variation in maternal style has previously been studied in primates (reviewed by Fairbanks 1996). These studies have shown that variation in primate maternal behaviour is not unidimensional but can be described using multiple dimensions: maternal protectiveness and maternal rejection (Fairbanks 1996) and maternal care or warmth (Maestripieri 1998). Protectiveness is characterised by high loadings on contact-promoting and nurturant behaviours, rejection by high loadings for mother rejection, breaking contact and leaving the infant. Maternal care or warmth is associated with cradling and grooming and accounts for less variation than the other two dimensions. This raises the possibility that variation in ovine maternal behaviour may also be multi-dimensional. If this is the case, it may be possible to classify ovine maternal behaviour in terms of maternal rejection, protectiveness and care/warmth and to use these dimensions to describe the maternal style of the two breeds.

6.1.2. Consistency in the expression of maternal behaviour by individual ewes.

Whilst conducting the observations on maternal behaviour between parturition and weaning it became apparent that individuals within the two breeds differed in the consistency of their expression of maternal care. Individual Blackface ewes appeared to be stable in their expression of maternal behaviour, showing a similar level of maternal care at each stage of the lactation period. In contrast, individual Suffolk ewes appeared to be more erratic in their expression of maternal care, performing some maternal behaviours 'well' or frequently and others 'poorly' or infrequently. The behaviour of individual ewes can be examined statistically to determine whether or not these apparent differences in the consistency of maternal care are real.

6.2. Methods.

6.2.1. Animals and measures of maternal behaviour.

Twenty-two ewes (10 Suffolk, 12 Blackface) had complete behavioural records for the whole lactation period. The husbandry and data collection methods have already been described in previous chapters. Eleven measures of behaviours were selected from previous analyses to represent maternal care from birth to weaning (Table 6.1). These measures were chosen as they relate to maternal behaviours important for the formation and maintenance of the ewe-lamb relationship, and the survival of the lamb. Behaviours expressed during later lactation, in the field, were presented as values for the whole time period (not fortnightly values as in previous chapters). For the purpose of this analysis, behaviours described as occurring during the immediate post-partum period were observed during the first 3 hours after birth, behaviours associated with later lactation were recorded when the ewes were in the field, between 3 days and 10 weeks post-partum.

Table 6.1.
Definitions and measurement details for behaviours used to investigate maternal style and consistency in the expression of maternal behaviour.

Behaviour	Definition and measurement
Immediate post-partum period:	
Latency to groom neonate	Time (s) between birth and first grooming of neonate.
Grooming duration	Duration (s) spent grooming neonate during the first 30 minutes post-partum.
Non-co-operation with early suck attempts	Proportion of initial suck attempts to which ewe responds by circling, backing or walking forward.
Negative behaviour	Presence or absence of negative behaviour (ewe leaves, retreats from or butts her lamb) during the first 30 minutes post-partum.
First 3 days post-partum:	
Ewe-lamb distance	Distance (m) between ewe and lamb.
Lie together	Proportion of scans in which ewe and lamb are lying within 1m of each other.
Nose lamb	Proportion of scans ewe seen to touch her lamb with her muzzle.
Later lactation:	
Ewe-lamb distance	Distance (m) between ewe and lamb.
Performance of head-ups	Frequency of head-up postures (ewe stands with head held vertically to her body) per observation.
Proportion of successful suck attempts	Proportion of suck attempts ewe accepts.
Nose lamb	Frequency of nosing (ewe touches lamb with her muzzle) per observation.

6.2.2. Statistical analysis for general maternal style.

A factor analysis, with an orthogonal Varimax rotation, was used to look for relationships between the various ewe behaviours and to examine the dimensions responsible for variation in maternal behaviour. The behaviour 'lie together' was excluded from this analysis as it was closely related to ewe-lamb distance during the first 3 days post-partum. The inclusion of closely related data can distort factor analysis. T-tests were used to assess if the individual scores of the two breeds were significantly different on each of the factors.

6.2.3. Statistical analysis for consistency in individual maternal style throughout lactation.

Kendall's Coefficient of Concordance (Siegel and Castellan 1988) was used to examine the consistency of individual ewes' behaviour between birth and weaning. This test measures the association and agreement between sets of rankings. By ranking the ewes in their performance of each of the maternal behaviours listed in Table 6.1, the consistency in the expression of maternal behaviour throughout lactation can be determined by assessing the association between these ranks. A strong association between ranks represents consistency in the expression of maternal behaviour, a weak association denotes variability, or a lack of consistency in expression. Ewes were ranked on their performance of each behaviour within their respective breed groups. The performance of negative behaviour during the immediate post-partum period was not used in this analysis as it could not be ranked; ewes either did or did not perform negative behaviour. The analysis then compared rankings across the behaviours and gave an indication of consistency between them.

6.3. Results.

6.3.1. General maternal style.

Three factors had eigen values above 1 and accounted for 70.2% of the total variation. The loadings of behaviours on the first 2 factors are shown in Figure 6.1. The first factor accounted for 32.0% of the variance. Duration of grooming during the immediate post-partum period and nosing the lamb in later lactation had strong positive loadings on this factor, the proportion of successful sucks during later lactation had a weak positive loading. The performance of negative behaviour during the immediate post-partum period, the latency to begin grooming and the ewe-lamb distance in later lactation had strong negative loadings, failure to co-operate with initial suck attempts had a weak negative loading. This factor therefore appears to represent the ewe's willingness to interact with, or maternal affiliation for, her lambs at different stages of the lactation period.

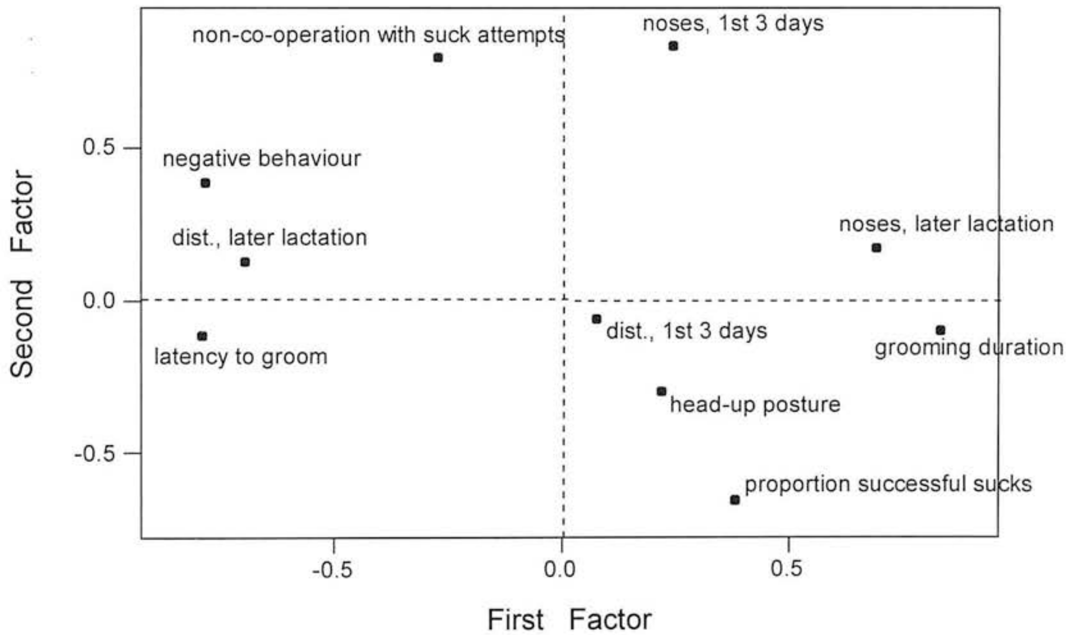

Figure 6.1. Loading plot for Factors 1 and 2 in the factor analysis of maternal care throughout lactation.

Factor 2 accounted for 20.3% of the variation in maternal behaviour between the ewes. Nosing the lamb during the first three days post-partum and non-co-operation with initial suck attempts had strong positive loadings on this factor, the performance of negative behaviour had a weak positive loading. The proportion of successful sucks during later lactation had a strong negative loading. This factor therefore appears to represent the ewe's reaction to her lamb's attempts to interact physically with her throughout lactation, especially during sucking interactions.

Factor 3 accounted for 17.9% of the variance. The ewe-lamb distance during later lactation had a strong positive loading on this factor and nosing the lamb during later lactation had a strong negative loading. Ewe-lamb distance is often taken as an indicator of the strength of the ewe-lamb bond (Morgan and Arnold 1974) and nosing during later lactation is thought to have a role in the maintenance of association between ewes and their lambs (Shillito and Hoyland 1971, Arnold et al. 1979). Factor 3 therefore appears to relate to the ewe-lamb bond during later lactation.

Figure 6.2 shows the score plots on the first two factors for individual ewes. Blackface ewes had mostly positive scores for Factor 1 whereas Suffolk ewes showed mostly negative scores for this factor (mean score (with s.e.m.): Suffolk= -0.647 (0.21), Blackface=0.539 (0.27), T-test T=3.49, d.f.=19, $p<0.01$). On Factor 2, Blackface ewes had mostly negative scores and Suffolk ewes had mostly positive scores (mean score (with s.e.m.): Suffolk=0.563 (0.28), Blackface=-0.469 (0.25), T=-2.76, d.f.=18, $p<0.05$). On the third factor there was a non-significant tendency for the two breeds to differ in their scores. Suffolk ewes showed mostly positive scores and Blackface ewes showed mostly negative scores (mean score (with s.e.m.): Suffolk=0.416 (0.25), Blackface=-0.350 (0.31), T=-1.94, d.f.=19, $p=0.067$). Individuals in both breeds showed more variation on the second two factors, compared to the first factor.

The scores of the Blackface ewes suggest that their behaviour was mostly associated with affiliative behaviour: grooming during the immediate post-partum period,

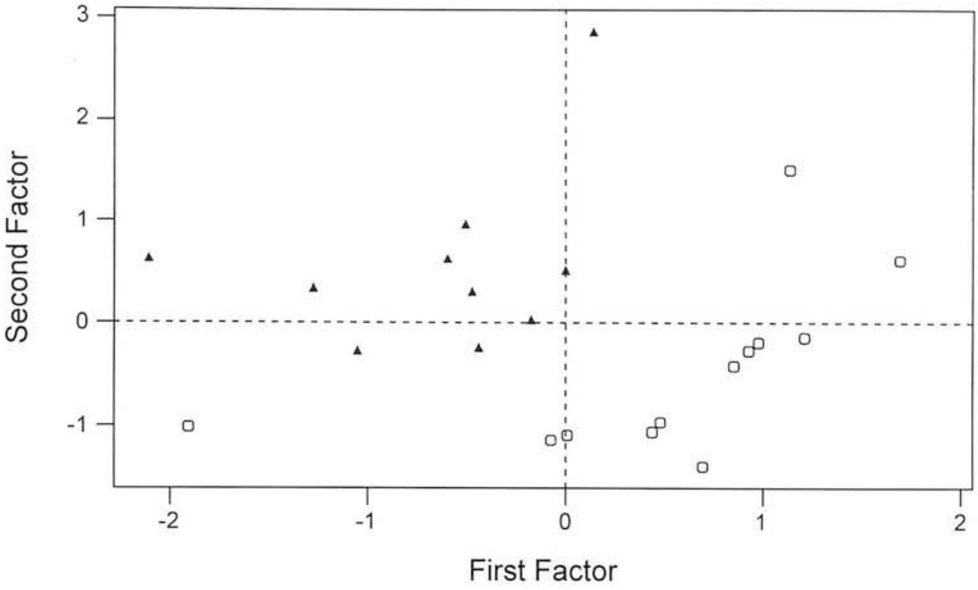

Figure 6.2. Score plot for individual ewes in the factor analysis of maternal behaviour throughout lactation. Blackface ewes are represented by open squares, Suffolk ewes by solid triangles.

performance of the head-up posture, nosing and the proportion of successful suck attempts during later lactation. The only Blackface ewe showing a strong negative loading on Factor 1 failed to groom her lamb during the immediate post-partum period but subsequently expressed similar maternal behaviour to the other Blackface ewes.

The scores of the Suffolk ewes suggest that their behaviour was more associated with rejecting behaviours than Blackface ewes, for example the latency to begin grooming the neonate, the performance of negative behaviour and non-co-operation with suck attempts during the immediate post-partum period, and ewe-lamb distance in later lactation.

6.3.2. Individual maternal style.

Table 6.2 shows the rankings for individual ewes within their breed groups. Blackface ewes showed consistency in their maternal behaviour throughout lactation (Kendall's Coefficient of Concordance $W=0.205$, $N=12/\chi^2=22.57$, d.f.=11, $p<0.05$). Suffolk ewes were variable in their expression of maternal behaviour and did not behave consistently over time ($W=0.132$, $N=11/\chi^2=11.88$, d.f.=9, NS).

Table 6.2.
Individual ewe rankings for maternal behaviours throughout lactation, ewes ranked within breed.

Ewe	Latency to groom	Duration of grooming	Non-co-op	Distance- 1st 3 days	Nose- 1st 3 days	Lie together	Distance- later lact.	Head-up posture	Nose- later lact.	Prop. succ. sucks
Suffolk ewes										
1	8	3	3	3	9.5	3	5	4	9	5
3	2	2	10	1	1	8	3	1	1	7
6	1	9	1	5	3	9	6	7	3	9
7	9	10	9	2	6.5	2	10	10	8	2
9	3	7	5	10	4	10	8	5.5	10	8
11	5	6	6	9	9.5	5	2	9	2	1
13	6	1	2	8	2	6	9	8	7	3
17	7	8	8	7	6.5	7	1	5.5	4	10
18	10	5	7	6	8	4	4	3	5	6
23	4	4	4	4	5	1	7	2	6	4
Blackface ewes										
26	3	6	12	8	1	11	2	11	5	12
30	4	3	10	9	3	3	4	4	4	4
32	8	9	7	12	11.5	10	3	9	12	11
34	7	7	9	10	6	7	5	7	3	5
35	10	10	8	7	8.5	8.5	11	1	11	10
38	2	5	2	1	4	1	6	12	2	7
39	5	8	6	11	8.5	8.5	9	6	10	9
40	12	12	3	2	6	5	12	3	9	3
41	6	4	11	6	2	4	1	2	1	8
42	9	1	1	3	11.5	2	8	8	6	1
43	1	2	5	5	6	12	7	10	7	6
44	11	11	4	4	10	6	10	5	8	2

6.4. Discussion.

6.4.1. General maternal style.

6.4.1.1. The dimensions describing maternal behaviour in the ewe.

The factor analysis showed that variation in maternal behaviour in the ewe can be described using multiple dimensions. However the use of these two particular breeds, and these breeds only, is likely to have had a strong influence on the dimensions described by the factor analysis and therefore limits the application of its findings to ewes in general. Consequently the findings of this factor analysis should not be assumed to describe general ovine maternal behaviour.

Factor 1 in this study represented the affiliation of the ewe for her lamb, comprising behaviours which relate to her willingness to interact with, and show maternal behaviour towards her lamb. The duration of grooming the neonate and nosing during later lactation, which helps to maintain the bond (Shillito and Hoyland 1971), represent intentional interaction with the lamb and an affiliation towards it. Rejection or unwillingness to interact with the lamb is represented by negative behaviour during the immediate post-partum period, including leaving the neonate, backing away from it and behaving aggressively toward it. Latency to groom and the failure to co-operate with initial suck attempts also represent an unwillingness to interact with the lamb at this time. During later lactation ewe-lamb distance acts as an indicator of the strength of the ewe-lamb bond (Morgan and Arnold 1974) and this measure therefore represents attraction between ewes and lambs beyond the post-partum period. The proportion of successful sucks also gives a good indication of the type of interactions ewes have with their lambs and their willingness to interact with them during later lactation.

Factor 2 in this study related to the ewe's reaction to her lamb's attempts to interact physically with her, especially during sucking interactions. Non-co-operation with initial suck attempts represents a definite unwillingness to allow the lamb to interact physically as it involves backing away from, and walking over the top of, lambs attempting to contact the udder. Negative behaviour during the post-partum period usually occurs in response to lambs moving towards the ewe (Lynch et al. 1992) and again represents an unwillingness to allow the lamb to make contact. During later lactation physical interaction between the ewe and lamb is limited to sucking interactions and the proportion of successful suck attempts at this time gives a good indication of the ewe's response to lamb attempts to interact with her. Factor 3 related to the ewe-lamb bond during later lactation.

The three factors in the present study appear to represent different aspects of maternal behaviour in the ewe but not in the same way as the dimensions described in the primate literature (Fairbanks 1996). The differences between the dimensions describing maternal behaviour in sheep and primates could be explained by differences in the nature of their mother-young relationship. Young primates appear to be much more dependent on their mothers than lambs, maintaining close contact and using her as a secure base from which to explore the surrounding environment (Fairbanks 1996). Whilst the primary social relationship of the lamb is with its dam (Morgan and Arnold 1974), and lambs return to their dams when stressed or disturbed (Hinch et al. 1987), lambs have less interaction with their dams for the majority of the lactation period, compared to young primates. The potential for maternal rejection, breaking contact and contact-promoting behaviour is therefore reduced in ewes, compared to female primates. In addition grooming is only seen in sheep during the immediate post-partum period.

6.4.1.2. Comparison with a study on early maternal behaviour in the ewe.

In a study of the consistency of initial maternal behaviour across parities in individual Suffolk and Scottish Blackface ewes, Dwyer and Lawrence (2000b) also found that variation in ewe maternal behaviour could be described multi-dimensionally. They classified their two dimensions using descriptive terms from the primate literature: maternal rejection and maternal care/warmth.

Maternal care or warmth is associated with cradling and grooming behaviour in primates. In sheep this dimension comprised behaviours associated with grooming and nosing the lamb and the reaction of the ewe to initial suck attempts (Dwyer and

Lawrence 2000b). During the immediate post-partum period the mother-young relationship between ewes and lambs shows greater similarity to that seen in primates, compared to the ewe-lamb relationship over the whole lactation period. The ewe and lamb remain in close proximity during the initial post-partum period, with grooming of the neonate and lamb suck attempts being predominant activities (Lynch et al. 1992). At this time the lamb is still uncoordinated and grooming by the ewe facilitates righting, standing and successful sucking (Vince et al. 1984). The ewe also follows straying lambs and prevents them leaving the birth site (Bareham 1976). Consequently the ewe has much potential to influence the behaviour of her lamb and to show rejection or promote contact with her lamb. Therefore the use of dimensions from the primate literature is appropriate for Dwyer and Lawrence's study (2000b) of the initial maternal behaviour of ewes, but not for the current study, which examined the whole lactation period.

6.4.1.3. Breed differences in maternal style.

Variation in ewe maternal behaviour over the whole lactation period has been described using three dimensions, maternal affiliation for her lamb, ewe response to her lamb's attempts to interact with her and the ewe-lamb bond during later lactation. The score plots of the individual ewes show that Suffolk and Blackface ewes differ in the style of their maternal behaviour according to these three dimensions. Maternal affiliation with the lamb, Factor 1, accounted for most of the variation in behaviour between the two breeds. The maternal behaviour of Blackface ewes was mostly characterised by behaviours with positive loadings on this factor, including grooming duration, nosing the lamb and the proportion of successful sucking attempts during later lactation. In contrast, the behaviour of Suffolk ewes was mostly associated with behaviours with negative loadings, for example the performance of negative behaviour during the immediate post-partum period, failure to co-operate with initial sucking attempts and ewe-lamb distance during later lactation. Blackface ewes may therefore be described as being more willing to interact with their lambs, compared to Suffolk ewes.

Factor 2 also accounted for some of the variation between breeds. The majority of Suffolk ewes had a positive loading on the second factor, indicating low co-operation with interactions from their lambs, especially during the immediate post-partum period. Blackface ewes had mostly negative scores, which suggests a more positive response to lambs' attempts to interact with their dam. The third factor, relating to the ewe-lamb bond during later lactation, accounted for the least amount of variation between the breeds and there was only a tendency for the two breeds to differ in their scores on this factor.

Variation between individual animals within the two breeds appeared to be greater on the second two factors, compared to Factor 1. Animals within the two breeds were quite tightly grouped on Factor 1, with the vast majority of Blackface ewes showing a positive score and the vast majority of Suffolk ewes showing a negative score. On the second two factors animals within the two breeds showed a wider range of scores, especially on Factor 3, and showed both positive and negative loadings on both factors. Variation between individuals within the two breeds therefore appears to receive more influence from the willingness of the ewe to accept interactions from her lamb (Factor 2) and their ewe-lamb bond during later lactation (Factor 3), compared to her own propensity to interact with it (Factor 1). Individuals within each of the two breeds can therefore be expected to show similarities in their levels of maternal affiliation, but to vary in their co-operation with their lamb's attempts to interact with them and their bond during the later lactation period.

6.4.1.4. Comparison of long-term maternal behaviour with initial maternal behaviour.

The results of this factor analysis can also be compared with the factor analysis of the first 3 days post-partum in Chapter 3. During the post-partum period the scores of the majority of Blackface ewes were tightly clumped together and their behaviour was associated with affiliative behaviours such as grooming the neonate and low pitched vocalisation. Suffolk ewes showed greater variation, especially on the negative loadings for both factors, and their behaviour was characterised more by negative behaviours and the latency to begin grooming the neonate. It therefore appears that Blackface ewes show increasing variation in their maternal behaviour as their lambs become older, although the general nature of this behaviour, affiliative rather than rejecting, does not change. Suffolk ewes show variation in their expression of maternal care from birth onwards but their behavioural style also shows similarities as their lambs age and is characterised by more rejection and a lower willingness to interact with their lambs than Blackface ewes.

The continued greater expression of affiliative maternal behaviour of the Blackface ewes, compared to the Suffolk ewes, may be facilitated by their greater attraction to their lambs, and subsequent closer interaction, during the early post-partum period. The hormonal control of maternal behaviour declines rapidly after birth and during later lactation maternal behaviour is regulated via sensory information from the lamb (Poindron and Le Neindre 1980). Maintenance of maternal behaviour at this stage is sustained through learning and reinforcement (reviewed by Fleming et al. 1999). A closer relationship with their lambs after birth will provide the Blackface ewes with more sensory stimulation from their lambs than Suffolk ewes, and this may result in a greater likelihood of expressing affiliative behaviour as their lambs age. In contrast, the Suffolk ewes' lower attraction towards lambs will provide less stimulation from their young, leading to less reinforcement of their maternal behaviour and a subsequent decline in maternal motivation. More work is needed to investigate this further.

6.4.2. Individual maternal style.

Analysis of consistency in maternal behaviour throughout lactation showed that individual Blackface ewes were consistent in their maternal behaviour but Suffolk ewes were not. This agrees with the predictions made from observations of maternal behaviour throughout lactation. Taking into account breed differences in maternal behaviour, individual Blackface ewes can therefore be described as having a consistent maternal style involving affiliative behaviour towards their lambs. The maternal style of Suffolk ewes is characterised by inconsistency, with less affiliative behaviour and more rejection than Blackface ewes.

6.4.2.1. Implications of consistency in maternal style for the ewe-lamb relationship.

The differences in the consistency of expression of maternal behaviour throughout lactation are likely to have implications for the ewe-lamb relationship in the two breeds. The consistent maternal style of Blackface ewes results in their lambs having a predictable relationship with their dams over time. Similar rankings in each of the maternal behaviours indicate that the level of maternal care and response of individual Blackface ewes to their lambs remains comparable as their lambs become older. Accordingly, the maternal behaviour of the ewe as her lamb ages can be predicted by her previous behaviour towards it and ewes are likely to show similar levels of maternal care in behaviours occurring at the same lamb age.

In contrast, lambs with Suffolk mothers do not have a predictable relationship with their dam. A Suffolk ewe's interaction with her lamb at any age can not be predicted by her previous behaviour toward it and her performance of different behaviours at the same stage of lamb development are less likely to be similar, compared to Blackface ewes. Consequently Suffolk-raised lambs are likely to have a less stable relationship with their dams and be less attracted to them than lambs with a Blackface dam. As lambs are primarily responsible for the physical maintenance of proximity in later lactation this could contribute to the greater ewe-lamb distance seen in Suffolk ewes.

6.4.3. Consequences of variation in maternal care for offspring development.

The observations of maternal behaviour between parturition and weaning show that the two breeds exhibit much variation in maternal care throughout lactation. This is likely to have consequences for their offspring. The implications for the ewe-lamb relationship have already been discussed in relation to specific maternal behaviours and consistency in the expression of maternal care. However variation in maternal care also has the potential to influence offspring development. In primates (reviewed by Fairbanks 1996) rejecting mothers were found to have more enterprising offspring, which developed independence at an earlier age than those with less rejecting mothers and appeared more resourceful than their more protected peers. These animals spent more time away from the mother, exploring the environment and interacting with social companions. In contrast, infants with protective, restrictive mothers were more cautious in response to novelty and challenging situations. This was because infants with anxious or protective mothers were generally held back in their early exploration of the physical and social world. However the influence of maternal rejection on the infant was not completely benign. Offspring with rejecting mothers had more tantrums, gave more distress cries and appeared more disturbed by forced separation from the mother.

Differences in maternal behaviour between the two ewe breeds therefore have the potential to influence lamb behaviour in the long-term, as well as in the present. The effect of variation in maternal care in the two breeds is explored further in Chapter 7, (influence on the stress response and learning ability of offspring) and in Chapter 8, (influence on traits relevant for production).

6.5. Summary.

Factor analysis of selected maternal behaviours between birth and weaning showed that variation in ewe maternal behaviour can be described using three dimensions, maternal affiliation, the ewe's reaction towards her lamb's attempts to interact with her and the ewe-lamb bond during later lactation. The two breeds differ most in their expression of maternal affiliation whereas variation between individual animals within the two breeds is primarily due to the ewe's response to her lamb interacting with her and their ewe-lamb bond during later lactation. Individuals within the two breeds were also shown to differ in the consistency of their expression of maternal care throughout lactation. Blackface ewes showed consistency in their maternal care whereas Suffolk ewes did not. This is likely to result in a stable ewe-lamb relationship in Blackface ewes but an unstable relationship in Suffolk ewes. Variation in maternal behaviour is likely to have consequences for the development of offspring and this is explored further in subsequent chapters.

Chapter 7: The effect of variation in dam and sire breed on the stress response and learning ability of offspring.

7.1. Introduction.

The aim of this chapter is to investigate the effect of variation in maternal care on the stress response and learning ability of offspring in sheep. The influence of maternal care on offspring development has received extensive investigation in laboratory rodents (reviewed by Meaney et al. 1996, Francis and Meaney 1999, Francis et al. 1999a).

7.1.1. The influence of maternal care in laboratory rodents.

During the 1950's and 1960's it was found that early stimulation of rat pups, in the form of removal from the nest for 3 minutes, results in accelerated development. This includes the earlier appearance of body hair and opening of the eyes (Levine 1959), earlier maturation of the Hypothalamic-Pituitary-Adrenal (HPA) axis (Levine et al. 1958) and earlier myelination of the central nervous system (Levine and Alpert 1959). In addition, physiological responses to environmental conditions in later life are markedly changed by infantile stimulation (Levine 1962). Rats which have been stimulated as pups are more reactive than non-stimulated animals to distinctly noxious and threatening stimuli, such as electric shock, but less reactive to milder stressors such as an open field test. In contrast, non-stimulated animals appear to be reactive to a greater variety of environmental changes and to require less extreme changes to elicit physiological stress responses. Stimulated animals have a greater ability to respond rapidly to a perceived noxious stimuli, and to return to normal levels more quickly, whereas non-stimulated rats respond more slowly and show a prolonged response (Levine 1962).

The responses of stimulated animals in the open field test, high activity and low defecation, are interpreted by Levine et al. (1967) as being indicative of low emotionality and a rat which is less responsive to novelty on both a physiological and behavioural level. Combined with the greater response of pups to distinctly noxious stimuli, it appears that infantile stimulation imparts the capacity for making finer discrimination of the relevance of environmental stimuli. Non-stimulated animals appear to have much less discrimination and react with a large corticosterone response, regardless of the specific aspects of the test situation (Levine et al. 1967).

The influence of infantile stimulation has since been found to be mediated via the maternal care received by the pups (e.g. Moore and Power 1992, Liu et al. 1997, Caldji et al. 1998, Francis et al. 1999b). The handling of the pups during the stimulation process alters the behaviour of their dams and results in them showing more licking/grooming when the pups are returned to the nest. The effect of increased licking/grooming on the stress response of offspring was initially tested by artificially manipulating the level received (Birke and Sadler 1987, Moore and Power 1992). Differences in the level of ano-genital licking received as a pup affect the behaviour of offspring in an open field test. Male pups receiving a reduced level of ano-genital licking show increased play and decreased exploration in the open field test compared to those receiving a normal level.

The importance of grooming has been further demonstrated during observations involving prolonged separation from the dam (12-24 hours) and, the resultant, deprivation of maternal care. During these separations simulated grooming, using a paintbrush, reduces the adverse effects of maternal separation (Kuhn et al. 1990, van Oers et al. 1998, Gonzalez et al. 2001). Kuhn et al. (1990) also found that passive sensory cues associated with the dam, provided by passive contact with an anaesthetised dam, reduce the corticosterone response to maternal separation. However the same study showed that active maternal care is necessary for growth hormone production and pups receiving only passive care show the same decrease in secretion as separated pups (Kuhn et al. 1990).

Zaharia et al. (1996) proposed that naturally occurring poor maternal behaviours may have effects comparable to those of repeated transient separations from the dam. Recent investigation of the influence of maternal care has used natural variation in maternal behaviour already present in the population (e.g. Liu et al. 1997, Caldji et al. 1998, Francis et al. 1999b). These studies use dams which show either high or low levels of licking/grooming and another positive maternal behaviour, arched back nursing, (LG-ABN). The adult offspring of dams showing high levels of LG-ABN have been found to show a decreased plasma ACTH and corticosterone response to restraint stress compared with those receiving low levels of LG-ABN, even though the basal hormonal levels of these two groups do not differ (Liu et al. 1997). Offspring of high LG-ABN mothers also show reduced behavioural fearfulness in response to novelty as adults, compared to the offspring of low LG-ABN (Caldji et al. 1998). Cross-fostering of pups between dams of high and low LG-ABN shows that this is a function of the maternal care received, rather than the genetics of the animals.

The maternal care received as a neonate does not just affect the stress response of adult animals. Learning ability (Zaharia et al. 1996, Anisman et al. 1998, Liu et al. 2000) and the offspring's own maternal care (Francis et al. 1999b) have also been found to be affected by the maternal care received as pups. Rats receiving high levels of LG-ABN are quicker to learn a spatial memory task and also show more searching of the relevant area if the goal is removed, compared to rats which have received low levels of LG-ABN (Liu et al. 2000). High reactivity to stressors and a failure to acquire a spatial learning response in a water maze in mice has been found to be reversed by early handling or cross-fostering to high grooming mothers (Anisman et al. 1998). In addition, female rats receiving high levels of LG-ABN as pups also show high levels of LG-ABN towards their own pups (Francis et al. 1999b), this is discussed further in Chapter 8.

7.1.2. The influence of maternal care in other species.

The influence of maternal care on the development of offspring has also received some investigation in species other than rodents. In primates (reviewed by Fairbanks 1996) the influence of general maternal style rather than specific maternal behaviours, as in laboratory rodents, has been studied. Primate mothers showing a more rejecting maternal style produce offspring which, provided they survive, are more enterprising and resourceful than their more protected peers. The offspring of more protective or restrictive mothers are more cautious in response to novelty, for example entering a new enclosure or approaching a new food container, and show more fearfulness in response to novel and challenging situations. The maternal style of female offspring is also influenced by the mother-infant contact received from their own mothers (Fairbanks 1996). Parental care is also known to influence cognitive development in human and non-human primates (Ammerman et al. 1986, Suomi 1997) and Reiss et al. (1995) found that inconsistent parenting of adolescent humans is linked to antisocial behaviour and depressive symptoms.

7.1.3. The influence of maternal care in sheep.

In sheep, the study of maternal care in relation to the development of offspring has been rare and mostly confined to the comparison of isolation raised, peer raised and dam raised lambs. In an open field test performed at 14 days of age, isolation reared lambs exhibit withdrawal behaviour. They are slow to initiate movement or investigate a novel object and show little ambulatory behaviour in comparison to the other groups. Peer raised animals also differ in their behaviour from dam raised lambs, being slow to initiate movement on first entering the arena and maintaining a close proximity to the novel object (a hobby horse) once they have approached it (Moberg and Wood 1982). These behavioural differences attenuate with age, with the exception of the greater attraction to the novel object shown by the peer raised lambs. These animals continue to show a greater interaction with the hobby horse than the other two groups. Despite their differences in behaviour, the three groups show no differences in adrenocortical response during the test (Moberg and Wood 1982).

More recent studies comparing artificially (peer) and dam raised lambs have shown that maternal deprivation soon after birth (between 18 and 48 hours post-partum), can cause impaired immune function and decreased weight gain over the first month post-partum. The behaviour of maternally deprived lambs during social isolation is also indicative of a greater stress response, compared to dam raised animals. Maternally deprived animals therefore show a reduced ability to cope with emotional and nutritional stresses, compared to dam raised animals (Napolitano et al. 1995, Sevi et al. 1999). However, if the artificially reared animals are given a gradual transition from maternal to reconstituted milk, after separation from the dam, the detrimental effects of artificial rearing on the behavioural, endocrine and immune responses of the lambs are minimised (Sevi et al. 1999).

Napolitano et al. (2003) studied the effect of reduced maternal care in the ewe by artificially preventing lambs from suckling their dam. Lambs which are raised with their dam but prevented from suckling, by covering the udder, appear less secure and display more withdrawal behaviour during social isolation, compared to dam or artificially raised animals. It is therefore possible that the frustration arising from being unable to feed from an available food source results in emotional disturbance in these lambs (Napolitano et al. 2003).

Ewe breed, rather than specific maternal care, has also been shown to influence general lamb behaviour in studies using cross-fostering or embryo transfer. Patterns of grazing and resting (Key and MacIver 1980) and the spatial relationships with other sheep, environmental location and general activity levels of lambs (Dwyer and Lawrence 2000a) are determined by the breed of dam rearing the lamb, rather than the breed of the lamb itself. Rearing environment has also been shown to affect the aggressiveness of lambs during pre-weaning social encounters but this does not appear to be linked to maternal care (Zito et al. 1978).

7.1.4. Potential for variation in maternal care to influence offspring development in the sheep.

Based on previous research and the close relationship between the ewe and her lamb, variation in maternal care appears to have much potential to influence the development of offspring in sheep. The naturally occurring differences in maternal behaviour between the two breeds of ewe can be used to investigate the influence of maternal care on offspring stress response and learning ability, in a similar way to that employed in the later rat studies (e.g. Liu et al. 1997). Although these studies used extremes in maternal behaviour to study its influence on offspring development, studies on normal rat mothers (without any experimental intervention) have shown that even subtle changes in the mother's tactile contact with her pups govern their future reaction to stress (reviewed by Winberg 1998). Therefore, even though the two breeds are not at opposite extremes in their expression of maternal care, it is still valid to use them in the exploration of the influence of maternal care on offspring development in the sheep.

In rats high LG-ABN is regarded as a positive maternal trait as it provides the young with increased stimulation and implies greater investment from the dam. In the two breeds of ewe in this study, the behaviour of the Blackface ewes is most similar to the high LG-ABN rat dams. Blackface ewes show more grooming during the immediate post-partum period, have a closer spatial relationship with their lambs and show more interaction with them during the later lactation period, implying greater maternal investment. It was therefore hypothesised that lambs with a Blackface dam would show a reduced stress response, compared to lambs with a Suffolk dam, and also show greater learning ability as young adults. As male lambs were left entire it was not possible to keep them after weaning and these tests were conducted on ewe lambs only.

7.2. Methods.

7.2.1. The stress response of lambs.

7.2.1.1. Animals and husbandry methods.

The stress response of 26 ewe lambs (17 with a Suffolk dam: 9 pure Suffolk and 8 crossbred, and 9 with a Blackface dam: 4 pure Blackface and 5 crossbred) was tested after weaning, when the animals were approximately 5 months old. Observations on the maternal behaviour of their dams during the lactation period had previously been conducted to provide information on the maternal care received, the methodology for these observations has been described in previous chapters (Chapters 3, 4 and 5). The animals were maintained as a single flock between weaning and testing, in the field in which they were raised and weaned. Testing of the stress response was conducted in the same shed as that used for lambing (Chapter 2). The ewe lambs were moved inside 2 weeks before testing to allow acclimatisation to the new environment. The animals were fed hay ad libitum and had free access to drinkers. The animals were tested in groups of 3 or 4 animals (2 groups of 3) and groups were balanced for the 4 different genotypes of lamb. The ewe lambs were maintained in their experimental groups throughout the testing period. The order of testing, both within groups and between groups, was randomised between the different stress response tests.

7.2.1.2. Testing the stress response of the lambs.

Three tests were used to assess the stress response of the lambs: a social separation test, a restraint test and a novel object test. These tests were chosen to examine different elements of the stress response and to mimic some of the procedures which occur during normal farm husbandry. The first test, the social separation test, involved placing the ewe lamb in a small pen within sight and hearing of the rest of the group. This lasted for 5 minutes. The aim of this test was to assess the reaction of the animals to being separated from their flock mates for a short period of time. This occurs quite frequently during normal sheep husbandry, for example during sorting or segregation for special procedures such as injections. The second test, the restraint test, involved separating the ewe lamb from the group, turning her on her back and holding her for 3 minutes. This was an extension of the social separation test and designed to mimic the handling involved during procedures such as feet trimming or shearing. Finally the ewe lambs' reaction to a novel object was tested in a similar way to that described in Chapter 3. This is a standard test of fear in animals (e.g. Romeyer and Bouissou 1992).

Tests were presented in the same order for all the ewe lambs, and were presented in order of increasing interaction with handlers and training to acclimatise the animals to social separation. This was due to the training and handling necessary for some of the tests potentially interfering with the other tests. The social separation involved in the restraint test and training and testing for the novel object test would have interfered with the social separation test, the handling involved in training for the novel object test may have interfered with the restraint test.

7.2.1.3. Measurement of the stress response.

The stress response of the ewe lambs was assessed during each test using behavioural observation, salivary cortisol measurements and heart rate measurements. Elevated cortisol and heart rate levels can indicate activation of the HPA axis and sympathetic autonomic nervous system respectively, which are involved in physiological stress responses.

The behavioural observations are described separately for each test. Changes in salivary cortisol were measured by collecting saliva before the test, to provide a baseline, and after the test, to assess any changes in cortisol levels caused by the test procedure. The baseline sample was collected 12 minutes before the start of the test. The 'test' sample was collected 10 minutes from the middle of the test period as there is a lag between cortisol being released and appearing in the saliva (Fell et al. 1985). Saliva was collected using veterinary swabs. These were inserted into the mouth of the ewe lambs, which then chewed them for approximately 30 seconds. This required a minimum of restraint, holding the head of the animal during insertion and removal, and the animals received training so that they readily accepted the swabs (described below). Once the swab had been removed it was placed in a collection tube. Swabs were then centrifuged after the end of testing for each group, to extract the saliva from the swab. Saliva samples were then frozen until assayed.

Saliva samples were analysed by the Endocrinology Section at SAC, Aberdeen using radioimmunoassay (Tunn et al. 1992). The samples were frozen at -20°C to precipitate the mucins. Once thawed they were centrifuged at 5000rpm in a micro-centrifuge for 5 minutes and the supernatant transferred to clean containers. Coat-A-Count Cortisol In-vitro Diagnostic Test Kits (Diagnostic Products Corporation UK, Euro/DPC Limited, Glyn Rhonway, Llanberis, Gwynedd) were used for the radioimmunoassay. Samples were assayed in duplicate along with low, medium and high quality controls. 200μl of each sample was incubated with 1000μl of tracer (^{125}I-cortisol) at room temperature for 3 hours. The sample tubes were emptied of supernatant and then counted for 1 minute on a gamma counter. The inter-assay coefficient of variation was 9.6%, the intra-assay coefficient of variation was 7.2%, and the minimum level of detection of the tests was 0.5 ng/ml.

Differences between the baseline and the test samples were used to assess the effect of the test procedure on the animal's cortisol levels. An average of the 3 baseline samples was also calculated to provide information on the baseline cortisol level for animals in the different breed groups. An average of all 3 samples was calculated as sheep show diurnal rhythms in their normal secretion of cortisol (Fulkerson and Tang 1979).

Changes in heart rate were measured using Polar heart rate monitors. Heart rate monitors were fitted round the chest of the animal, with the fleece removed from the area in contact with the monitor sensor. The fleece was removed 1 week before testing by clipping the relevant area and then applying Boots own brand hair removal cream. This ensured that all fleece was removed from the area and the monitor could make a good contact with the skin. Henley's Medical Ultrasound gel (Welwyn Garden City, Herts, UK) was also used when the monitors were fitted for experiments, to improve the contact between the sensors and the animal's skin. Monitors which did not make a good contact were bound to the animal using a crepe bandage to hold the monitor in place and prevent slipping. This was very rare, occurring only once in the social separation test and three times in the restraint test. All monitors made a good initial contact in both novel object tests (control and 'novel object present'). Training was also given to accustom the animals to wearing the heart rate monitors and having them fitted (described below).

Heart rate monitors were fitted 30 minutes before the start of each test to allow the animals to settle down after fitting. The monitors were then removed after the second saliva sample was taken. The monitors were programmed to take a reading of the animal's heart rate at 15 second intervals. The average heart rate was calculated for the test period and for a baseline period of the same duration, measured before the start of the test. The baseline period was started 10 minutes before the start of the test. The difference between these two measurements was then used to assess the effect of the test procedure on the heart rate of the animal.

Heart rate monitors were not 100% reliable and some monitors stopped working during the testing procedure. Data from 21, 19 and 18 animals were collected during the social separation, restraint and novel object test respectively.

7.2.1.4. Training for saliva collection and heart rate monitoring.

The ewe lambs were trained to accustom them to saliva sampling and to wearing the heart rate monitors. One week before testing they were trained to chew on the veterinary swabs for saliva sampling. To aid insertion of the swab into the mouth during training, the animals were held collectively in small pens within the larger home pen. The animal was backed into a corner, the head held lightly but firmly and a swab placed in its mouth, towards the back. The automatic reaction of the ewe lambs was to chew the swab. After 30 seconds the swab was removed. This period of time was found to be sufficient to saturate the swab with saliva. The process was repeated until the animals readily accepted the insertion of the swab and chewed on it (approximately 2 or 3 swabs). Training was repeated the following day but as the ewe lambs readily accepted the swabs, only 1 swab was used per animal.

Following training for saliva collection, the fleece was removed for the heart rate monitors. The ewe lambs were turned on their backs, an area just bigger than the sensor area clipped and the hair removal cream applied. The ewe lambs were then released for 5 minutes before being restrained in a standing position whilst the hair removal cream was wiped off and the area washed with water. Fitting of the heart rate monitors for the first time was delayed until the following day to prevent the ewe lambs associating the heart rate monitors with the potentially aversive procedure of fleece removal. During training the ewe lambs were turned on their back and the monitor fitted as quickly as possible. The monitors were then left in place for an hour. Monitors were removed by reaching under the animals whilst they were standing and unclipping the strap. Training was repeated on two further days before the testing began.

Although the training for saliva collection and heart rate monitoring involved interaction with, and handling of, the ewe lambs, which could have potentially interfered with the subsequent tests, this was felt to be necessary and unavoidable. Removal of the fleece for the heart rate monitors was essential to allow the monitors to detect the heartbeat of the animals. In addition, a lack of training was felt to have greater potential to interfere with the reaction of the animals during the test procedures than the controlled training given to all the animals. Without training animals would be experiencing unfamiliar handling directly before a test, which would be expected to alter their reaction during the test. Individual animals are likely to react differently to this handling and therefore the effect on their behaviour during the test would be different. The provision of prior training and the training of individuals until they appeared acclimatised to the procedure helped to counter this.

7.2.1.5. Test procedures.

7.2.1.5.1. Social separation test.

Testing took place over 2 days with 4 experimental groups tested on the first day and 3 groups tested on the second day. Each group was tested separately using apparatus connected to their home pen (Figure 7.1).

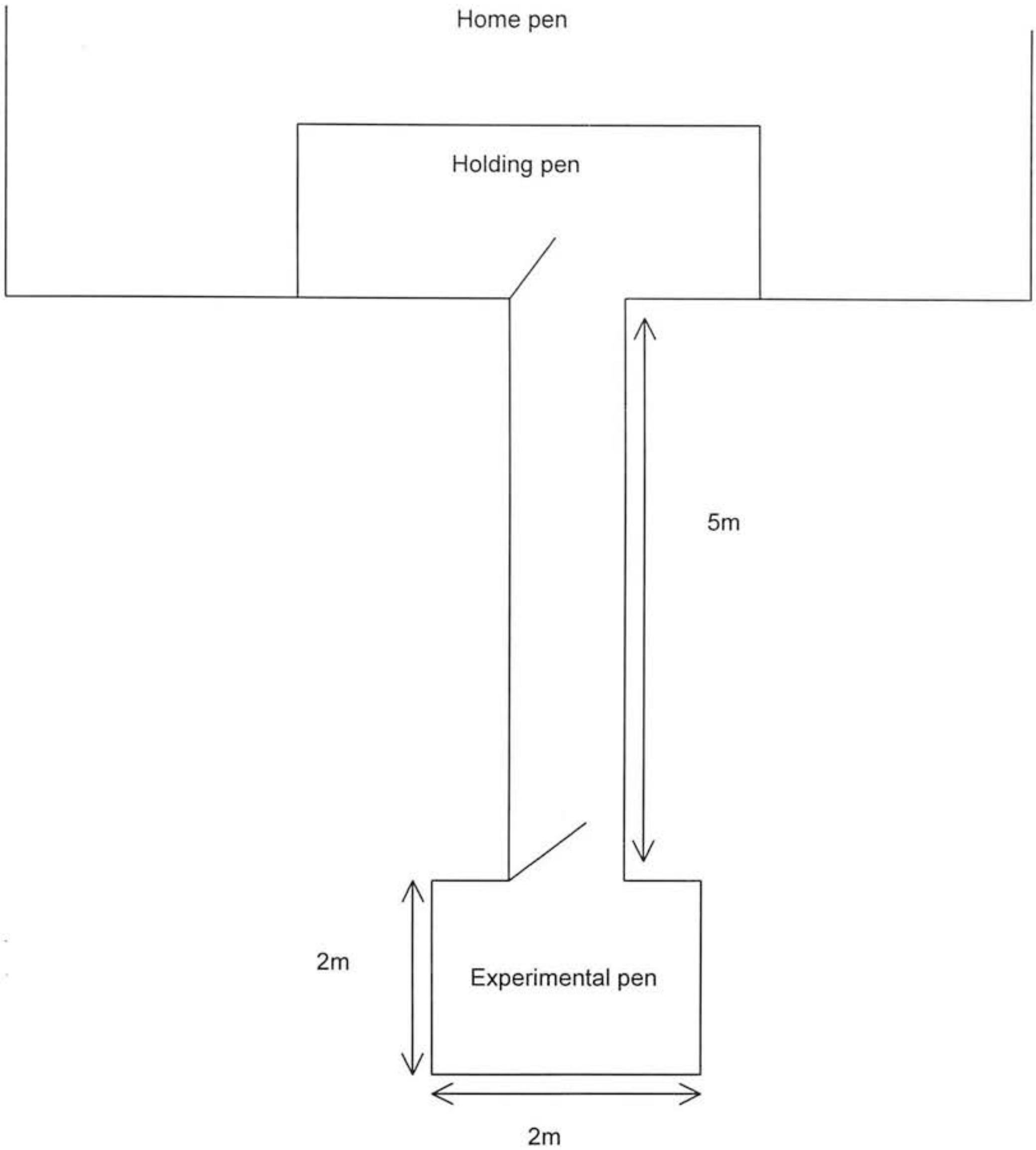

Figure 7.1. Apparatus used in the social separation test (not to scale).

Before the start of the test the ewe lambs were moved into the holding pen. Heart rate monitors were fitted to all the animals 30 minutes before the first animal was tested. A handler walked through the animals 5 minutes before the first saliva sample was due to be taken to mimic the events that were likely to happen before the other animals in the group had baseline saliva samples taken. A saliva sample was taken from the first ewe lamb 12 minutes before it was due to be tested.

During testing the animal was separated from the rest of the group and moved from the holding pen to the experimantal pen via the interconnecting run. The ewe lamb was held in the experimental pen for 5 minutes before being moved back to the holding pen. The second saliva sample was collected 7.5 minutes after the end of the test and the heart rate monitor removed. The procedure was repeated for the other members of the group in an overlapping fashion, resulting in all animals being tested in as short a time as possible.

The behaviour of the ewes in the test pen was recorded using videotape and subsequently analysed using The Observer software (Noldus et al. 2000). Table 7.1 shows the behaviours recorded, with their definitions. The duration of time spent sniffing the straw and pen, walking, standing still and the frequency of escape attempts were recorded from the videotape. Data on the frequency of vocalisations, urination and defecation were recorded live by tallying the number made by each animal during the test.

Table 7.1.
Definitions of behaviours recorded during social separation test

Behaviour	Definition
Vocalise	Ewe lamb makes high pitched 'baaaa' vocalisation.
Urinate	Ewe lamb urinates.
Defecate	Ewe lamb defecates.
Sniff straw	Ewe lamb stands still with nose in contact with the straw, begins when nose touches straw, ends when nose is no longer in contact with the straw.
Sniff pen	Ewe lamb stands still with nose in contact with side of pen, head may be moving, begins when nose touches pen, ends when nose is no longer in contact with the pen.
Standing still	Ewe lamb stands still, does not interact with the straw or pen sides.
Walking	Ewe lamb moves forward.
Escape attempt	Ewe lamb jumps at pen, burrows under pen or actively pushes head through pen.

7.2.1.5.2. Restraint test.

Testing took place over the 2 days following the social separation test. Four experimental groups were tested on day 1, 3 groups on day 2. Ewe lambs were tested in apparatus connected to their home pen (Figure 7.2). Ewe lambs were held in the holding pen during the test to assist saliva collection. The pre-test routine was the same as for the social separation test: animals were moved into the holding pen and heart rate monitors fitted 30 minutes before testing, the handler walked through the animals 5 minutes before the first saliva sample. The first saliva sample was collected 12 minutes before the start of the test.

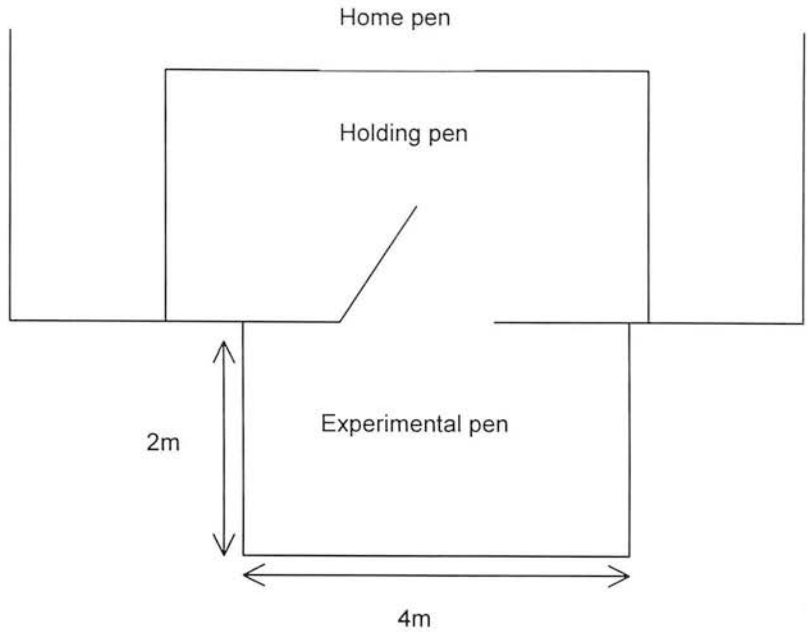

Figure 7.2. Apparatus used in the restraint test (not to scale).

During a test the animal was released into the experimental pen, caught up as quickly as possible and moved to the middle of the pen. The ewe lamb was then turned onto her back and held by the front legs for 3 minutes. The following behavioural measures were taken during the test: time taken (from being caught) to turn the ewe lamb onto her back, the proportion of time spent struggling and the frequency of struggles during the restraint period. Struggling was defined as the ewe tensing and moving her front legs or body. A struggle was considered to have started when the ewe tensed her front legs or body and began to push against the handler's grip. It was considered to have ended when the ewe relaxed and stopped pushing against the handler. The same handler was used in all restraint tests and care was taken to standardise the strength of the grip used to hold the animals on their backs. The grip was tightened if the animals struggled but softened as soon as they stopped struggling. Behaviour was recorded by a second person, using a Psion Workabout handheld computer and The Observer software (Noldus et al. 2000). As struggling may have been hard to assess from observation alone the handler quietly informed the recorder when the animal began or ceased struggling.

At the end of the test the ewe lamb was guided back into the holding pen and a second saliva sample taken 8.5 minutes after the end of the test. This was repeated for the other members of the group in an overlapping fashion, to ensure that animals were tested in the minimum period necessary.

7.2.1.5.3. Novel object test.

7.2.1.5.3.1. Test apparatus.

Figure 7.3 shows the apparatus used in the novel object test. This had the same design as that used in Chapter 3. Ewe lambs had visual, olfactory and auditory contact with their flock mates during the test to minimise the effects of social isolation. Training was also given to familiarise the ewe lambs with the apparatus and to accustom them to being alone in the test run. The same traffic cone as that used in Chapter 3 was used as the novel object, and this was placed in the centre of the 1m area in front of the holding pen. The ewe lamb was considered to be present

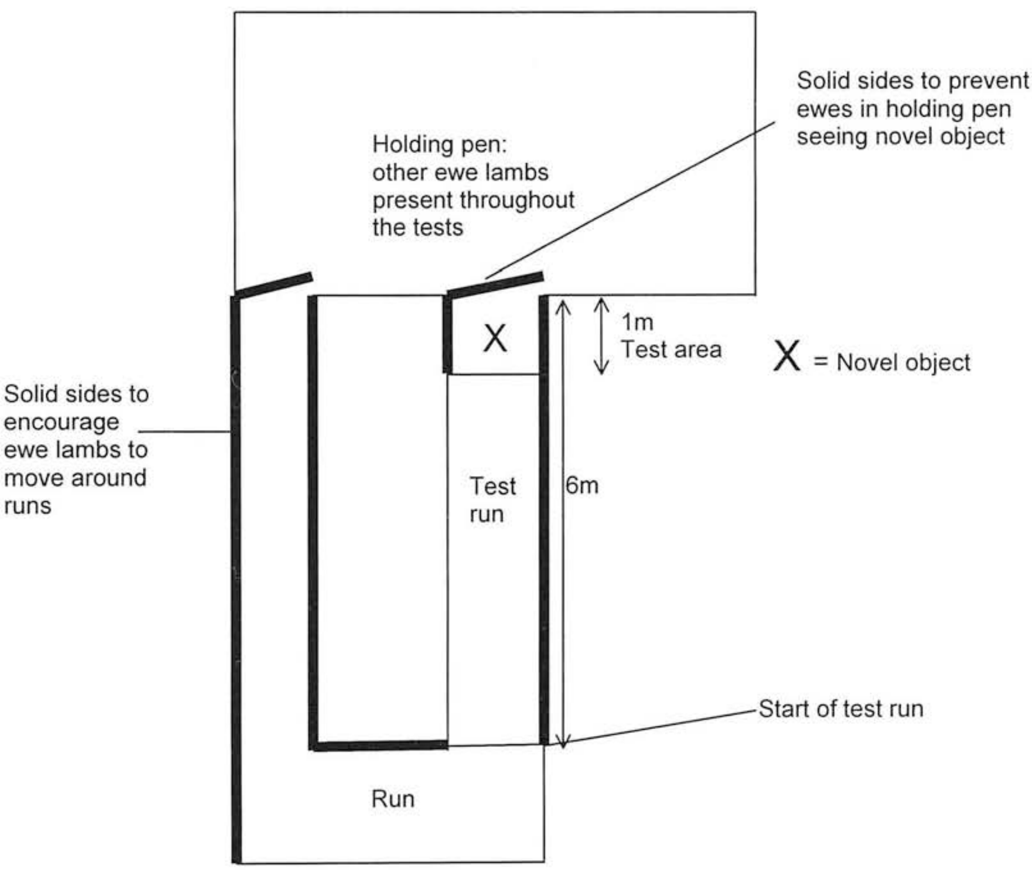

Figure 7.3. Apparatus used in the novel object test (not to scale).

in this area if at least two legs and half her body length were inside the area.

7.2.1.5.3.2. Training.

Following the restraint test the ewe lambs were trained to use the novel object test apparatus. On the first day the ewe lambs were given group training in their experimental groups. The animals were penned in the apparatus for 20 minutes, during which time all the gates were open and the animals had full access to the whole apparatus. The ewe lambs were walked slowly round apparatus 5 times during the group training period. Ewe lambs were trained to walk around the apparatus individually over the following 2 days. The group was penned in the holding pen. An individual animal was then released into the start of the run and left to walk round to the other end. If she did not voluntarily walk round within 30 seconds she was gently walked round by the handler. Once she reached the end of the run the gate was opened, allowing her to return to her flock mates. This was repeated for each ewe until she voluntarily walked round the run and rejoined the group.

7.2.1.5.3.3. Testing.

Testing took place over 4 days. The control test (without the novel object) was conducted first, with 4 groups tested on the first day and 3 groups on the second. The test was repeated with the novel object present on the third and fourth days. Groups which had received their control test on the first day were tested with the novel object present on the third day, animals given the control test on the second day were tested with the novel object on the fourth day.

The pre-test routine was the same as for the previous two tests: animals were moved into the holding pen and heart rate monitors fitted 30 minutes before testing, the handler walked through the animals 5 minutes before the first saliva sample. The first saliva sample was collected 12 minutes before the test.

Each ewe lamb was tested for 3 minutes in the test apparatus and their behaviour was recorded using a Psion Workabout handheld computer and The Observer software (Noldus et al. 2000). During both tests the following measures were taken: latency to reach the test area in front of the holding pen; duration spent in the test area;

number of times the ewe lamb walked away from the test area (from any location in the test run) and the number of times the ewe lamb backed away. During the 'object present' test the additional measures of time spent sniffing the novel object and the number of aggressive behaviours (bites, kicks and head butts) directed at the novel object were also recorded. Table 7.2 gives definitions for the behaviours recorded during the tests. After each animal the traffic cone was washed with water to remove any odours that may have altered the significance for subsequent ewe lambs. A second saliva sample was taken 8.5 minutes after the end of each test. This was repeated for the other members of the group in an overlapping fashion, to ensure that animals were tested in the minimum period necessary.

Table 7.2.
Definitions of ewe lamb behaviours recorded during the novel object test.

Behaviour	Definition
Walk away	Ewe lamb turns and walks away (makes at least two steps) from the test area with her head facing away from the holding pen.
Backing	Ewe lamb walks backwards away (makes at least two steps) from the test area with her head still facing towards the holding pen.
Sniffs cone	Ewe lamb contacts cone with end of muzzle, does not use teeth or top of head, begins when muzzle touches cone, ends when muzzle is no longer in contact with cone, each individual sniff recorded.
Bites cone	Ewe lamb contacts the top of the cone with her teeth and grips it between them, each individual bite recorded.
Kicks cone	Ewe lamb briefly contacts cone with foot, each individual kick recorded.
Head butts cone	Ewe lamb briefly contacts cone with top of head, causes cone to move, each individual head butt recorded.

7.2.1.6. Statistical analysis.

The data were analysed to assess the influence of dam breed on the stress response of offspring.

7.2.1.6.1. Salivary cortisol.

Data were checked for normality, average baseline salivary cortisol levels were square root transformed but all other data were normally distributed. The baseline value collected before each test and the test sample were compared within the two dam groups using Paired T-tests to assess if the test caused a change in salivary cortisol. The average baseline cortisol level and changes in cortisol levels caused by the three tests were compared between the two dam groups using REML. Sire breed and dam breed were fitted in the model.

7.2.1.6.2. Heart rate.

Data were checked for normality and found to be normally distributed. The baseline value collected before each test and the test sample were compared within the two dam groups using Paired T-tests to assess if the test caused a change in heart rate. Changes in heart rate caused by the three tests were analysed using REML. Sire breed and dam breed were fitted in the model.

7.2.1.6.3. Behavioural measures.

Data were checked for normality. The number of vocalisations and the duration spent standing still during the social separation test, the proportion of time spent struggling during the restraint test and the duration spent sniffing the cone during the novel object test were found to be skewed, all other data were normally distributed. The duration spent standing still, the proportion of time spent struggling and the duration spent sniffing the cone were square root transformed, the number of vocalisations could not be transformed. REML was used to analyse the behaviour of the ewe lambs during the tests, sire breed and dam breed were fitted in the model. The non-parametric Mann-Whitney test was used to analyse the number of vocalisations as these data could not be transformed. The effects of both sire breed and dam breed were examined.

7.2.2. Relationship between maternal behaviour and stress response.

The data were also used to examine the influence of maternal behaviour on the stress response of young.

7.2.2.1. Classification of the ewe lambs' stress response.

Data from the social separation and restraint tests were analysed using a factor analysis, with an orthogonal Varimax rotation. Due to the number of animals in the study it was not possible to use all the data and the results from the novel object test were not included as this was the least conclusive of the tests. Heart rate data were also omitted as a substantial number of animals had these data missing. The score plots for the individual animals on the various factors were taken as a representation of the response of the ewe lambs to putatively stressful situations.

7.2.2.2. Relationship between maternal behaviour and stress response.

In Chapter 6 a factor analysis showed that ovine maternal behaviour could be described using multiple dimensions. The two largest sources of variation in maternal care were due to the willingness of ewes to interact with their lambs throughout lactation (Factor 1) and their reaction to their lambs' attempts to interact with them (Factor 2). The scores of the ewes on these two factors were used as a representation of these two aspects of their maternal behaviour. The relationship between maternal behaviour and stress response was investigated by correlating the ewe lambs' scores in the factor analysis exploring stress response (above) with the scores of their dams on the first two factors investigating maternal behaviour (Chapter 6). The use of multiple correlations increases the chances of obtaining a spurious correlation. Consequently the third factor in the factor analysis of maternal behaviour, representing the ewe-lamb bond in later lactation, was not included in this analysis.

All data were normally distributed and Pearsons correlation was used in the analysis of stress response and maternal care received as a lamb. It was not possible to assess the relationship between maternal behaviour and stress response for all the ewe-lambs as not all of their dams were included in the factor analysis in Chapter 6. Eight Suffolk raised and 6 Blackface raised lambs were included in this analysis.

7.2.3. Learning ability.

7.2.3.1. Animals and husbandry methods.

The ability of the same animals to learn a spatial memory task was tested when they were approximately 16 months old. Due to fatalities during the previous year only 23 animals remained in the flock (15 with a Suffolk dam: 6 pure Suffolk, 9 crossbred and 8 with a Blackface dam: 4 pure Blackface and 4 crossbred). Testing took place in the same shed as that used for lambing (Chapter 2). The animals were brought inside

2 weeks before testing to acclimatise them to being indoors. They were fed ad libitum hay and had free access to drinkers.

7.2.3.2. Test apparatus and protocol.

The experimental protocol was based on a previous study by Edwards et al. (1996) which had successfully shown that ewes could learn a spatial memory task. Figure 7.4 shows the experimental apparatus. The bowls used were 30 cm deep and submerged to 20 cm in the straw. This meant that the ewes could see the location of the bowls from a distance but could not see whether or not they contained food. The

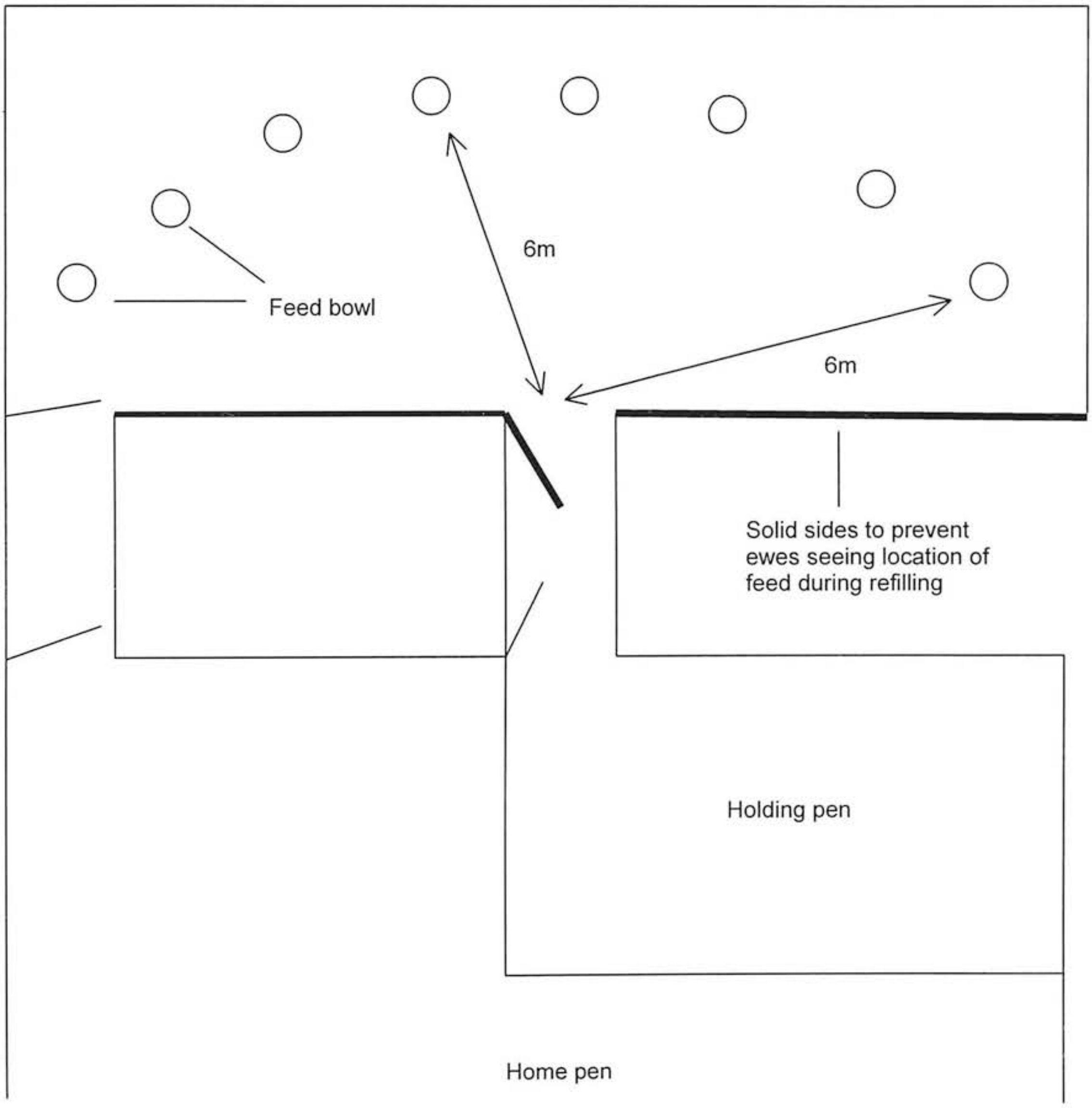

Figure 7.4. Apparatus used in the spatial memory task (not to scale).

sheep were first trained to associate the bowls with food. Each ewe was then randomly assigned one bowl which contained food and their ability to learn the location of this bowl tested. Learning ability was assessed by counting the number of trials taken for the ewe to walk directly to the bowl containing food, without visiting any other bowls. The food used was standard concentrated pellet feed.

7.2.3.3. Training.

The ewes needed to be trained to not only associate the bowls with food but to also enter the test area alone. During the first stage of training 100g feed was placed in each bowl and groups of 3 ewes (one group of 2) were released into the apparatus. When all bowls had been visited the ewes were returned to the home pen. Each group received 3 training runs at 10 minute intervals. The following day 100g feed was placed in each bowl as before. Ewes were initially released in pairs for 3 training sessions and then individually for 2 training sessions. By this stage ewes were entering the arena and approaching food bowls without apparent agitation.

7.2.3.4. Testing.

The ability of the ewes to learn the location of their food bowl was tested over 10 trials, with a 24 hour interval in between each trial. Testing took place each day between 8 and 9am, before the hay was replenished in the hayracks of the home pen. Hay was available ad libitum but only replenished twice a day, at 9am and 4pm. Therefore at 8am the hayracks were almost empty. This gave a slight degree of food deprivation but, as the attraction of ewes towards pellet feed is so high, it was not felt necessary to completely food deprive the animals before the trial. The ewes were tested in the same order during each trial to standardise the time interval between trials. One bowl was randomly assigned to each ewe and this bowl only contained 100g feed during each trial. Between each test the bowl of the previous ewe was checked to make sure that she had eaten all the food, any remaining was removed. Although the opaque plastic sheeting prevented the ewes seeing where the food was placed there was the potential for them to use auditory cues when the food was placed in the bowl. To counter this the food was placed in the bottom of the bowl as quietly as possible, rather than being dropped into the bowl.

During each trial the ewe was released into the experimental pen and the number of each bowl visited was recorded. The ewe was considered to have visited a bowl if she paused by the bowl and lowered her head towards it or placed her head in it. A maximum time of 3 minutes was set and if the ewe had not located the food by this time the trial was stopped and the ewe gently guided to the bowl containing the food. Two ewes with a Suffolk dam had to be guided to the food bowl during the first trial. Once the ewe had eaten the food the trial was stopped and the ewe returned to the home pen by the far gate.

A ewe was determined to have learnt the location of the food bowl once she proceeded straight to a food bowl on 3 successive trials. The trial number of the first successful trial was taken as the measure of learning ability. The minimum trial number was trial 2 as a direct approach during the first trial must have been made by chance and not through learning. Animals which did not directly approach the food bowl during any of the trials were given a maximum value of 10.

7.2.3.5. Statistical analysis

Data were tested for normality and found to be normally distributed. REML was used to analyse the effect of dam breed on the learning ability of the ewes, sire breed and dam breed were fitted in the model.

7.2.4. Relationship between maternal behaviour and learning ability.

The data were also used to look for relationships between maternal behaviour and learning ability. The scores of the ewes' dams on the first two factors of the factor analysis investigating maternal behaviour (Chapter 6) were used as a representation of the maternal care received as a lamb. Data were tested for normality and found to be normally distributed. The number of trials taken to learn the location of the food was correlated with the scores of the ewes' dams using Pearsons correlation. Seven Suffolk raised and 6 Blackface raised ewes were included in this analysis.

7.3. Results.

Unless otherwise stated, sire breed did not have an effect on the ewes' behavioural or physiological responses in the stress tests, or on their ability to learn the spatial memory task.

7.3.1. The stress response of lambs.

7.3.1.1. Social separation test.

7.3.1.1.1. Lamb behaviour.

Figure 7.5 shows the duration of time spent in various behaviours by the ewe lambs. There was no effect of dam breed on the amount of time spent sniffing the pen (REML Wald=2.18, d.f.=1, NS). Ewe lambs with a Suffolk dam spent longer sniffing the straw (Wald=13.48, d.f.=1, $p<0.001$) and standing still (Wald=7.93, d.f.=1, $p<0.01$) than ewe lambs with a Blackface dam. Ewe lambs with a Suffolk sire also spent longer standing still than ewe lambs with a Blackface sire (mean duration (s) (with 95% confidence interval): Suffolk sire=25.44 (14.07-40.16), Blackface sire=8.27 (2.50-17.37), Wald=4.21, d.f.=1, $p<0.05$).

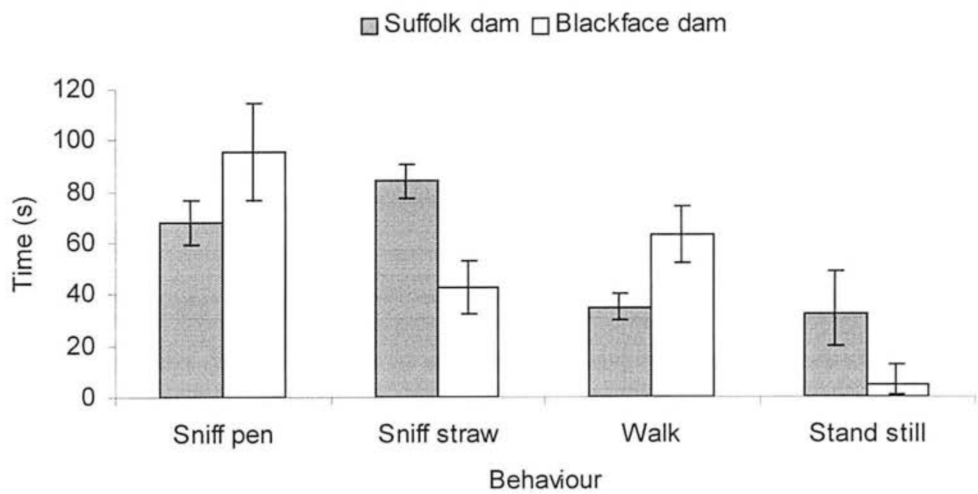

Figure 7.5. Mean duration spent in various behaviours during the social separation test (with s.e.m.).

Ewe lambs with a Blackface dam spent longer walking than those with a Suffolk dam (Wald=10.03, d.f.=1, $p<0.01$), ewe lambs with a Blackface sire also spent longer walking than those with a Suffolk sire (mean duration (s) (with s.e.m.): Suffolk sire=35.16 (4.92), Blackface sire=56.00 (9.74), Wald=5.86, d.f.=1, $p<0.05$).

There were non-significant tendencies for ewe lambs with a Blackface dam to urinate more frequently than ewe lambs with a Suffolk dam (mean number during test (with s.e.m.): Suffolk dam=0.24 (0.11), Blackface dam=0.56 (0.18), Wald=3.61, d.f.=1, $p=0.057$) and for ewe lambs with a Blackface sire to urinate more frequently than ewe lambs with a Suffolk sire (mean number during test (with s.e.m.): Suffolk sire=0.21 (0.11), Blackface sire=0.50 (0.15), Wald=3.01, d.f.=1, $p=0.083$).

There was no effect of dam breed on the frequency of vocalisation during the test (median number during test (with Q1, Q3): Suffolk dam=1.0 (0, 3.5), Blackface dam=1.0 (0, 13.5), Mann Whitney U=131.5, NS). Escape attempts and defecation during the test were very rare. Only 3 animals attempted to escape during the test (1 crossbred with a Blackface dam and 2 pure Blackface) and 6 animals defecated (4 crossbred with a Suffolk dam and 2 pure Blackface).

7.3.1.1.2. Heart rate.

Figure 7.6 shows the difference in heart rate between the baseline average, collected before the test, and the test average. Social separation caused an increase in the ewe lambs' heart rate (Suffolk dam: Paired T-test T=-3.00, $p<0.05$, Blackface dam: T=-2.95, $p<0.05$). Ewe lambs with a Blackface dam showed a greater increase in heart rate during the test than ewe lambs with a Suffolk dam (Wald=6.02, d.f.=1, $p<0.05$).

7.3.1.1.3. Salivary cortisol.

Figure 7.7 shows the difference in salivary cortisol between the baseline value, collected before the test, and the test value. Social separation did not cause a significant change in the ewe lambs' salivary cortisol (Suffolk dam: T=-1.49, NS, Blackface dam: T=-0.19, NS). As neither dam group showed a significant change in salivary cortisol levels the two dam groups were not compared.

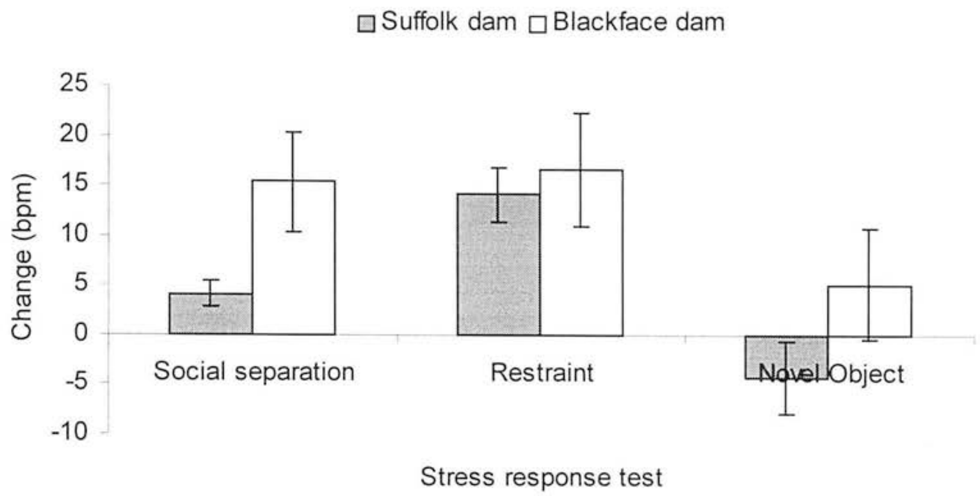

Figure 7.6. Mean change in heart rate during each of the stress response tests (with s.e.m.).

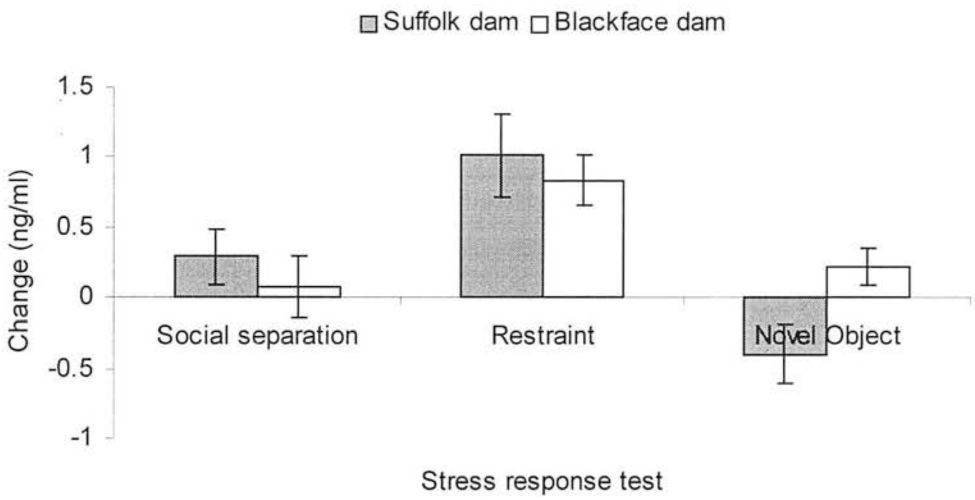

Figure 7.7. Mean change in salivary cortisol during each of the stress response tests (with s.e.m.).

7.3.1.2. Restraint test.

7.3.1.2.1. Lamb behaviour.

Table 7.3 shows the time taken to restrain the animal, the proportion of time spent struggling during the restraint and the frequency of struggles. Dam breed had no effect on the time taken to restrain the animal (Wald=0.00, d.f.=1, NS). There was a non-significant tendency for ewe lambs with a Blackface dam to struggle for longer

Table 7.3.
Behavioural measures recorded during the restraint test.

	Suffolk Dam	Blackface Dam
Time to restrain (s)	8.62 (0.54)	8.61 (1.24)
Proportion of test spent struggling (%)	1.72 (0.39, 3.99)	6.39 (3.40, 10.32)
Frequency of struggles	2.94 (0.89)	5.67 (1.67)

Table shows the mean time taken to restrain the ewe lamb from capture (with s.e.m.), the mean proportion of the test spent struggling (with 95% Confidence Interval) once ewe lamb was restrained and the mean number of struggles during the restraint period (with s.e.m.).

than ewe lambs with a Suffolk dam (Wald=3.02, d.f.=1, p=0.082). There was no effect of dam breed on the frequency of struggles during the test (Wald=2.57, d.f.=1, NS) but there was a non-significant tendency for ewe lambs with a Suffolk sire to struggle more frequently than ewe lambs with a Blackface sire (mean number of struggles during test (with s.e.m.): Suffolk sire=5.21(1.36), Blackface sire=2.33 (0.71), Wald=3.34, d.f.=1, p=0.068).

7.3.1.2.2. Heart rate.

Figure 7.6 shows the difference in heart rate between the baseline average, collected before the test, and the test average. Restraint caused an increase in the ewe lambs' heart rate (Suffolk dam: T=-5.24, $p<0.001$, Blackface dam: T=-2.99, $p<0.05$). There was no effect of dam breed on the heart rate change during the test (Wald=0.17, d.f.=1, NS).

7.3.1.2.3. Salivary cortisol.

Figure 7.7 shows the difference in salivary cortisol between the baseline value, collected before the test, and the test value. Restraint caused an increase in the salivary cortisol of the ewe lambs (Suffolk dam: T=-3.46, $p<0.01$, Blackface dam: T=-5.03, $p<0.01$). There was no effect of dam breed on the change in salivary cortisol levels of the animals (Wald=0.26, d.f.=1, NS) but ewe lambs with a Suffolk sire showed a bigger increase in salivary cortisol than ewe lambs with a Blackface sire (mean increase in salivary cortisol (ng/ml) (with s.e.m.): Suffolk sire=1.32 (0.34), Blackface sire=0.52 (0.10), Wald=4.56, d.f.=1, $p<0.05$).

7.3.1.3. Novel object test.

7.3.1.3.1. Lamb behaviour.

Table 7.4 shows the behaviour of the ewe lambs during the novel object test. There was a non-significant tendency for ewe lambs with a Blackface dam to be quicker to reach the test area when the novel object was present than ewe lambs with a Suffolk dam (Wald=2.95, d.f.=1, p=0.086). There was no effect of dam breed on the duration of time spent in the test area (Wald=1.38, d.f.=1, NS) or on the number of times the ewe lambs walked away from the test area (Wald=2.65, d.f.=1, NS). There was a non-significant tendency for ewe lambs with a Suffolk dam to show more backing away from the test area when the novel object was present, compared to ewe lambs with a Blackface dam (Wald=3.52, d.f.=1, p=0.061). There was no effect of dam breed on the duration of time spent sniffing the novel object (Wald=0.44, d.f.=1, NS).

Table 7.4.
Behavioural measures recorded during the novel object test.

	Suffolk Dam	Blackface Dam
Time to reach test area (s)	-6.65 (2.67)	-20.80 (9.73)
Duration in test area (s)	18.50 (13.40)	44.40 (17.30)
Frequency of walking away	-1.88 (0.58)	0.00 (1.12)
Frequency of backing	0.59 (0.34)	-0.67 (0.67)
Duration sniffing novel object (s)	21.60 (15.51, 28.70)	24.90 (18.33, 32.48)

Table shows the mean differences (with s.e.m.) in the time to reach the test area, the duration spent in the test area, the frequency of walking away and the frequency of backing away when the novel object was present, compared to the control test. The mean duration of time spent sniffing the novel object (with 95% Confidence Interval) is also shown.

Only 3 animals showed aggression towards the novel object (1 crossbred with a Suffolk dam and 2 crossbreds with a Blackface dam).

7.3.1.3.2. Heart rate.

Figure 7.6 shows the difference in the change in heart rate between the control test and the test with the novel object present. The presence of the novel object did not affect the ewe lambs' heart rate (Suffolk dam: T=1.15, NS, Blackface dam: T=-0.49, NS). As there were no significant changes in heart rate the two dam groups were not compared.

7.3.1.3.3. Salivary cortisol.

Figure 7.7 shows the difference in the change in cortisol levels between the control test and the test with the novel object present. The presence of the novel object did not affect the ewe lambs' salivary cortisol levels (Suffolk dam: T=1.91, NS, Blackface dam: T=-1.83, NS). As there were no significant changes in cortisol levels the two dam groups were not compared.

7.3.1.4. Baseline salivary cortisol levels.

There was no effect of dam breed on baseline salivary cortisol levels (mean (ng/ml) (with 95% Confidence Interval): Suffolk dam=0.68 (0.52, 0.86), Blackface dam=0.46 (0.33, 0.62), Wald=2.29, d.f.=1, NS) but ewe lambs with a Suffolk sire had a higher baseline level than ewe lambs with a Blackface sire (mean (ng/ml) (with 95% Confidence Interval): Suffolk sire=0.79 (0.61, 0.98), Blackface sire=0.38 (0.26, 0.52), Wald=9.61, d.f.=1, $p<0.01$).

7.3.2. Relationship between maternal behaviour and stress response.

7.3.2.1. Classification of the ewe lambs' stress response.

Four factors had eigen values above 1, accounting for 70.1% of the variance. The factor analysis was therefore run with 4 factors. Figure 7.8 shows the loading plot for the first two factors. Factor 1 accounted for 26.8% of the variance. The duration of time spent sniffing the straw and standing still in the social separation test both had a strong negative loading on the first factor, the duration of time spent walking and sniffing the pen and the frequency of vocalisation and urination in the social separation test all had strong positive loadings. Factor 1 therefore appears to represent the behavioural response of lambs during the social separation test, specifically whether they responded actively or passively to the situation.

The second factor related to the response of the lambs to restraint and accounted for 22.9% of the variance. The time taken to restrain the animal had a high positive loading on this factor, whereas the proportion of time spent struggling, the frequency of struggles and the salivary cortisol response all had strong negative loadings.

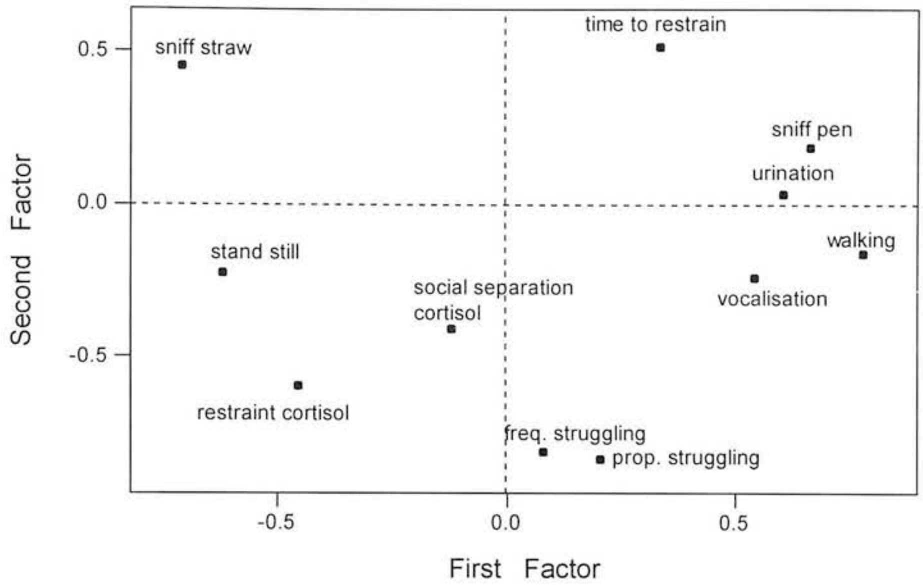

Figure 7.8. Loading plot for Factors 1 and 2 in the factor analysis of ewe lamb response to social separation and restraint.

Factor 3 accounted for 10.8% of the variance. The cortisol response of the ewe lambs during the social separation test had a strong negative loading on this factor, there were no strong positive loadings. Factor 4 accounted for 9.6% of the variance but there were no strong loadings on this factor.

The first two factors provided most information about the response of the ewe lambs during the tests. Accordingly only the first two factors were used in the assessment of the relationship between maternal behaviour and the ewe lambs' response to a putatively stressful situation.

Figure 7.9 shows the score plot for the individual animals on the first two factors. There was some overlap between the two groups but in general lambs with a Blackface dam had a positive loading on Factor 1 and lambs raised by a Suffolk dam had a negative loading (mean score (with s.e.m.): Suffolk=-0.405 (0.18), Blackface=0.770 (0.35), T=3.02, d.f.=12, $p<0.05$). This suggests an active response in the Blackface-raised ewe lambs and a more passive response in the Suffolk-raised animals. There was wide variation between animals in their response to restraint (Factor 2) and no obvious patterns emerged (mean score (with s.e.m.): Suffolk=0.190 (0.25), Blackface=-0.358 (0.28), T=-1.44, d.f.=19, NS).

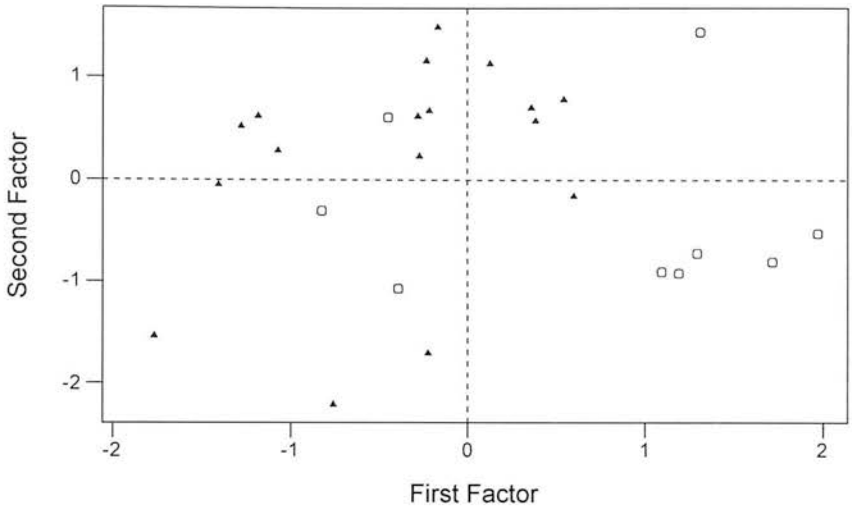

Figure 7.9. Score plot for individual animals on Factors 1 and 2 in the factor analysis of ewe lamb response to social separation and restraint. Animals with a Blackface dam are represented by open square symbols, animals with a Suffolk dam are represented by solid triangles.

7.3.2.2. Relationship between maternal behaviour and stress response.

There was no significant correlation between the ewe lambs' behavioural response during the social separation test and the willingness of their dams to interact with their lambs (Pearsons correlation r=0.238, NS) or their dam's response to their lambs' attempts to interact with them (r=-0.235, NS). There was also no significant correlation between the ewe lamb's response to restraint and the willingness of their dams to interact with their lambs (r=0.332, NS) or their dam's response to their lambs' attempts to interact with them (r=-0.250, NS).

7.3.3. Learning ability.

Figure 7.10 shows the average number of bowls visited in each trial by the two groups of ewes. Ewes with a Blackface dam were quicker to learn the location of the food bowl than ewes with a Suffolk dam (mean number of trials to show learning (with s.e.m.): Suffolk dam=6.93 (0.621), Blackface dam=4.50 (1.04), Wald=4.01, d.f.=1, $p<0.05$). There was no effect of dam breed on the time taken to reach the food bowl during the first trial in which the animals demonstrated learning (mean time to reach food bowl (s) (with s.e.m.): Suffolk dam=8.53 (1.19), Blackface dam=7.75 (1.26), Wald=0.01, d.f.=1, NS).

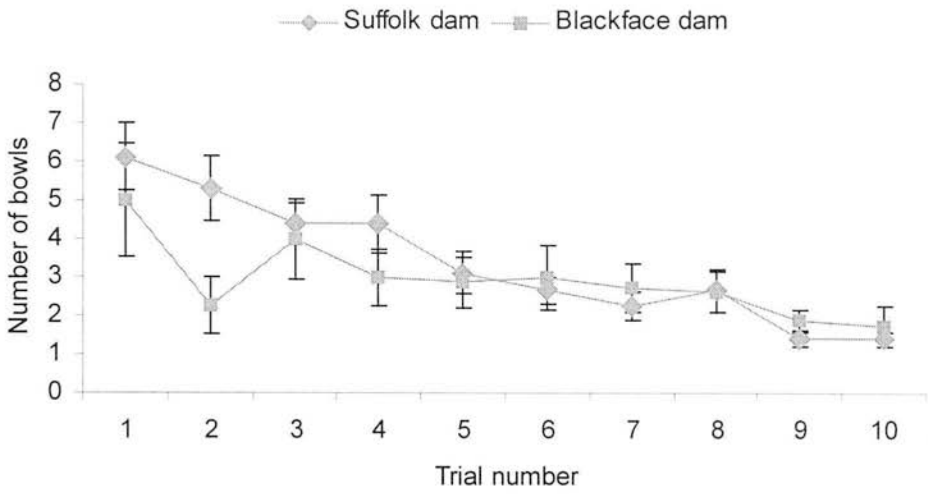

Figure 7.10. Mean number of bowls visited during each trial (with s.e.m.).

Four of the Blackface raised ewes which learnt the location of the food bowl early in the test were observed to begin visiting other food bowls before proceeding to the correct bowl in later trials. This was not observed in the Suffolk raised ewes.

7.3.4. Relationship between maternal behaviour and learning ability.

There was no significant correlation between the number of trials taken to learn the location of the food bowl and the willingness of the ewes' dams to interact with their lambs (r=0.162, NS) or their response to their lambs' attempts to interact with them (r=-0.142, NS).

7.4. Discussion.

7.4.1. The stress response of lambs.

7.4.1.1. Social separation test.

During the social separation test lambs raised by a Blackface dam showed more walking than those raised by a Suffolk ewe and had a tendency to urinate more frequently. Suffolk raised lambs spent a longer duration sniffing the straw than Blackface raised lambs, which may indicate exploration of the pen. They also spent more time standing still than Blackface raised lambs. Some of these behavioural differences may also be related to breed genetics as sire breed was found to have some influence on walking, standing still and urinating behaviour. The lambs' salivary cortisol levels were not affected by social separation but all lambs showed an increase in heart rate during the test. This was significantly higher in lambs with a Blackface dam, compared to lambs with a Suffolk dam. However, as heart rate also rises during activity, this could be due to the greater time spent walking by the Blackface raised lambs and the longer duration spent inactive and sniffing the straw by Suffolk raised lambs. It may not therefore indicate a greater stress response in the Blackface raised lambs.

Urination and defecation are gross indicators of an autonomic response (Levine 1962), and walking and bleating have been identified as being indicative of a panic or active defensive reaction in sheep (Vandenheede et al. 1998). The higher frequency of urination in Blackface raised lambs and their more frequent walking could therefore indicate a greater stress response in these animals, compared to lambs raised by Suffolk ewes. In addition, escape attempts are also indicative of a fear reaction (Vandenheede et al. 1998) and, although they were rare, they were only observed in Blackface raised animals. However it should also be considered that Blackface raised lambs did not show more frequent vocalisation than lambs with a Suffolk dam and their salivary cortisol levels did not increase in response to social separation. This suggests that any stress response in Blackface raised lambs was very slight. In addition, the behavioural and physiological responses of all the lambs suggest that the test was not very stressful for any of the animals. The differences in behaviour between the two groups of lambs may therefore reflect an active response to separation in Blackface raised lambs and a passive response in Suffolk raised lambs, rather than a greater stress response in the Blackface raised lambs.

7.4.1.2. Restraint test.

During the restraint test there was a tendency for Blackface raised lambs to struggle for longer than lambs with a Suffolk dam. Both groups showed an increase in heart rate and salivary cortisol, indicating that they found the test stressful, but there was no effect of dam breed on these physiological changes. The tendency for Blackface raised lambs to struggle for longer than Suffolk raised lambs, with the absence of a difference in cortisol or heart rate levels, gives further evidence of an active response to stressful situations in Blackface raised lambs and a passive response in Suffolk raised animals.

Lambs with a Suffolk sire showed a tendency to struggle more frequently and also showed an elevated cortisol response, compared to lambs with a Blackface sire. Sire breed therefore appears to have a greater effect than dam breed on the lamb stress response to restraint. As sires made only a genetic contribution to their offspring, this must be due to the animal's genetics rather than its previous experience, its nature rather than its nurture.

7.4.1.3. Novel object test.

The novel object test was the least conclusive of the three tests. There were no significant differences in behaviour between the two groups, although Blackface raised lambs showed a tendency to reach the novel object faster than Suffolk raised lambs and to back away from it less frequently. This suggests that lambs raised by a Blackface dam found the novel object less aversive than lambs raised by a Suffolk ewe. However, neither group showed a change in salivary cortisol level or heart rate, indicating that none of the ewe lambs found the presence of the novel object very stressful.

7.4.1.4. Significance of the three tests for the ewe lambs.

The lambs appear to have found the restraint test the most aversive of the three treatments. This agrees with a previous finding that sheep find this type of handling (inversion in isolation) more aversive than other handling treatments, for example isolation, close proximity of a human and capture (held in an upright position) in isolation (Rushen 1986). The greater aversion to the restraint test may be due to the nature of the perceived stress in this test being different to that experienced in the other two tests. During the social separation and novel object tests the lambs had greater control, being able to actively respond to social separation or to remove themselves from the presence of the novel object. In the restraint test the lambs were held firmly and were unable to remove themselves from the situation, effectively leaving them with no control.

7.4.1.5. Baseline cortisol levels.

There was no effect of dam breed on the baseline cortisol level of lambs but lambs with a Suffolk sire had a higher baseline level than lambs with a Blackface sire. This suggests that genotype influences an animal's baseline cortisol level. Plasma cortisol concentrations in lambs have also been found to be dependent on genotype, rather than rearing system (Goddard et al. 2000). This highlights the need for caution when comparing animals with different genotypes.

7.4.1.6. Classification of the lambs' stress response.

The factor analysis used to classify the lambs' stress response gives further evidence for a difference in the behavioural response of lambs raised by Suffolk and Blackface dams. The behaviour of lambs during the social separation test, specifically whether they behaved actively or passively, was the greatest source of variation between the animals. In addition, the scores for the individual animals showed that the behaviour of the majority of lambs raised by a Blackface dam was associated with an active response, whereas the majority of Suffolk raised lambs showed an association with a passive response.

The second largest source of variation was the response of lambs to restraint, the test which appeared to be most aversive to the lambs. There was no clear pattern relating to dam breed on this factor. Coupled with the comparisons between the two dam breed groups this suggests that dam breed does not have a major influence on the stress response of lambs.

7.4.1.7. The influence of dam breed on the later behaviour of offspring.

Although the results of these tests did not indicate an effect of dam breed on the stress response of lambs, they did reveal differences in the behavioural response of lambs in the tests. Lambs raised by Blackface dams showed a more active response, moving around more during the social separation test and tending to struggle for longer in the restraint test. Suffolk raised lambs were more passive during both tests. The results of the novel object test were harder to interpret in terms of an active or passive response, although lambs raised by a Blackface ewe appeared to find the novel object less aversive than lambs raised by Suffolk ewes.

The influence of dam breed on the behaviour of their lambs has the potential to be both genomic and non-genomic. Consequently the different responses of the ewe lambs during social separation and restraint may be due to the genetics they inherited from their mother, and/or to the maternal care they received from her during lactation. The significant influence of sire breed on some aspects of the lambs' behaviour during the two tests suggests that breed genetics do have a role in the response of sheep to putatively aversive situations. However this does not rule out the possibility that previous experience also plays a role, as seen in the rat (e.g. Meaney et al. 1996), and that the maternal care received as a lamb has some influence on later behaviour.

In order to investigate this further the potential influences of genetics and previous experience must be separated. In the rat this was achieved by cross-fostering pups soon after birth. However cross-fostering is unlikely to be successful in the sheep, due to the short period of time during which ewes are receptive to newborn lambs (e.g. Lynch et al. 1992). An alternative approach is embryo transfer. Ewes of the two breeds could be implanted with embryos that were not genetically related to themselves, but were genetically similar to each other. This would remove the dam's genetic influence and allow more accurate exploration of the influence of maternal behaviour on the behavioural response of sheep in putatively aversive situations.

The two aspects of maternal behaviour examined in this study, the willingness of the dam to interact with her lamb and the dam's response to her lamb's attempts to interact with her, did not have a significant association with the ewe lambs' behavioural response during social isolation. Consequently these two aspects of maternal behaviour do not appear to account for the differences in behaviour between Blackface and Suffolk raised lambs. However these were not the only differences in the maternal care received by the two groups of lambs. Blackface and Suffolk ewes also differed in the consistency of their expression of maternal behaviour over the lactation period and the way in which they controlled their lambs' sucking behaviour during later lactation. These aspects of maternal care also have the potential to shape the behavioural response of the lambs.

Suffolk ewes were inconsistent in their performance of maternal care and were not proactive in their control of their lambs' sucking behaviour. Consequently lambs raised by Suffolk ewes had an unstable relationship with their dams, sometimes experiencing a positive interaction, sometimes a negative one. This may have taught them that they had no ability to predict or control the outcome of their behaviour and thus caused them to assume a passive response to external factors. If proactive behaviour did not always result in a positive outcome these lambs may have gradually been discouraged from making active responses. In contrast, the use of communication in the control of lamb sucking behaviour and a consistency in the general expression of maternal care meant that lambs raised by Blackface ewes were able to predict the outcome of their interactions with their dam and usually received positive feedback to their actions. This could encourage their lambs to be proactive in response to aversive situations.

7.4.1.8. Differences in the influence of maternal care in rodents and sheep.

Maternal care in the ewe does not appear to have the same influence on offspring development as maternal care in rodents. In rats, variation in the performance of two specific maternal behaviours, licking/grooming and arched back nursing, were found to influence the stress response of young (e.g. Liu et al. 1997, Francis et al. 1999b). In the sheep, dam breed, and therefore maternal care, did not have an influence on the lambs' stress response. There are a number of possible explanations for this difference.

7.4.1.8.1. Differences in the development of the neonate in the two species.

Neonatal rats are altricial. Most of their behavioural capacities are undeveloped at birth and are limited to simple sensorimotor co-ordination. Consequently the mother must play the most active role in the interactions between mother and young (Rosenblatt and Lehrman 1963). In contrast, newborn lambs are precocial, rapidly standing and interacting with their dam after birth (Lynch et al. 1992). Newborn lambs are therefore more developed at birth than newborn rats and this may result in the maternal care received during the neonatal period having less potential to shape offspring development in the precocial lamb, compared to the altricial rat.

The influence of maternal care in rodents is thought to be mediated through its influence on the developing central nervous system. The reduced plasma ACTH and corticosterone responses to restraint stress seen in the offspring of high LG-ABN mother are thought to be caused by increased hippocampal glucocorticoid receptor (GR) expression and the resultant increase in glucocorticoid feedback sensitivity (Liu et al. 1997). These measures are significantly correlated with the frequency of maternal licking/grooming, suggesting that the tactile stimulation derived from maternal licking and grooming regulates pup physiology and affects central nervous system development, programming the HPA response to stress in the offspring (Liu et al. 1997). Early life events (handling or maternal separation) also influence the development of the $GABA_A$ receptor system, altering the expression of fearfulness in adulthood (Caldji et al. 2000b).

Weaver et al. (2000) found that repeated handling of neonatal pigs did not produce the same changes in HPA function as those seen in the rat. They suggested that this could be due to the fact that adult levels of hippocampal GR are present by the time of birth in the pig, and therefore not altered by neonatal handling. In contrast, in the rat, GR levels are low over the first two weeks of life and do not increase to adult levels until the third week of life (Weaver et al. 2000). As neonatal lambs are more similar in terms of development to neonatal pigs than neonatal rats, this may also be the reason for the differences between rats and sheep. However, there may be an alternate explanation for the differences between pigs and rats. The pig study did not examine the maternal behaviour received by the piglets after handling. As the effects of handling in the rat are mediated via changes in maternal behaviour, the failure to find an effect of neonatal handling in pigs could be due to there being no differences in the maternal care received by the handled and non-handled piglets.

7.4.1.8.2. Differences in the early environment of the two species.

Another explanation for differences in the potential influence of maternal care in the rat and sheep are differences in the early environment of the two species. Under natural conditions development in the rat typically occurs in the dark, tranquil confines of a burrow, where the major source of stimulation is the mother and littermates (Caldji et al. 1998). The mother serves as the primary link between the outer environment and the developing animal, appearing to transduce information pertaining to this environment to her young. Therefore it seems reasonable that variation in mother-pup interaction has great importance on pup development in the rat (Caldji et al. 2000a). In contrast, lambs are born in the open environment and, although the ewe seeks isolation from the rest of the flock before birth, she soon rejoins her flock mates, creating the potential for social interactions with a range of other animals (Lynch et al. 1992). Consequently lambs are exposed to a wide variety of stimuli from birth onwards and experience the 'outer environment' directly, rather than through their interactions with their dam. Environmental factors such as changes in temperature, the weather, and the day and night cycle, all create a varied environment for the lamb. The presence of the rest of the flock also provides a range of social stimulation. As a result, their dam is a comparatively smaller source of stimulation for young lambs, compared to neonatal rats, and her behaviour has less potential to be the principal, or exclusive, influence on their development. These differences in the potential to influence the development of their offspring may account for the differences in the nature of maternal influence between the two species.

7.4.2. Learning ability.

Ewes raised by Blackface dams were quicker to learn the spatial memory task than ewes raised by Suffolk dams. The fact that there were no differences in the time taken to reach the food bowl, once its location had been learnt, suggests that this was due to the ewes' learning ability, rather than their motivation to search for food. There is a possibility that the ewes were using olfaction in their location of the food and the design of the study does allow this to be ruled out. However the rapid and direct approach of the ewes to the bowl containing the feed suggests that the use of olfaction at a distance was limited. In addition, all the food bowls contained feed during several trials on each test day and they were not washed between trials. As a result, although only one food bowl actually contained feed during each trial, all the bowls were likely to have at least a residual odour of feed, reducing the efficacy of relying solely on olfaction to locate the feed. Therefore, although the use of olfaction cannot be ruled out, it is more likely that Blackface-raised ewes were quicker to learn the task, rather than having better olfaction than Suffolk-raised ewes, and that dam breed does not have a significant influence on the cognitive development of offspring. As discussed above the influence of dam breed may be mediated via genetics, maternal care or a combination of both and further work is needed to separate these two potential influences.

7.4.2.1. The influence of maternal care on the learning ability of sheep.

Previous work on rats has suggested that maternal care influences adult learning ability via stimulation of hippocampal synaptogenesis in early development (Liu et al. 2000). Variation in maternal care causes differential levels of sensory experience for the developing pups, in the form of tactile stimulation, and this may result in altered levels of hippocampal synaptic development. In adult rats spatial memory and learning depend on hippocampal integrity, thus the increased hippocampal synaptogenesis caused by high levels of LG-ABN result in enhanced spatial learning in adulthood (Liu et al. 2000). As discussed above, the potential for the ewe to have a major influence on the development of her offspring's central nervous system is reduced, compared to the rat dam. Therefore, although the relationship between learning ability and maternal care has the potential to be the same in both species, the mechanisms for the influence of maternal behaviour may differ.

Neither of the two aspects of maternal care examined in this study, the willingness of the dam to interact with her lamb and the dam's response to her lamb's attempts to interact with her, had a significant association with the ewes' learning ability. Therefore these aspects of maternal behaviour do not appear to account for the differences in the learning ability of Blackface and Suffolk raised lambs. However, as discussed above, other aspects of maternal care also have the potential to influence later behaviour. The inconsistent maternal behaviour of the Suffolk ewes is likely to result in an unstable ewe-lamb relationship, compared to the more consistent Blackface ewes. In addition, the continued use of postural communication by Blackface ewes may give their lambs more stimulation than lambs raised by Suffolk ewes.

Human studies have shown that neglected children show delays in their cognitive development (Trickett and McBride-Chang 1995) and exhibit a greater prevalence of intellectual deficits and academic underachievement compared to non-maltreated children (Ammerman et al. 1986). The processes through which abuse and neglect lead to intellectual deficit is difficult to determine but suggestions have included environmental impoverishment and a lack of stimulation (Ammerman et al. 1986), or an insecure attachment with the parent which is not conducive to appropriate cognitive development (Trickett and McBride-Chang 1995). When examining the role of variation in maternal care on offspring development it is not necessary to look to extreme conditions of abuse and neglect for evidence of the importance of parental care. Variations in maternal care falling within the normal range of the species can still have a profound influence on development (Francis and Meaney 1999). Therefore, whilst it is invalid to describe the maternal behaviour of Suffolk ewes as neglecting or abusive, their unstable ewe-lamb relationship and the lower stimulation

received by their lambs, compared to lambs raised by Blackface dams, could contribute to these animals being slower to learn the spatial memory task.
In addition, Sneddon et al. (2000) found that pigs reared in an enriched environment were faster at learning a spatial memory task than pigs reared in a barren environment. The authors proposed that the influence of enrichment on cognitive development may have been mediated via social factors. The greater maternal stimulation received by Blackface raised lambs may have the same positive influence on learning ability as environmental enrichment in the pig study. More frequent maternal stimulation may make Blackface raised lambs more socially enriched than lambs raised by Suffolk ewes.

7.4.2.2. Behaviour of the ewes during the trials.

All ewes were included in all ten trials, regardless of when they learnt the location of the food bowl. During later trials, four of the Blackface raised ewes which had learnt the location of the food early in the test began to visit other bowls before reaching the correct bowl. The ewes were observed to briefly look in other bowls, often only lowering their head to the bowl without placing it inside, before walking quickly and deliberately to the correct bowl and consuming the food. The deliberate approach to the correct bowl after looking in the other bowls suggests that these ewes had not forgotten the location of the food, but were looking to see if the other bowls contained food. During training all bowls contained food, therefore the ewes had previous experience of food being in other bowls.

Grazing animals often forage from plant communities where plant species or characteristics of the vegetation (e.g. sward height or biomass) are distributed in patches within the general vegetation (Grieg-Smith 1983). Once these patches have been grazed they take time to recover and return to their previous state. Consequently it is not beneficial for animals to keep returning to the same patches and they will have a better chance of finding good grazing if they explore a wide area containing multiple patches. The behaviour of the Blackface raised ewes in the spatial memory task may reflect this and represent another difference in the behaviour of ewes raised by the two dam groups. This would be an interesting area for further exploration.

7.5. Summary.

Dam breed did not influence the stress response of offspring in sheep but did influence the behavioural response of lambs in the stress response tests. Blackface raised lambs showed a more active response, moving about more in the social separation test and struggling for longer in the restraint test compared to Suffolk raised lambs, which tended to react more passively. The influence of dam breed has the potential to be genomic, non-genomic (via maternal care) or a combination of both. The influence of sire breed on the response of lambs to aversive situations suggests that genetics do play a role, but this does not exclude the possibility of maternal care also influencing later behaviour in the sheep. The willingness of the ewe to interact with her lamb and her response to her lamb's attempts to interact with her did not show a significant association with lamb response to social separation. However other aspects of maternal behaviour in the two breeds, for example the consistency of their expression of maternal care or their control of sucking interactions also have the potential to influence whether their lambs have an active or a passive behavioural response to social separation. Dam breed also influenced the cognitive ability of adult offspring. Ewes raised by Blackface dams were quicker to learn a spatial memory task than ewes raised by Suffolk dams. It is possible that this is also related to the maternal style of the two ewe breeds. The reduced maternal stimulation of Suffolk raised lambs (especially via postural communication) and the weaker mother-young attachment seen in Suffolk ewes may impair their lambs' cognitive development.

Chapter 8: The effect of variation in dam and sire breed on traits relevant to sheep production.

8.1. Introduction.

The aim of this chapter is to look at the effect of variation in maternal care on offspring traits which may have consequences for the sheep farming industry. The relationship between the ewe and her lambs has the potential to affect many aspects of lamb development. The lambs' stress response and learning ability have already been explored in Chapter 7 but maternal care also has the potential to influence lamb development in ways which are more obviously relevant to the farming industry.

The maternal care of the ewe may have consequences for the lamb in both the short and long term. As discussed in previous chapters, a poor ewe-lamb relationship is likely to result in lambs that are not strongly bonded to their dam and do not interact frequently with her. Infrequent interaction with the dam and a lack of co-operation with early suck attempts may potentially affect the development of dam recognition by the neonatal lamb, leading to poor dam recognition and a weak attraction towards the dam. A weak ewe-lamb bond may also have consequences for lamb growth rate. For example, a poor suckling relationship between the ewe and her lambs could result in a reduced lamb growth rate compared to lambs that have a good relationship with their dam. In the long term, the maternal behaviour of female offspring may also be affected by the maternal care they received as a lamb. Previous studies in rats have highlighted the potential for the dam's maternal behaviour to have a non-genomic influence on an offspring's own maternal care (e.g. Francis et al. 1999b). In this chapter three aspects of lamb development were investigated, the ability of lambs to recognise their dam at 24 hours old, the growth rate of lambs over the lactation period and the maternal behaviour of the ewe lambs at their first parturition.

8.1.1. Dam recognition in the young lamb and attraction towards the dam.

Young lambs have been shown to have the ability to recognise their dam at close quarters from 12 hours post-partum (Nowak et al. 1987, Nowak et al. 1990) and from a distance of several metres from 24 hours post-partum (Nowak 1990b, Nowak and Lindsay 1990, Nowak et al. 1990, Terrazas et al. 2002). Establishment of dam recognition and a preference for her is dependent on the nature of the initial sucking interactions between the ewe and her lamb (Nowak 1996, Nowak et al. 1997). Newborn lambs which have been prevented from sucking lose interest in their mothers (Alexander and Williams 1966a, Nowak et al. 1997) and are not attracted to her in a choice test (Nowak et al. 1997). Therefore unrestricted sucking from birth is required in order for a preferential relationship to develop.

Previous studies investigating the development of dam recognition, and attraction towards her by the lamb, have involved the artificial prevention of sucking during the post-partum period and have not examined the influence of natural variation in maternal care. The level of dam co-operation with suck attempts during the immediate post-partum period may also affect the development of a preference for the dam, resulting in a delay in recognition for lambs experiencing a low rate of co-operation. The different rates of co-operation with initial sucking attempts in the Suffolk and Blackface ewes can be used to investigate this further. As Blackface ewes show more co-operation than Suffolk ewes it was hypothesised that Blackface lambs would be better able to recognise their dams and show a stronger preference for them than Suffolk lambs. The lambs were tested at 24 hours old as this is the age at which distance recognition starts to become established.

8.1.2. Lamb growth rate.

The nature of the ewe-lamb relationship may also affect the growth rate of lambs. Lambs begin to show some grazing as early as 2 days after birth (Morgan and Arnold 1974) but continue to suck from their dam until they are weaned between 3 and 6 months old (Arnold et al. 1979). During the first few weeks of life the milk supply provided by the ewe is the main determinant of the growth rate of lambs (Ewbank 1967). Lambs that have a poor suckling relationship with their dams will therefore have to rely more heavily on grazing at an earlier age than lambs with more co-operative dams, and this may affect their growth rate. The poorer reaction of Suffolk ewes towards sucking attempts from their lambs, compared to Blackface ewes, may therefore result in their lambs growing at a slower rate than lambs with a Blackface dam.

To test this the growth rate of lambs was measured over the lactation period. As the Suffolk breed has been subject to greater selection for rapid growth than the Blackface breed (Simm 1998) the genotype of the lambs will also influence their growth rate. To take this into account, only crossbred lambs were included in the study of lamb growth rate. It was hypothesised that lambs with a Suffolk dam would grow comparatively more slowly than lambs with a Blackface dam. The small number of crossbred lambs (25) will almost undoubtedly confound results and only large effects are likely to be detected in such a small sample size. Consequently the influence of natural variations in maternal behaviour may be too subtle to be detected and this investigation should be viewed as a pilot study for future work.

8.1.3. Own maternal behaviour.

A ewe's own maternal behaviour has the potential to be affected by the maternal care she received as a lamb, as well as by her genetics. Although individual genes have been found to regulate maternal behaviour in rodents (e.g. Li et al. 1999) previous experience of the maternal care received as neonates has also been shown to influence subsequent maternal behaviour. Rats which are temporarily separated from their dam as neonates (between day 4 and day 20 post-partum), and therefore experience disrupted maternal care, show deficits in their own maternal care, licking and crouching over their pups less frequently than non-separated rats (Lovic et al. 2001).

In addition, females whose mothers perform high rates of licking and grooming and arched-back nursing (LG-ABN) also show high levels of licking and grooming of their own pups, compared to those which receive a low frequency of LG-ABN (Francis et al. 1999b). Cross-fostering shows this to be due to the maternal care received as a neonate, rather than the genetic make-up of the rats. Pups born to low LG-ABN mothers which are then fostered to high LG-ABN mothers at birth, show higher rates of LG-ABN themselves, as mothers, compared to females raised by low LG-ABN mothers. The opposite is also true of pups born to high LG-ABN mothers, and subsequently fostered to low LG-ABN mothers. These animals show lower rates of LG-ABN themselves, compared to females raised by high LG-ABN mothers (Francis et al. 1999b).

The mode of transmission of maternal style in rats has been shown to be behavioural as handling of female pups in early life causes them to become high LG-ABN mothers, regardless of their parentage (Francis and Meaney 1999). Handling of pups leads to high levels of LG-ABN in the dam, which subsequently leads to high LG-ABN offspring. Artificial rearing produces dams which are responsive to pups, and do not show abusive or neglectful behaviour, but have a lower frequency and shorter duration of response to their pups compared to maternally reared females (Gonzalez et al. 2001). This effect can be ameliorated to a certain extent by the provision of tactile stimulation but these animals do not reach the same level of responsiveness as maternally reared animals. Further evidence of the behavioural transmission of maternal style lies in the fact that daughters of artificially raised dams also show deficits in the time spent licking and crouching over their pups (Gonzalez et al. 2001).

To investigate the potential influences of genetics and previous experience of maternal care on maternal behaviour in sheep, the maternal behaviour of the female young from the earlier part of the study (Chapters 3-6) was examined during the first few hours following parturition in their first parity. Although the number of animals available was too low to allow sound conclusions to be drawn, observation of the ewes' own maternal behaviour in relation to the maternal care they received as lambs and their genetic make-up (sire breed and dam breed) was used to provide a pilot study for future work.

8.2. Methods.

8.2.1. Ability of day old lambs to recognise their dam.

8.2.1.1. Animals.

Twenty pure bred lambs (10 Blackface, 10 Suffolk) were used to investigate the ability of lambs to recognise their dams at approximately 1 day old. Lambs were tested as close to 24 hours after parturition as possible, the average age of Blackface lambs was 26.21 hours (s.e.m.=0.45), the average age of Suffolk lambs 25.90 hours (s.e.m.=0.67). Six first born twins and 4 single lambs were tested in each breed.

8.2.1.2. Test apparatus and procedure.

Lambs were given a choice between their dam and a ewe of the same breed with lambs of a similar age. They were tested in the apparatus shown in Figure 8.1, the construction of which has been described in Chapter 3. The distance between the holding pen and the area comprising the contact zone was reduced to take account of the smaller size of the lambs, compared to ewes. The ewes were placed in the holding pens in a pseudo-random fashion to ensure that presentation of ewes was balanced for both groups and to prevent data being biased towards side preferences.

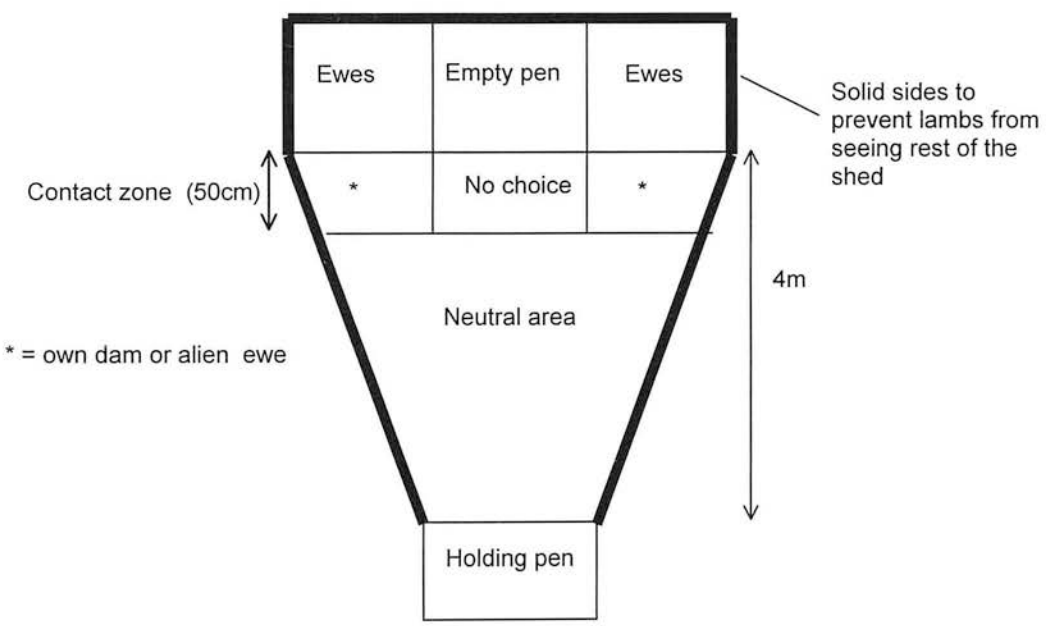

Figure 8.1. Diagram of the test apparatus used for the lamb choice test (not to scale).

A lamb was considered to be present in an area in front of the holding pens if its front legs and at least half its body length were in that area. Lambs standing in the area directly in front of a ewe were considered to be choosing to associate with that ewe.

Each lamb was tested for 3 minutes. During a test the ewe and her lamb(s) were removed from their home pen. The ewe was placed in a 'ewe' pen and the lamb(s) placed in a holding pen that was separate from the experimental apparatus. This pen was located on the opposite side of the shed, approximately 6m from the holding pen of the experimental apparatus. The alien dam and her lambs were then removed from their home pen, the ewe placed in the remaining 'ewe' pen and her lambs placed in the same holding pen as the other lambs. During the test second born twins and the alien ewe's lambs were left in this pen. The location of the pen meant that if the ewes in the test were calling for their lambs and responding to lamb bleats they would remain facing towards the centre of the test apparatus and the lamb being tested. The test lamb was then placed in the experimental holding pen and left for 30 seconds to allow the lamb the opportunity to see and hear both ewes before entering the pen. The test was started by opening the gate on the holding pen. The lamb's behaviour was recorded using a Psion Workabout handheld computer and the Observer software (Noldus et al. 2000). The location of the lamb and the number of lamb vocalisations were recorded continuously during the test. From this it was possible to calculate the time taken to reach own dam, the time spent with own dam and the time spent with the alien ewe. The high pitched vocalisation rates of the ewes were also recorded to provide some measure of ewe behaviour during the test.

8.2.1.3. Statistical analysis.

Data were tested for normality, all data were normally distributed. The number of lambs reaching their dam during the test and the number of lambs approaching their dam first in each breed were analysed using Fisher's Exact test (numbers were too low for Chi-squared analysis). Latency to reach own dam, the time spent with dam, time spent with the alien ewe and vocalisation rates were analysed using REML.

Litter size and lamb breed were fitted in the model. The vocalisation rates of own dam and the alien ewe were compared using T-tests.

8.2.2. Lamb growth rate.

8.2.2.1. Animals and weight recording.

Animals and husbandry methods are described in Chapter 2. Only crossbred animals were used in the study (Table 8.1). Lambs were initially weighed at approximately 24 hours old. Subsequent recordings of weight were made at 4 week intervals, calculated from the birth of the last lambs to be born. Recording continued until weaning, when the youngest lambs were 12 weeks old. The initial recording was made using a hand-held scale from which the lambs were suspended by their front legs. Subsequent weight recording in the field used a portable weigh crate. Each interval between weight recordings corresponded approximately to a month long period and the intervals were referred to as the first month, second month and third month post-partum. Growth rate was then measured by calculating the percentage increase in body weight over the period. Growth rate was measured over each month and over the whole period between birth and weaning.

Table 8.1.
Number of lambs used in the investigation of growth rate.

	Blackface dam	Suffolk dam
By litter size		
Single	3	2
Twin	6	14
By lamb sex		
Male	4	8
Female	5	8

This table shows the number of lambs in each group, broken down by litter size or lamb sex. Data on growth rate were collected from a total of 25 lambs.

8.2.2.2. Relationship between maternal behaviour and lamb growth rate.

The relationship between lamb growth rate and maternal behaviour was also examined. As described in Chapter 7, the scores from the first two factors of the factor analysis exploring maternal behaviour (Chapter 6) were used as a representation of maternal behaviour. As the factor analysis of maternal behaviours related to the whole lactation period, the relationship between maternal behaviour and lamb growth over the whole lactation period only was investigated.

8.2.2.3. Statistical analysis.

Data were checked for normality, all were normally distributed. The growth rates of the two groups of lambs (Suffolk dam or Blackface dam) were compared for each month and for the whole lactation period using REML. Litter size, lamb sex and dam breed were fitted in the model. The relationship between maternal behaviour and lamb growth rate was examined using Pearsons correlation.

8.2.3. Own maternal behaviour.

8.2.3.1. Animals.

The maternal behaviour of the female lambs born during the first part of the study was observed during their first parturition. Table 8.2 shows the number of ewes in each group and the litter sizes they gave birth to.

Table 8.2.
Number of ewes in each group giving birth to single and twin litters.

	Suffolk dam		Blackface dam	
	Pure Suffolk	Crossbred	Pure Blackface	Crossbred
Twin	2	4	3	1
Single	4	4	2	1
Total	6	8	5	2

8.2.3.2. Behavioural observations.

Ewes lambed over a week long period, the husbandry procedures have been described in Chapter 2. During this period ewes were kept under 24-hour surveillance, backed up by continuous video recording. Ewes were observed for the first 30 minutes after the birth of a lamb, followed by three 10 minute observations every 30 minutes, finishing at 2 hours post-partum. For twin litters the observation schedule was restarted after the birth of the second lamb. Vocalisation data were collected live using Psion Workabout handheld computers and The Observer software (Noldus et al. 2000). Data on maternal behaviour were collected from videotape using The Observer software (Noldus et al. 2000). Table 8.3 gives the definitions of the behaviours recorded during the post partum period. Data on

Table 8.3.
Definitions of behaviours recorded during the first 3 hours post-partum.

Behaviour	Definition
Ewe	
Standing still	Ewe stands immobile without interacting with the lamb.
Grooming	Ewe licks or nibbles her lamb, includes chewing the remnants of foetal membranes during a grooming bout. A grooming bout ends when the ewe stops licking her lamb and is not chewing foetal membranes.
Circling [1.]	As the lamb investigates/contacts the udder or makes a suck attempt (interacts) the ewe walks around the lamb, usually finishing with the lamb at her head.
Backing [1.]	As the lamb interacts with the ewe she walks backwards away from the lamb.
Walking forward [1.]	As the lamb interacts, especially when attempting to reach the udder, the ewe walks forward, away from the lamb.
Butts	Ewe pushes the lamb with downwards or sideways movement of her head.
Retreats [2.]	Ewe walks backward away from the lamb when the lamb is not attempting to interact.
Leaves [2.]	Ewe walks away from her lamb(s), facing away from it/them, the lamb(s) is/are not attempting to interact.
Low pitched Vocalisation	'mmm' bleat, low rumbly bleat made with the mouth closed.
High pitched Vocalisation	'baa' vocalisation, made with the mouth open.
Lamb	
Investigate dam [3.]	Lamb makes contact, with its head, with any part of the ewe's body except the udder region.
Contact udder [3.]	Lamb places its head underneath ewe in the udder region, in any position apart from inverse parallel (standing parallel to ewe but facing in opposite direction).
Suck attempt [3.]	Lamb places its head underneath the ewe in the udder region, in the inverse parallel position.

[1.] The performance by the ewe of any of these three behaviours, circling, backing and walking forward, usually resulted in the lamb losing contact with the dam or being removed from the udder region. These behaviours were considered to indicate that the ewe was not co-operating with the lamb's attempts to find the udder and suck. Co-operation with a suck attempt was indicated by the ewe standing still, grooming the lamb, or grooming the other lamb in twin litters. [2.] The behaviours 'retreat' and 'leave' were considered to indicate a withdrawal from the lamb. [3.] As the newborn lamb rarely goes straight to the udder and makes a suck attempt, three lamb behaviours were combined to represent the lamb's early attempts to find the udder and suck: investigate dam, contact udder and suck attempt.

grooming behaviour, ewe vocalisations and negative behaviour (aggression and withdrawal from the lamb) were collected during the first 30 minute observation period only. Data on co-operation with sucking attempts were collected during all 4 observation periods and an average calculated.

Over the first 3 days post-partum scan samples were made of lambed ewes at 2 hourly intervals. The ewe-lamb distance and the behaviours of the ewe and her lambs were recorded. During analysis only ewe-lamb distance and interactions between the ewe and her lamb(s) were examined, Table 8.4 gives definitions of these interactions. In twin litters the ewe could be lying with, or suckling, one or both of her lambs, by definition she could only nose one lamb at a time. One single-bearing pure Suffolk ewe was excluded from these data as she was confined in a foster crate.

Table 8.4.
Definitions of ewe and lamb behaviours recorded during scans.

Behaviour	Definition
Lies	Ewe or lamb lying down.
Stands	Ewe or lamb stands with all 4 feet on the ground.
Noses lamb	Ewe touches lamb with her muzzle.
Sucking interaction	Lamb has head underneath the ewe in the udder region, ewe is standing or lying still.

8.2.3.3. Influence of specific maternal behaviours on the ewes' own maternal care.

The relationship between the maternal care received as a lamb and the ewes' own maternal care was also investigated. The latency for the dam to begin grooming, duration of grooming received as a neonate and the co-operation of the dam with initial suck attempts was compared with the ewes' own expression of the same behaviours.

8.2.3.4. Statistical analysis.

Data were checked for normality. Latency to groom was log (base 10) transformed, all other behaviours were normally distributed. REML was used to investigate differences in the maternal behaviour of the different groups (Blackface dam or Suffolk dam). Litter size, sire breed and dam breed were fitted in the model. Pearsons correlation was used to look at the relationship between the ewes' own maternal behaviour and the maternal care they received during the initial post-partum period. Spearmans ranked correlation was used for the 'latency to groom' data.

8.3. Results.

Unless otherwise stated, sire breed and litter size did not have an effect on the behaviour or growth rate of the animals.

8.3.1. Ability of day old lambs to recognise their dam.

8.3.1.1. Ability to locate and recognise the dam.

Nineteen out of the 20 lambs reached a ewe during the test, the exception being 1 Suffolk lamb. All Blackface lambs found their dam during the test but only 6 Suffolk lambs reached their dam (Fishers Exact p=0.043). When approaching a ewe for the first time, 7 Blackface lambs and 3 Suffolk lambs approached their own dam first (p=0.089).

Figure 8.2 shows the latency to reach own dam, the time spent with the dam and the time spent with the alien ewe during the test. Blackface lambs were significantly quicker than Suffolk lambs to reach their dam (REML Wald=10.32, d.f.=1, p<0.001). Blackface lambs also spent longer with their dam than Suffolk lambs (Wald=9.4, d.f.=1, p<0.001) but there was no significant difference between the two breeds in the time spent with the alien ewe (Wald=2.59, d.f.=1, NS). Single lambs spent longer

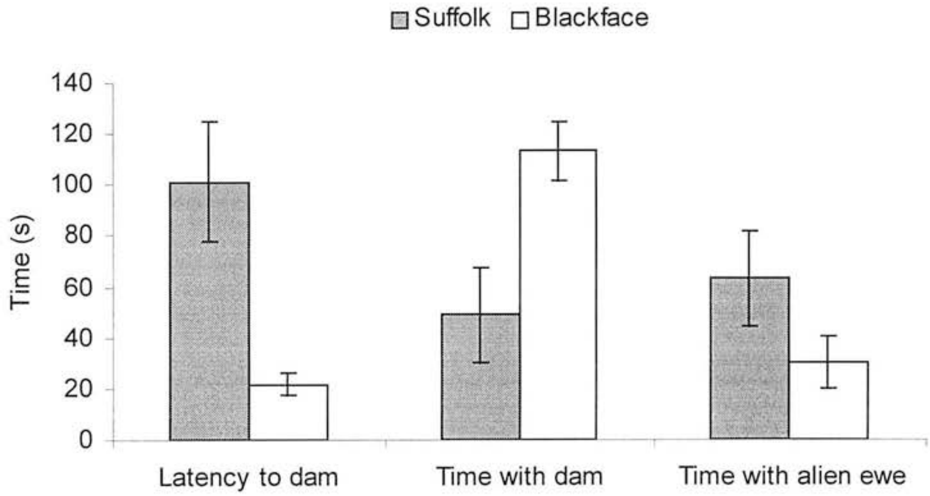

Figure 8.2. Mean latency to reach own dam and the mean amount of time spent with own dam and the alien ewe during the lamb choice test (with s.e.m.).

with their dam than first born twin lambs (mean (s) (with s.e.m.): single=106.6 (21.9), twin=64.3 (14.9), Wald=3.9, d.f.=1, p<0.05) but litter size did not affect the latency to reach own dam or the time spent with the alien ewe.

8.3.1.2. Vocalisations during the test.

Figure 8.3 shows the vocalisation rates recorded during the test. There were no differences in the rate of lamb vocalisations during the test (Wald=0.77, d.f.=1, NS) or in the rate of dam vocalisations (Wald=0.86, d.f.=1, NS) and alien ewe vocalisations (Wald=1.75, d.f.=1, NS) received by lambs during the tests.

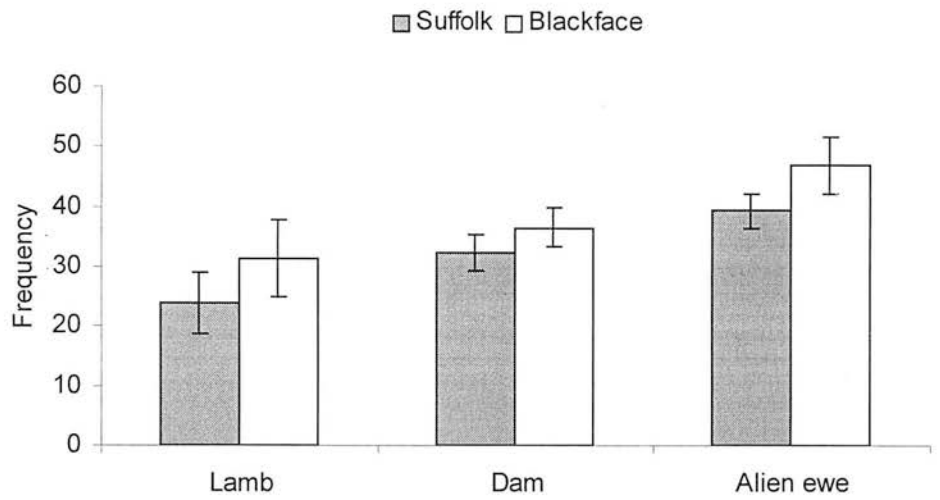

Figure 8.3. Mean number of vocalisations made by each animal during the 3 minute lamb choice test (with s.e.m.).

Dam vocalisation rate was also compared with alien ewe vocalisation rate within the two lamb breeds, to assess the behaviour of the ewes during the test. There were no significant differences between the vocalisation rates of the alien ewe and the lambs' own dam in either breed (Suffolk: T-test T=-1.71, d.f.=17, NS, Blackface: T=-1.80, d.f.=16, NS,). There were also no significant differences between the vocalisation rates of the alien ewe and the lambs' own dam in twin litters (mean number of vocalisations (with s.e.m.): alien ewe=42.2 (4.3), dam=36.7 (2.9), T=-1.06, d.f.=19, NS). However within the single litters, the alien ewe vocalised more frequently than the lambs' own dam (mean number of vocalisations (with s.e.m.): alien ewe=44.88 (3.3), dam=31.33 (3.5), T=-2.86, d.f.=13, p<0.05).

8.3.2. Lamb growth rate.

8.3.2.1. Comparison of lamb groups.

Table 8.5 shows the growth rate of the 2 lamb groups (Suffolk dam, Blackface dam) over the lactation period. Dam breed did not influence lamb growth rate over the first, second or third month post-partum (Wald=0.61, d.f.=1, NS, Wald=0.93, d.f.=1, NS and Wald=0.39, d.f.=1, NS respectively) or over the entire lactation period (Wald=0.16, d.f.=1, NS).

Table 8.5.
Proportion increase in lamb body weight during the lactation period.

	Suffolk dam	Blackface dam
First month	364.60 (21.50)	348.85 (12.60)
Second month	162.36 (2.27)	163.51 (4.07)
Third month	125.35 (1.67)	126.00 (1.74)
Birth to weaning	740.70 (45.30)	716.90 (27.90)

Table shows the mean proportion increase in weight (with s.e.m.).

8.3.2.2. Relationship between maternal behaviour and lamb growth rate.

The willingness of the ewe to interact with her lamb was significantly correlated to her lamb's growth rate between birth and weaning (Pearsons correlation r=0.591, p<0.05), but there was no significant correlation between the reaction of the ewe to her lamb's attempts to interact with her and its growth rate (r=0.031, NS).

8.3.3. Own maternal behaviour.

8.3.3.1. Immediate post-partum period

There was no significant influence of dam breed on the ewes' latency to begin grooming their lambs (mean latency to begin grooming (s) (with 95% Confidence Interval): Suffolk dam=84.92 (38.74-186.12), Blackface dam=53.95 (24.62-118.25), Wald=0.46, d.f.=1, NS). However dam breed did have an effect on the duration spent grooming the neonate during the first 30 minutes post-partum. Ewes with a Blackface dam groomed their lambs for longer than ewes with a Suffolk dam (mean duration of grooming (s) (with s.e.m.): Suffolk dam=858.37 (117.23), Blackface dam=1331.14 (123.79), Wald=8.74, d.f.=1, p<0.01). Sire breed and litter size also influenced the duration of grooming. Ewes with a Blackface sire groomed their lambs for longer than ewes with a Suffolk sire (mean (s) (with s.e.m): Suffolk sire=791.41 (170.76), Blackface sire=1153.44 (109.25), Wald=7.22, d.f.=1, $p<0.01$) and ewes with single lambs groomed their lambs for longer than ewes with twin lambs (mean (s) (with s.e.m.): single=1160.78 (154.33), twin=855.32 (108.76), Wald=4.11, d.f.=1, $p<0.05$).

There was a non-significant tendency for ewes with a Blackface dam to show more co-operation with suck attempts than ewes with a Suffolk dam (mean proportion of suck attempts resulting in circling, backing or walking forward (%) (with s.e.m.): Suffolk dam=35.03 (4.21), Blackface dam=20.17 (5.82), Wald=3.04, d.f.=1, $p=0.081$). Only one ewe (a pure Suffolk) was observed to show negative behaviour towards her lamb. The ewe made two retreats from her lamb.

The significant influence of sire breed on the duration of grooming the neonate indicates a genetic influence on this maternal behaviour. The duration of grooming in the four genotype groups (pure Suffolk and crossbred with a Suffolk dam, pure Blackface, crossbred with a Blackface dam) was also analysed to investigate this further (Figure 8.4). There was a significant effect of lamb genotype on the duration of grooming the neonate (Wald=16.73, d.f.=3, $p<0.001$).

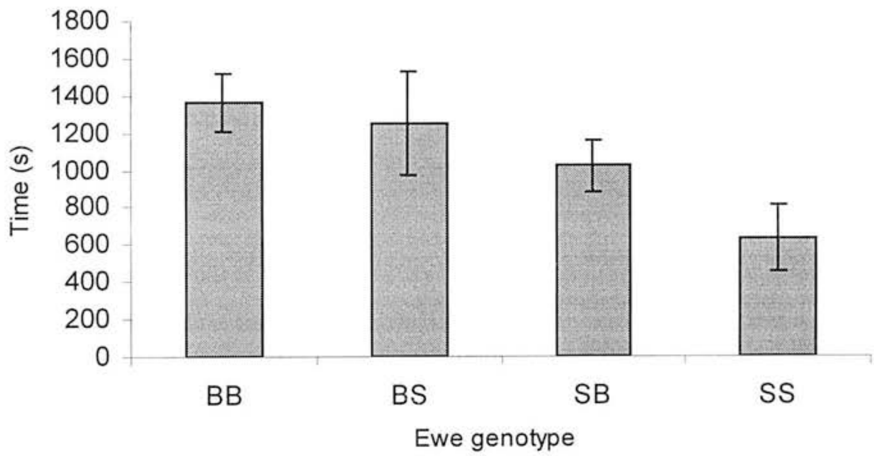

Figure 8.4. Mean duration spent grooming the neonate by ewes in the four genotypes (with s.e.m.). BB= pure Blackface, BS= crossbred with a Blackface dam, SB= crossbred with a Suffolk dam, SS= pure Suffolk.

8.3.3.2. First 3 days post-partum.

Table 8.6 shows the data collected from scans during the first 3 days postpartum. Dam breed had no influence on the average ewe-lamb distance (Wald=0.06, d.f.=1, NS) or on the proportion of scans in which the ewes and lambs were lying within 1m of each other (Wald=0.30, d.f.=1, NS). There was a non-significant tendency for ewes with a Blackface sire to be closer to their lambs during the first three days post-partum than ewes with a Suffolk sire (mean distance (m) (with s.e.m.): Suffolk sire=1.25 (0.11), Blackface sire=1.05 (0.07), Wald=3.14, d.f.=1, p=0.076).

Table 8.6.
Ewe behaviour over the first 3 days post-partum.

	Suffolk dam	Blackface dam
Ewe-lamb distance (m)	1.13 (0.06)	1.09 (0.13)
Proportion of scans:		
Lying within 1m (%)	33.97 (2.65)	36.51 (4.13)
Nosing lamb (%)	11.11 (1.30)	9.52 (1.80)
Suckling lamb (%)	10.04 (1.56)	9.92 (2.90)

Table shows information collected in scan samples. Mean values are shown (with s.e.m.).

The proportion of scans where ewes were observed to nose their lambs was not influenced by dam breed (Wald=0.3, d.f.=1, NS), nor was the proportion of scans where the ewe was observed to be suckling at least one lamb (Wald=0.31, d.f.=3, NS). Litter size had an effect on the nosing and suckling behaviour of the ewes. Ewes with single lambs were observed to nose their lambs more than ewes with twin lambs (mean proportion of scans (%) (with s.e.m.): single=13.06 (1.31), twin=8.06 (1.2), Wald=10.01, d.f.=1, p<0.01). Twin-bearing ewes were observed to suckle their lambs more frequently than single-bearing ewes (mean proportion of scans (%) (s.e.m.): single=6.11 (1.93), twin=13.89 (1.01), Wald=13.22, d.f.=1, p<0.001).

8.3.3.3. Relationship between received maternal care and the ewe's own maternal behaviour during the initial post-partum period.

There were no significant correlations between the latency of the dam to begin grooming and the ewes' own latency to begin grooming the neonate (Spearmans correlation r=0.231, NS) or the received co-operation with initial suck attempts and the ewes' own co-operation with suck attempts (Pearsons correlation r=-0.038, NS). However there was a non-significant tendency for the received duration of grooming as a neonate to be correlated with the ewes' own duration of grooming during the immediate post-partum period (r=0.424, p=0.063).

8.4. Discussion.

8.4.1. Ability of day old lambs to recognise their dam and show a preference for her.

All but one lamb approached a ewe during the test, indicating that the lambs were attracted to the ewes and sought proximity with them. Blackface lambs were more likely than Suffolk lambs to reach their dam during the test and they were also quicker to reach her and spent longer with her, compared to Suffolk lambs. Blackface lambs were therefore better able than Suffolk lambs to recognise their dams and were more attracted to them.

8.4.1.1. The role of maternal behaviour in the development of a preference for the dam.

As described in the introduction this could be due to the maternal care experienced by the lambs during the immediate post-partum period. Initial sucking interactions have a two-part role in the development of dam recognition, creating an attraction between the lamb and its dam (Nowak et al. 1997) and also allowing the lamb to learn her physical characteristics (Shillito-Walser 1978b). Unrestricted sucking during the immediate post-partum period is important for the lamb to become attracted to its dam (Alexander and Williams 1966a, Nowak and Lindsay 1990, Nowak et al. 1997) and well-fed lambs are thought to be more motivated to find their dam than poorly fed lambs (Nowak and Lindsay 1990). Previous studies (Dwyer and Lawrence 1998, Dwyer and Lawrence 1999a, Chapter 3) have shown that Blackface ewes show more co-operation with their lamb's initial sucking attempts than Suffolk ewes. The greater ability of the Blackface lambs to discriminate their dams, compared to Suffolk lambs, may therefore result from a greater motivation to seek proximity with their dams.

The close contact involved in a sucking interaction also allows the lamb to learn the smell, appearance and voice of its dam (Shillito-Walser 1978b) and this learning is reinforced by the reward of obtaining milk (Nowak et al. 1997). Consequently lambs which have experienced poor co-operation with early suck attempts may have had less opportunity to learn the physical characteristics of their dam than lambs which have received a positive response to suck attempts. This could also explain the differences in the ability of Blackface and Suffolk lambs to recognise their dams. However, at the age of testing lambs may be relying more on the ewes' behaviour for discrimination between their dam and alien ewes, as they may not have fully learnt the physical characteristics of their dam (Terrazas et al. 2002). In the maternal choice test described in Chapter 3 Blackface ewes were shown to be more motivated to associate with their lambs and to have a stronger ewe-lamb bond with them than the Suffolk ewes. It is therefore possible that during the current test of dam recognition the Blackface ewes showed more accepting behaviour as their lambs approached than the Suffolk ewes, making it easier for their lambs to recognise them.

A ewe's behavioural cues of acceptance have not been fully investigated but may involve the type of vocalisations she emits or her general restlessness when the lamb is in close proximity (Nowak et al. 1987). Terrazas et al. (2002) proposed that low pitched vocalisations could be involved in acceptance behaviour and ewes have been shown to bleat more in response to recordings of their own lambs, compared to recordings of alien lambs (Shillito-Walser et al. 1981). The high pitched vocalisation rates of the dam and alien were not found to differ within the two breed groups during the current tests and the lambs could not have been using vocalisation rate as a cue for recognition. However low pitched vocalisations were not recorded and it is possible that there may have been differences in the rate of these vocalisations, once the lambs reached the ewes. The ewes may also have differed in their postural behaviour but, as this was also not recorded, it is not possible to discern whether or not the two breeds showed differences which could have been used by their lambs as an aid for recognition.

In conclusion it appears that natural variation in early maternal care does have an influence on the attraction of lambs towards their dams and their ability to recognise them, although it is not clear when this is manifest. Further investigation is needed to determine whether or not the two breeds differ in their acceptance behaviour toward their young lambs and whether this contributes to the better ability of the Blackface lambs to recognise their dams at 24 hours old.

8.4.1.2. The influence of lamb development on their ability to recognise their dam.

Blackface lambs have been found to more advanced in their development at birth compared to Suffolk lambs (Dwyer and Lawrence 1998), being quicker to stand, reach the udder and to suck successfully than Suffolk lambs. This raises the possibility that the differences in dam recognition between the two breeds result from Blackface lambs being more developmentally advanced than Suffolk ewes at 24 hours old. However, in a study comparing pure Merino lambs with Border Leicester X Merino lambs, Nowak and Lindsay (1990) found that the speed of the lamb to stand and suck at birth was not related to its subsequent ability to discriminate its dam from an alien ewe. Pure Merino lambs were quicker than crossbred lambs to stand and suck after birth but crossbred lambs were better able recognise their dam at 12 hours old. Therefore, the greater speed of the Blackface lambs to stand and suck after birth, compared to the Suffolk ewes, may not predispose them to be better able to recognise dams at 24 hours old. In order to clarify this the test should be repeated using crossbred lambs, thereby controlling for any breed differences in lamb development.

8.4.1.3. The influence of litter size on the time spent with the dam during the test.

Litter size had an effect on the time spent with the dam, with single lambs spending longer with their dam than twin lambs. In previous studies single lambs have been found to be better able to recognise their dams, compared to twin lambs (Nowak et al. 1990, Nowak 1990b). However in this study litter size did not effect the lambs' latency to reach their dam or the time they spent with the alien ewe, suggesting that both singles and twins were able to identify their dam. The behaviour of the ewes during the test may account for single lambs spending longer with their dam than twin lambs. As described above, young lambs potentially use behavioural cues, as well as visual, auditory and olfactory cues, to recognise their dam. Once a lamb has reached its dam during the test a single-bearing ewe will be able to see her whole litter and is likely to change her behaviour and become less restless. However a twin-bearing ewe will only be able to see one lamb and may persist in 'looking' for her second lamb, continuing to use high pitched vocalisations and appearing restless. Consequently her lamb may not receive behavioural cues of acceptance, resulting in confusion and a decreased likelihood of staying with the dam. In agreement with this, alien ewes vocalised more frequently than dams in the tests of single lambs but there was no significant difference in vocalisation rates in the tests of twin lambs.

8.4.2. Lamb growth rate.

8.4.2.1. The influence of maternal behaviour on lamb growth rate.

Dam breed was not shown to have a significant effect on lamb growth rate at any time during lactation or over the whole period. However the willingness of the ewe to interact with her lamb was found to have a positive correlation with lamb growth rate. Positive scores on this factor are associated with the proportion of successful sucks in later lactation and also grooming the neonate and nosing the lamb during later lactation, both of which are important in the formation and maintenance of the ewe-lamb bond. Negative scores are associated with ewe-lamb distance during later lactation, the latency to begin grooming and the performance of negative behaviour towards the neonate. Consequently, lambs showing a faster growth rate are also likely to have experienced a high degree of affiliative maternal care and a close ewe-lamb bond. Lambs showing a slower growth rate are likely to have experienced more rejection from their dams. Although the positive association between affiliative maternal care and lamb growth does not imply a causal relationship there are several ways in which maternal care has the potential to influence lamb growth.

8.4.2.2. Potential influences of maternal behaviour on lamb growth rate.

The behaviour of the ewe during sucking interactions is likely to have a direct effect lamb growth during the early lactation period, when lambs are nutritionally dependent on their dams. However other aspects of the ewe-lamb relationship, for example the general nature of the ewe-lamb bond and the resultant ewe-lamb distance, may have an indirect effect, especially as the lamb becomes less dependent on milk for nutrition. As lambs grow older they begin to show more grazing and rely less heavily on their dams for nutrition (Morgan and Arnold 1974). At this stage the ewe-lamb distance may have an influence on the grazing behaviour of the lambs and, consequently, their growth rate. Sward composition within a paddock is not uniform and some plants may be more digestible than others or contain a higher nitrogen concentration. Grazing animals therefore forage from plant communities where plant species or characteristics of the vegetation (e.g. sward height or biomass) are distributed in patches (Grieg-Smith 1983). In response to this sheep employ selective grazing, using their relatively narrow muzzle to select plants or plant parts with great precision (Lynch et al. 1992). These behavioural patterns of grazing are not innate and lambs must acquire them, either from previous experience or via social learning, by grazing next to their dam, other adult ewes or their peers (Lynch et al. 1992).

Lambs learn which foods to eat or avoid by grazing with their dam (Thorhallsdotir et al. 1990b) and have been shown to acquire long-term grazing preferences by copying the habits of their dam (Key and MacIver 1980). The ewe-lamb distance can therefore have a profound effect on how the lamb learns about grazing. A lamb grazing close to its dam has more opportunity to learn from her about the selection of more favourable plants or plant parts, and to graze in an area of the paddock where these plants are more abundant. A lamb which is far from its dam is less likely to be influenced by her grazing habits and will have to learn diet selection by other means, by copying other animals or learning through its own experience.

Learning from experience takes time, resulting in a longer period to acquire an efficient grazing technique. Social learning from animals other than the dam is also likely to be less successful than learning from the dam. A lamb's peers are unlikely to be as experienced as adult animals and the exclusive nature of the ewe-lamb bond results in lambs rarely associating closely with ewes other than their dam. Although lambs can learn about feeding behaviour from other ewes, the process is more efficient when they learn from their dam (Thorhallsdotir et al. 1990a).

Good diet selection should lead to better nutrition and, subsequently, to a faster growth rate. Lambs with a closer ewe-lamb bond therefore have greater opportunity to learn from their dams and may consequently graze more efficiently at an earlier age, gaining weight faster. In addition, the grazing preferences formed during the first six months of life are very persistent (Arnold and Maller 1977), and it is therefore important for lambs to develop good grazing habits early in life.

8.4.2.3. The potential for future study.

As discussed in the introduction this investigation was just a pilot study. However the results indicate that this is an area with much potential for investigation on a larger scale, utilising many more animals than was possible in the current study.

8.4.3. Own maternal behaviour.

8.4.3.1. Differences in maternal behaviour.

Ewes with a Blackface dam groomed their newborn lambs for longer than ewes with a Suffolk dam and also had a tendency to react negatively to fewer of their lambs' initial suck attempts. Sire breed also affected maternal behaviour: ewes with a Blackface sire groomed their lambs for longer than ewes with a Suffolk sire. The grooming behaviour of the four ewe genotypes was examined in order to investigate this further. Pure Blackface ewes groomed for the longest duration, closely followed by crossbred ewes with a Blackface dam. Pure Suffolk ewes groomed their lambs the least, approximately half of the duration seen in ewes with a Blackface dam. Although crossbred lambs with a Suffolk dam were intermediate between the two groups, they appeared to be closer in duration to the Blackface raised ewes than the pure Suffolk ewes.

8.4.3.2. Relationship between own and received maternal care.

These results suggest that the dam does have an influence on her female offspring's maternal behaviour. Ewes with a Blackface dam received a higher rate of grooming as neonates and more co-operation with their initial suck attempts compared to ewes with a Suffolk dam (Chapter 3). Blackface-raised ewes subsequently groomed their lambs for longer than ewes with a Suffolk dam and showed a tendency to be more co-operative with early suck attempts, mirroring their dams' behaviour. In addition, the duration of grooming received as a neonate had a tendency to be associated with the ewes' own duration of grooming. However, although these results indicate that dam breed influences a ewe's own maternal behaviour, it is not possible to distinguish whether this is due to genetics or the maternal care received as a lamb and further investigation is needed.

8.4.3.3. The influence of genetics on the maternal behaviour of ewes.

The influence of sire breed on maternal behaviour provides evidence of genetic inheritance as sires made only a genetic contribution to their offspring. Additionally, lambs with the same breed of sire received different experiences of maternal care as some had a Suffolk dam and some had a Blackface dam. An influence of paternal genetics on the offspring's maternal behaviour has previously been shown in other species. In rodents a number of imprinted genes originating from the father (paternally expressed) have been linked to the expression of maternal care. These genes are inherited from both parents but only the paternal allele is expressed in the offspring. For example, in mice the paternally expressed M*est* and *Peg3* genes are known to function in regulating maternal behaviour (Li et al. 1999). Primiparous mothers with a mutation in the *Peg3* gene fail to exhibit any of the normal maternal behaviours of building a nest, gathering their pups together or keeping them warm by crouching over them (Li et al. 1999).

8.4.3.4. The potential for future study.

As discussed in the introduction the number of ewes available was too low to allow definite conclusions to be drawn from the exploration of this data. However the results do indicate that this is an area with the potential for further study. The use of embryo transfer would help to give a clearer idea of the different roles of genetics and previous experience on the expression of maternal behaviour in the ewe. Cross-fostering, as in the rat studies, is not very successful in sheep as the interval when ewes are willing to accept newborn lambs is so short, 30-60 minutes after birth.

8.4.4. Differences in early maternal behaviour between ewes and their dams.

An interesting difference between the maternal behaviour of the ewes in this chapter and the ewes in previous studies (Dwyer and Lawrence 1998, Dwyer and Lawrence 1999a, Chapter 3) is the performance of negative behaviour during the initial post-partum period. Ewes in previous studies, especially Suffolk ewes, have shown some negative behaviour towards their newborn lambs but the incidence of negative behaviour amongst these ewes was virtually nil. Only one pure Suffolk ewe showed any negative behaviour and this consisted of retreating from her lamb, one of the milder forms of negative behaviour (compared to head-butting). The explanation for this difference could lie in the background of the animals. Ewes in previous studies have been maintained in the same manner as standard flocks within the farming industry. Whilst every effort was made to do the same for the ewes studied in this chapter their inclusion in tests designed to examine their stress response and learning ability (Chapter 7) resulted in them having a greater experience of new situations and changing environments than 'normal' sheep.

The effect of previous exposure to novelty on the response of animals to new novel experiences was discussed in Chapter 3, as was the possibility that primiparous Suffolk ewes show more negative behaviour towards their newborn lamb than primiparous Blackface ewes because of a greater fear of novelty. Greater exposure to new experiences is thought to aid animals in learning about and classifying novel objects (Wood-Gush et al. 1990). Therefore the background of the ewes used in this study may have made them quicker to learn about their newborn lambs (the 'novel object') compared to ewes which have not been exposed to such a variety of experiences. This would be expected to reduce any fear due to novelty and any subsequent fear reaction. As discussed in Chapter 3, aggression and avoidance can be signs of fear (Boissy 1995) and the absence of negative behaviour in the ewes in this study could indicate that these ewes are less fearful of the neonate than 'normal' ewes. Whilst this conclusion differs from that drawn in Chapter 3 it is based on different facts and highlights the need for further investigation of this area. A controlled experimental design involving ewes raised 'normally' and ewes raised with exposure to new situations and a changing environment could be used to elucidate this matter.

8.5. Summary.

Maternal care has been shown to have the potential to influence various offspring traits that are relevant to sheep farming. Blackface lambs were better able than Suffolk lambs to recognise and locate their dams at a day old and this may be due to the more affiliative behaviour they receive from their dams during the post-partum period, compared to Suffolk lambs. However this may not be the only factor influencing dam recognition in lambs and further investigation is needed.

Although dam breed was not shown to influence lamb growth rate during the lactation period, the willingness of the ewe was found to have a positive association with her lamb's growth rate. Affiliative maternal care may have a positive influence on lamb growth via the behaviour of the dam during sucking interactions and also the general nature of the ewe-lamb bond and the resultant ewe-lamb distance. This may have an indirect affect on lamb growth as lambs learn their grazing behaviour from their dam, consequently lambs which are closer to their dams have more opportunity to learn and develop good grazing habits.

The breed of a ewe's dam was also shown to influence her own maternal care. Ewes showed similarities with their dams in their levels of neonate grooming and co-operation with initial suck attempts. It was not clear if the influence of the dam's maternal behaviour was manifest through genetic inheritance or via the maternal care received as a lamb. Sire breed was also found to influence grooming duration. This effect must occur via genetic inheritance as sires made only a genetic contribution to their offspring. The experimental design and low number of available ewes allowed only speculation rather than firm conclusions but the results show that this area has the potential for further study.

Chapter 9: A preliminary study of initial maternal behaviour in the horse.

9.1. Introduction.

The previous chapters in this study have demonstrated that maternal behaviour influences the development of offspring in sheep. The maternal behaviour experienced throughout lactation has important implications for the ewe-lamb relationship and this in turn influences the later behaviour of the offspring. These findings have the potential to be applied to other domestic species which have similar relationships with their young. One potential example is the horse, as it shares a number of characteristics with the sheep. Both species live in social groups and give birth to small litter sizes. The young remain with the dam for an extended period after birth and employ a following strategy (Lent 1974). By comparing previous studies on the maternal behaviour of both species' it is possible to assess whether or not they show similarities in their maternal care and their relationship with their young.

9.1.1. Parturition and post-parturient behaviour in the mare.

The behaviour of the mare during the immediate post-partum period has received very little study. Mares are known to give birth in the recumbent position and remain lying for up to 40 minutes after the birth (Rossdale 1967, Tyler 1972, Crowell-Davis and Houpt 1986). As with the ewe (e.g. Vince et al. 1985), foetal fluids play an important role in stimulating maternal behaviour (Rossdale 1967) and primiparous mares are prone to initially direct their attention at fluids rather than their young (Beaver 1981). Tyler (1972) appears to be the only study providing detailed observation of the initial maternal behaviour of the mare. She reports that once mares have risen to their feet they lick the foal vigorously for a period of 30 minutes. Subsequently the frequency of licking declines but still occurs intermittently over the first day post-partum. Tyler (1972) suggested that licking of the foetal fluids is important in the establishment of foal recognition in the mare. After licking their young, mares can discriminate between their own and alien foals and react aggressively towards the approach of the latter (Tyler 1972). This is similar to the expression and function of grooming behaviour in the ewe (e.g. Alexander 1988). However Tyler's observations of post-parturient maternal behaviour were qualitative descriptions, compiled from the field observations of only 3 New Forest Pony mares. Consequently they may not provide an accurate representation of initial maternal behaviour in the horse. In addition, the role of licking in the establishment of a selective bond between the mare and foal has not been formally tested.

9.1.2. Maternal behaviour during later lactation.

The maternal behaviour of the mare during later lactation has been studied more extensively than the initial post-partum period and more is known about the established mare-foal relationship.

9.1.2.1. The spatial relationship.

The horse, like the sheep, is a Follower species and there are many similarities in the spatial relationship each have with their young. During the first month post-partum the dam is a foal's most frequent nearest neighbour. This declines with increasing age, as the foal increasingly associates with other foals (Crowell-Davis et al. 1986) and the foal-mare distance becomes greater (Barber and Crowell-Davis 1994). Like the lamb, the foal assumes responsibility for maintaining proximity as it ages (Tyler 1972, Crowell-Davis 1986), and also shows increasing behavioural synchrony with its dam, especially when resting (Smith-Funk and Crowell-Davis 1992).

One difference between sheep and horses is the recumbency response of the mare. During the first few months post-partum the spatial relationship of the mare and foal is determined by the postural state of the foal (Barber and Crowell-Davis 1994). When the foal is lying the mare will graze in a circle around it, or stand still beside it (Crowell-Davis 1986). This response wanes with increasing age and by 3 to 4 months post-partum the mare may be over 100m away when her foal is sleeping (Crowell-Davis and Houpt 1986). A recumbency response has not been reported in the sheep, although the ewe may be closer to her lamb when it is recumbent, compared to other behaviours, after the first month post-partum (Chapter 4).

9.1.2.2. Mare-foal recognition.

Not much is known about recognition between mares and their foals. Vocalisation appears to be important in locating one another after separation. However this does not appear to be due to recognition of calls but rather attraction towards calling individuals, allowing orientation and then long distance visual recognition (Tyler 1972). In common with the sheep, visual cues appear to be used in distance recognition and olfaction is important in recognition at close proximity (Wolski et al. 1980). As proposed by Terrazas et al. (2002) for the lamb, foals may also rely on the mare's behaviour as a final cue of acceptance (Wolski et al. 1980).

The development of dam recognition in the foal appears to be slower than that of the lamb. Foals initially approach the nearest mare, regardless of size or colour, and only appear able to discriminate between their dams and mares at very short distances. By 2 to 3 weeks foals seem able to recognise and locate their mother by her coat colour and vocalisations, moving directly towards her in response to nickering by their dam (Tyler 1972). Lambs develop distance recognition of their dam during the first few days post-partum (Nowak 1990b).

9.1.2.3. Sucking interactions.

Unlike in the sheep, where the ewe controls the sucking behaviour of her lamb after the first few weeks post-partum (Trivers 1985), the majority of sucking interactions are initiated by the foal (Carson and Wood-Gush 1983, Barber and Crowell-Davis 1994). The foal also terminates the vast majority of bouts throughout lactation, although the mare shows a high proportion of terminations between day 1 and the end of week 1. Mares usually terminate these bouts by walking away (Crowell-Davis 1985) and it is probable that this functions to teach the foal following behaviour (Barber and Crowell-Davis 1994).

Sucking by the foal is initially very frequent during the first week but gradually decreases with age (Tyler 1972, Duncan et al. 1984). After the eighth month the frequency of sucking remains at a constant low level until weaning at approximately 1 year. Sucking generally occurs after the foal had been resting, after a period of separation or disturbance or after the mare has approached the foal. Some nursing interactions, consisting of nuzzling only, are thought to act as a comforting stimulus and are associated with disturbance or periods of separation (Tyler 1972, Crowell-

Davis 1985, Smith-Funk and Crowell-Davis 1992). This is also seen in the sheep (Shillito and Hoyland 1971). Mares wean their foals a few days or weeks before the birth of their next foal. Weaning is very rapid and is manifest by the mare avoiding her foal and threatening it during sucking attempts. If the mare does not have a new foal weaning may be delayed until the foal's second year (Tyler 1972).

9.1.3. Maternal aggression.

Another difference between the ewe and mare is the expression of aggression towards their young. In the sheep aggression towards own young appears to be confined to the immediate post-partum period whereas in the horse it is expressed throughout lactation. The vast majority of maternal aggression in the horse is associated with sucking interactions (Smith-Funk and Crowell-Davis 1992) and is probably due to the bunting behaviour of the foals being painful (Barber and Crowell-Davis 1994). Aggression is rare during the first month but increases to a peak at 3 to 4 months. Foals frequently show no response to maternal aggression but may respond by pausing or kicking their dam (Barber and Crowell-Davis 1994). Crowell-Davis (1985) proposed that aggression may be linked to the weaning process, negatively reinforcing sucking, but foals rarely terminate the bout or alter bout frequency or duration in response to aggression (Barber and Crowell-Davis 1994).

9.1.4. Aims of the study.

From the above discussion it can be seen that sheep and horses show some similarities in their maternal behaviour and their mother-young relationship, creating the potential for the findings of the sheep study to be applied to the horse. However, very little is known about the maternal behaviour of the mare during the immediate post-partum period. This is a very important time in the mother-young relationship as this is when the exclusive bond is formed between the mother and her young. In the sheep the behaviour of the dam at this time has been shown to have a lasting effect on her relationship with her young (e.g. Nowak et al. 1997, the current study). Consequently it is important to gain more information about the initial maternal behaviour of mares.

In order to do this formal observations of the maternal behaviour of Arab mares were conducted over the first 24 hours post-partum, with particular focus on the 3 hours following parturition. The propensity of the foal to follow its dam when first leaving the foaling stall was used to give an indicator of the relationship between maternal care and the developing mare-foal relationship.

9.2. Methods.

9.2.1. Location.

Observations took place at the 'Horse Teaching and Research Farm' at Michigan State University, East Lansing, Michigan, USA.

9.2.2. Animals.

Thirteen Arab mares of various parities were used in the study. Table 9.1 gives the details of each mare's parity, background and the sire and sex of her foal. Mares foaled over a 9 week period between April and June 2001.

Table 9.1.
Details of the mares used in the study.

Mare	Background	Parity	Foal sire	Foal sex
Brocade	MSU	4	Afire Bey	Female
Pasketta	MSU	9	Consensus	Male
Aurora	California	1	Ben	Male
Glytter	MSU	10	Ben	Female
Summer	MSU	5	Ben	Female
Likafire	MSU	6	Ben	Female
Goldy	MSU	2	Ben	Female
Showgirl	MSU	1	Ben	Male
Rosetta	California	1	Ben	Male
Foxy Lady	MSU	3	Legacy	Male
Aulana	California	1	Ben	Male
Beauty	California	5	Ben	Female
Eternity	MSU	4	Legacy	Male

The table shows the details of the mares used in the study. MSU did not own all the mares, the background of the mare indicates where they originated from.

9.2.3. Husbandry.

Mares were individually housed in foaling stalls (approximately 3mx4m) and moved out to a group paddock to exercise during the day. Stalls had natural ventilation and light, which was supplemented with overhead electric lighting as necessary. The stalls were bedded with straw and were mucked out daily. The time spent in the paddock varied according to the weather and other activities on the farm. When housed in the stalls mares had ad libitum access to hay and water and were given a daily ration of concentrates according to body condition.

As a mare's expected parturition date approached, or she showed signs of foaling (producing colostrum), a 24 hour watch was placed on the mare. The majority of mares gave birth during the night (only one mare, Showgirl, foaled during the day). Mares were attended during foaling. A similar intervention schedule to the one used in the sheep study was devised but, due to the value of the individual foals and management constraints, this was not strictly adhered to. Foaling assistance was given to a mare 1 hour after the appearance of fluids, with no parts of the foal and/or 2 hours after parts of the foal were seen at the vulva with no obvious progress being made.

9.2.4. Observations on maternal behaviour.

Mares remained in the stalls for at least 24 hours after parturition. A continuous video record was made of the first 24 hours post-partum. Data on the latency to begin vocalising, and the frequency of 'nicker' vocalisations during the first 3 hours post-partum were collected live using a Psion Workabout handheld computer with The Observer software (Noldus et al. 2000). The presence or absence of squealing by the mare in response to sucking interactions was also recorded. Observations on grooming and sucking behaviour were made using the video record, a Psion Workabout computer and The Observer software (Noldus et al. 2000). Table 9.2 gives the behaviours, with definitions, recorded during the first 24 hours post-partum. During the first 3 hours post-partum the following measures were recorded: the latency from birth to begin grooming and make nicker vocalisations; the latency

Table 9.2.
Behaviours, with definitions, recorded during the initial post-partum period.

Behaviour	Definition
Mare	
Recumbent	Mare lies inactive on the straw.
Stand	Mare stands inactive on all four legs, does not interact with her foal.
Groom foal	Mare licks her foal on any part of its body.
Nickers	Mare makes a soft vocalisation.
Squeals	Mare makes a high pitched squealing vocalisation in response to her foal investigating her body.
Circles	Mare walks forward and round as foal interacts with her.
Backs	Mare backs away from her foal as it interacts with her.
Foal	
Investigates body	Foal touches mare's body with its muzzle in any area other than the udder.
Udder directed behaviour	Foal touches the mare's udder with its muzzle but does not take the teat into its mouth.
Suck	Foal takes the teat into its mouth and sucks, swallowing can be seen in the foal's throat.

from standing to begin grooming; the duration of time spent grooming and the frequency of vocalisation in each of the first three hours post-partum; the mare's reaction to her foal's attempts to locate the udder and suck. The time taken for the umbilical cord to be broken was also recorded. During the 3rd, 4th, 6th, 12th, 18th and 24th hour post-partum the frequency, average bout length and proportion of sucking bouts ended by the foal were recorded to give information on sucking interactions during the first day.

9.2.5. Assessment of foal-dam attachment.

On the second day post-partum the mares and foals were led out of the stall for the first time. A follow score was assigned to each foal to measure its propensity to follow its dam as she left the stall. The score used was as follows:

1. Foal does not attempt to follow dam and shows no agitation at being left,
2. Foal does not attempt to follow dam but vocalises and moves around in stall,
3. Foal initially follows dam but remains in the stall, may vocalise and pace,
4. Foal hesitates before following dam at a distance, may vocalise,
5. Foal follows dam at a distance, may vocalise,
6. Foal follows dam in close proximity.

9.2.6. Statistical analysis.

Data on grooming, vocalisation and co-operation with initial nursing attempts are presented descriptively. Data were checked for normality, non-co-operation with initial suck attempts (second and third hour), the latency to begin vocalising and the frequency of vocalisations during the first, second and third hours post-partum were skewed, all other data were normally distributed. Pearsons correlation was used to investigate the relationships between the expression of various maternal behaviours during the first 3 hours post-partum. Spearmans ranked correlation was used for skewed data. During the first hour post-partum the relationships between the latency to stand, the latency to groom from standing, the latency to make nicker vocalisations, the duration of grooming and the frequency of vocalisations were assessed. During the second hour post-partum the relationships between grooming duration, frequency of vocalisations and co-operation with suck attempts were assessed. As these were the first suck attempts for the majority of the foals (11/13) the relationships between co-operation and the latency to stand and the latency to begin grooming after standing were also assessed. During the third hour post-partum the relationships between grooming duration, frequency of vocalisations and co-operation with suck attempts were assessed.

As previous experience may influence the maternal behaviour of the animals, the behaviour of the primiparous mares was compared with that of the multiparous mares. T-tests and Mann-Whitney tests were used to analyse the effect of dam parity on maternal behaviour during the first 3 hours post-partum.

9.3. Results.

9.3.1. First 3 hours post-partum.

Table 9.3 shows the data collected during the first 3 hours post-partum. Mares remained recumbent after parturition for an average of approximately 20 minutes and did not begin grooming their foals until after they had stood. The umbilical cord was broken approximately 15 minutes after parturition. Grooming was very infrequent in each of the 3 hours and the duration of grooming showed a decrease over the first 3 hours post-partum. Mares began vocalising (nickers) before standing but vocalisation was infrequent throughout the initial post-partum period. The majority of foals did not begin investigating their dam's body during the first hour post-partum. Circling and backing in response to foal's attempts to find the udder and suck was very rare. Aggression was also infrequent and seen in only two mares, Eternity and Foxy Lady, which bit their foals. 69% of mares squealed in response to their foals investigating their body.

Table 9.3.
Data collected during the first three hours post-partum

Behaviour	Range of expression	Mean/median value
Latency to stand (s)	35.7-2142.2	1245.0 (177.0)
Latency between birth and cord breaking (s)	60.0-1680.0	987.7 (149.7)
Latency to groom from birth (s)	44.9-2160.5	1360.0 (184.0)
Latency to groom from standing (s)	9.2-534.8	115.2 (41.6)
Duration of grooming, 1st hour (s)	8.8-740.5	364.90 (74.20)
Duration of grooming, 2nd hour (s)	37.8-347.8	114.40 (22.40)
Duration of grooming, 3rd hour (s)	0.0-87.6	35.08 (8.75)
Latency to vocalise (s)	4.7-5258.2	229.0 (22.0, 1187.0)
Number of vocalisations, 1st hour	0.0-181.0	7.50 (2.0, 84.20)
Number of vocalisations, 2nd hour	0.0-127.0	14.50 (1.30, 39.50)
Number of vocalisations, 3rd hour	0.0-49.0	5.0 (0.0, 8.0)
Proportion of non-co-operation with sucking attempts, 2nd hour (%)	0.0-14.81	0.0 (0.0, 4.71)
Proportion of non-co-operation with sucking attempts, 3rd hour (%)	0.0-23.53	0.0 (0.0, 9.27)

Mean values are given with s.e.m, median values with Q1, Q3. Non-co-operation with sucking attempts was defined as the mare circling or backing in response to a foal investigation, udder directed behaviour or suck. Only two foals showed investigation or udder directed behaviour during the first hour post-partum so data from the second and third hours only are presented.

9.3.2. Relationships between the various maternal behaviours expressed during the first 3 hours post-partum.

9.3.2.1. First hour.

Latency to stand did not show a significant correlation with the latency to begin grooming after standing (Pearsons correlation r=0.05, NS) but was significantly correlated with the latency to begin making nicker vocalisations (Spearmans correlation r=0.830, p<0.001). There was a non-significant tendency for the latency to stand to be correlated with the duration of grooming (r=-0.480, p=0.097) but there was no significant correlation between latency to stand and the frequency of nicker vocalisations (r=-0.288, NS) during the first hour post-partum. The latency to begin grooming after standing was not significantly correlated with the duration of grooming (r=-0486, NS), the latency to begin making nicker vocalisations (r=0.132, NS) or the frequency of nicker vocalisations (r=0.316, NS) during the first hour. The latency to begin making nicker vocalisations was not significantly correlated with the frequency of nicker vocalisations (r=-0.437, NS) or the duration of grooming (r=-0.308) during the first hour.

9.3.2.2. Second hour.

The mare's co-operation with suck attempts was not significantly correlated with her latency to stand (r=-0.176, NS) or her latency to begin grooming after standing (r=-0.161, NS). It also showed no significant correlation with the duration of grooming (r=-0.195, NS) or the frequency of nicker vocalisations (r=-0.035, NS) during the second hour. There was no significant correlation between the frequency of nicker vocalisations and the duration of grooming (r=0.232, NS) during the second hour.

9.3.2.3. Third hour.

There was no significant correlation between any of the behaviours measured during the third hour post-partum: duration of grooming and frequency of nicker vocalisations (r=0.353, NS), duration of grooming and co-operation with suck attempts (r=0.317, NS) or frequency of nicker vocalisations and co-operation with suck attempts (r=-0.144, NS).

9.3.3. Sucking interactions over the first 24 hours.

Table 9.4 shows the frequency, average bout length and proportion of sucking bouts ended by the foal during the first day post-partum. Nursing behaviour appeared fairly constant over the first day and foals terminated the vast majority of sucking bouts.

Table 9.4.
Sucking interactions during the first day post-partum

Number of hours post-partum	Number of sucking bouts	Average length of a sucking bout (s)	Proportion terminated by the foal
3	5.31 (1.37)	61.60 (19.10)	*
4	3.15 (0.48)	130.21 (10.50)	100.0 (100.0, 100.0)
6	4.84 (0.81)	125.70 (13.40)	100.0 (100.0, 100.0)
12	4.46 (0.83)	119.20 (11.20)	100.0 (100.0, 100.0)
18	5.09 (0.78)	99.30 (9.93)	100.0 (88.88, 100.0)
24	4.63 (0.39)	97.74 (6.50)	100.0 (66.67, 100.0)

Data for frequency and average length of sucking bout presented as means with s.e.m., data for the proportion of sucking bouts terminated by the foal presented as medians with Q1, Q3.

9.3.4. Effect of parity on maternal behaviour.

There was no significant difference between primiparous and multiparous mares in the latency to stand after birth (mean time (s) (with s.e.m.): primiparous=1123.4 (319.7), multiparous=1299.8 (223.2), T-test T=0.45, d.f.=6, NS). There were also no significant differences between the two groups in the latency between birth and grooming (mean time (s) (with s.e.m.): primiparous=1228.3 (305.4), multiparous=1419.4 (238.1), T=0.50, d.f.=6, NS) or the latency between standing and grooming (mean time (s) (with s.e.m.): primiparous=105.0 (52.7), multiparous=119.7 (57.3), T=0.19, d.f.=9, NS).

Figure 9.1 shows the duration of time spent grooming during each of the 3 hours post-partum. There were no significant differences between the 2 groups during any of the time periods (1^{st} hour: T=-1.63, d.f.=6, NS, 2^{nd} hour: T=0.94, d.f.=10, NS, 3^{rd} hour: T=-0.28, d.f.=5, NS).

Parity group did not affect the latency to begin making nicker vocalisations (median time (s) (with Q1, Q3): primiparous=266.9 (22.3, 1730.4), multiparous=190.8 (15.8, 943.2), Mann-Whitney U=57.0, NS). Figure 9.2 shows the frequency of nicker vocalisations during each of the 3 hours post-partum. There were no significant differences between the 2 groups during any of these time periods (1^{st} hour: U=24.0,

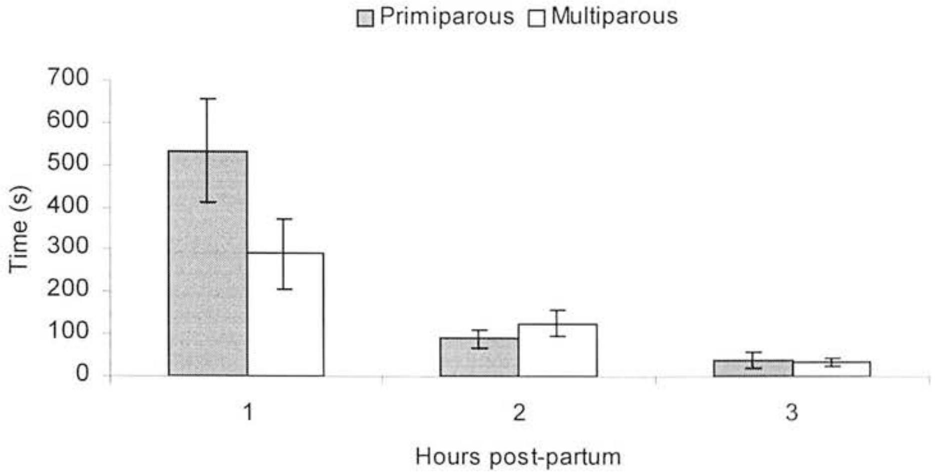

Figure 9.1. Mean duration of grooming shown by the primiparous and multiparous mares during each of the first three hours post-partum (with s.e.m.).

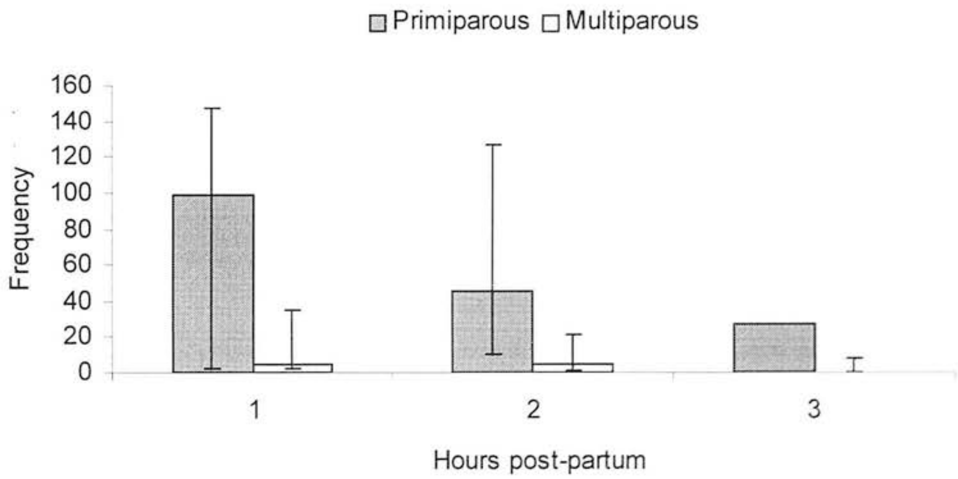

Figure 9.2. Median number of nicker vocalisations made by the primiparous and multiparous mares during each of the first three hours post-partum (with Q1, Q3).

NS, 2nd hour: U=50.0, NS, 3rd hour: U=49.0, NS).

There were also no significant differences between the 2 groups in the proportion of suck attempts in which the mares showed circling or backing, during the second hour (median proportion (%) (with Q1, Q3): primiparous=0.0 (0.0, 1.5), multiparous=0.0 (0.0, 9.0), U=62.0, NS) or the third hour post-partum (primiparous=3.7 (0.5, 11.24), multiparous=0.0 (0.0, 8.6), U=57.0, NS).

9.3.5. Association between maternal behaviour and foal follow score.

Eleven foals were assigned a follow score of 3, the other 2 foals were assigned a score of 1 and 2 respectively. Due to the lack of variation amongst these scores it was not appropriate to analyse the association between follow score and maternal behaviour.

9.4. Discussion.

9.4.1. Maternal behaviour in the mare during the immediate post-partum period.

In agreement with previous descriptions of early maternal behaviour (Rossdale 1967, Beaver 1981, Crowell-Davis and Houpt 1986) the mares in this study remained recumbent after parturition. However the duration of recumbency was much shorter than previous studies have reported: approximately 20 minutes, compared to around 40 minutes. The latency between birth and the umbilical cord breaking was approximately 15 minutes, indicating that some mares remained recumbent after the cord had broken.

The mares began vocalising (nickers) to their foals whilst still recumbent but did not begin grooming until after they had stood. Most mares began grooming their foals soon after standing and the longest latency between standing and grooming was just under 10 minutes (534.8 seconds). Both grooming and vocalising were very infrequent and some mares were virtually silent, making only 1 or 2 vocalisations during the entire observation period. The mares showed wide variation in their grooming behaviour, especially during the first hour post-partum. Grooming duration ranged from only 8.8 seconds to just over 10 minutes (740.5 seconds) in this time period. The duration of grooming decreased over the 3 hours after parturition and by the third hour post-partum mares had either ceased grooming or showed very infrequent grooming, the longest duration being 87.6 seconds.

The majority of foals did not begin investigating their dam's body until the second hour post-partum. A failure to co-operate with this was rare, although the majority of mares made some squealing noises in response to foal investigation. Over the first 24 hours foals nursed between 4 and 5 times an hour and terminated the majority of sucking interactions. Physical aggression towards the foals was only seen in 2 mares, both of which were multiparous. In both cases the mares bit their foals.

9.4.2. Relationships between the various maternal behaviours expressed during the first 3 hours post-partum.

In the majority of cases a mare's expression of a particular maternal behaviour was not significantly related to her expression of any of the other behaviours measured during the first 3 hours post-partum. However the latency to stand after parturition was positively correlated with the latency to begin making nicker vocalisations. In addition, there was a tendency for the latency to stand to be negatively correlated with the duration of grooming during the first hour after birth. This could be due to the fact that mares did not begin grooming their foals until after standing. A long latency to stand would therefore leave less time for grooming during the first hour post-partum. However mares did not show long bouts of grooming and the longest duration of grooming during the first hour was approximately 12 minutes. In addition, the mare with the longest latency to stand groomed her foal for just over 10 minutes, one of the longest grooming durations recorded. Therefore, although this does not rule out the possibility that a longer latency to stand was associated with a short grooming duration due to a reduced opportunity to groom the foal, it does reduce the likelihood that this is the reason for the relationship between the two behaviours.

All three of these behaviours potentially represent the mare's first interactions with her foal and her interest in the neonate. Nicker vocalisations can be expressed before the mare stands and, as the mare does not show any physical interaction with her foal before standing, vocalisation will be the only 'contact' she makes with her foal whilst still recumbent. The latency to vocalise may therefore represent the mare's willingness to interact with her newborn foal. Once the mares had stood they almost immediately groomed their foals (mean latency was just under 2 minutes). Consequently the latency to stand may give a good indication of their interest in their foal, as will the duration of grooming during the first hour.

Recumbency after birth does have a function, allowing blood to flow from the placenta to the foal via the umbilical cord (Rossdale and Mahaffey 1958), and the mare must therefore remain recumbent for a period of time to allow this to happen.

The cord is broken by the movements of the foal or the mare (Rossdale 1967). In the current study some mares remained recumbent long after the cord had broken and the latency to stand therefore still has the potential to represent the mare's interest in the neonate.

The relationship between the three behaviours indicate that mares with a short latency to stand also showed a short latency to vocalise and may have shown a long duration of grooming during the first hour post-partum, all indicative of an interest in their foal and a willingness to interact with it. The opposite is also true, long latencies to stand and vocalise and a short duration of grooming all suggest a disinterest in the foal. These behavioural measures may therefore make a good basis for future investigation of the development of the mare-foal bond.

9.4.3. Comparison with previous observations of initial maternal care.

These results differ from previous observations on early maternal behaviour in the horse. Tyler (1972) reported that New Forest Pony mares lick their foals almost continuously for a period of 30 minutes following standing. There are many differences between the animals in Tyler's study (1972) and the current study and this may account for the different findings. Tyler studied feral, forest-dwelling New Forest Ponies. The Arab breed is originally a desert-living horse and the different habitats of the two breeds may have an influence on their maternal behaviour, albeit an historical one for the Arab mares. Breeds associated with a desert habitat are adapted for heat dissipation, whereas northern breeds, such as the New Forest pony, show adaptations for energy conservation and survival in a cold climate (Goodwin 2002). One of the functions of grooming in the ewe is thought to be drying and warming the neonate (McGlone and Stobart 1986). If grooming has a similar function in the horse this would be expected to be less important in a desert habitat where animals are adapted to lose heat, not conserve it. Additionally, the more open environment of the desert, compared to the forest, may necessitate greater vigilance in desert-living animals, leading to a reduction in grooming. Long periods of grooming will make the mare more vulnerable to predation as her attention will be focused on her foal. The immediate post-partum period is an especially dangerous time, in terms of predation, as the uncoordinated neonate is an easy target for predators.

The actual environment of the animals may also have an influence on their behaviour. The mares in the current study were maintained in an intensively managed, highly structured environment with lots of interaction with humans. They were also housed in close proximity to other mares. In contrast the New Forest Ponies were free to roam in the forest and whilst they may have had some interaction with humans, this is likely to be minimal in comparison to the Arab mares. Human interaction and the close proximity of other mares may interfere with the normal expression of behaviour, causing the Arab mares to interact with their foals less frequently than they would without these potential distractions.

Human involvement may also influence maternal behaviour in the long term, altering the expression of maternal behaviour at the breed level. Domestic horse breeding is an intensively managed process with mating arranged to produce offspring with specific desirable traits. The resultant foals are often very valuable and human intervention at parturition is very common in order to ensure the foals survive. Over time this could lead to a change in maternal behaviour as traits which have low survival value (after Alexander 1988) will be compensated for by the human intervention and will persist in the population. Such traits are unlikely to be seen at a high frequency under extensive conditions as they would decrease the chances of successfully raising young.

The Arab breed has also been associated with a higher than expected level of foal rejection (Juarbe-Diaz et al. 1998). Although there was no foal rejection in this study, the low interaction of Arab mares with their foals during the post-partum period, compared to New Forest Ponies, may be related to this. In Juarbe-Diaz et al.'s study (1998) rejecting mares were found to exhibit less post-partum grooming and nickering than non-rejecting mares. Foal rejection was thought to be affected by inherited and environmental factors inherent in the breeding system (Juarbe-Diaz et al. 1998).

In conclusion, the differences in maternal behaviour between the New Forest Ponies (Tyler 1972) and the Arab mares highlight the potential for an influence of breed and/or environment on the expression of maternal behaviour in the horse. If environment is the main cause of the differences in behaviour the results of the current study are likely to be more applicable to intensively managed domestic horses than the results of Tyler's study (1972). In order to investigate this further the maternal behaviour of many more breeds should be studied under a range of different environments.

9.4.4. Effect of parity on maternal behaviour.

There were no significant differences in the expression of maternal behaviour between the primiparous mares and multiparous mares. However the low number of primiparous mares (4) reduces the accuracy of the analysis as it is not a very representative sample. Consequently it is not possible to definitely conclude that maternal experience does not affect the expression of maternal care in the horse. Further investigation, with a much larger sample size, is needed.

9.4.5. Relationship between maternal behaviour and foal attachment to the dam.

It was not possible to investigate the relationship between maternal behaviour and foal follow score as almost all foals were assigned the same score. Therefore, although the mares showed some variability in their maternal behaviour, their foals did not show variability in their propensity to follow their dam. This could have been due to flaws in the experimental procedure which may not have allowed accurate assessment of foal-mare attachment. During testing the foal was 'asked' to not only follow its dam, but to also leave a familiar environment and enter an area containing sights, sounds and smells which it had not encountered before. The test therefore involved an assessment of the foal's fear response as well as its attachment to its dam.

These two motivations are likely to be conflicting and the fear of entering a novel environment may have interfered with the following behaviour of the foals. Evidence for this is seen in the fact that the most common score assigned to foals was '3'. To be assigned this score foals initially followed their dam but then remained in the stall, possibly showing agitation by vocalising and pacing. The initial following behaviour of the foal therefore appeared to be disrupted as it reached the exit to the stall. Due to management constraints which prevented the animals being placed in a formal test situation, the Follow score was the best measure of foal-dam attachment available during the study and this confounding factor could not be avoided. Therefore, it is not possible to use the results of this study to conclude whether or not initial maternal behaviour has an influence on foal-mare attachment.

9.4.6. Comparison of maternal care in the ewe and mare.

In this study mares were found to show a similar type of initial maternal behaviour to the ewe but the expression was much less intensive in the mare, compared to the ewe, and mares showed less active interaction with their young than ewes. Grooming duration and vocalisation frequency in particular, were much lower in the mare, compared to the ewe. However this could just be a characteristic of the Arab breed as Tyler (1972) reported more intensive grooming in New Forest ponies. Mares showed much more co-operation with attempts to find the udder and suck than ewes and there was virtually no aggression directed towards the foals. Therefore, although they showed much less interaction with their young than ewes, they also showed less negative behaviour.

These differences in maternal behaviour during the initial post-partum period may result in different influences of maternal behaviour on the development of the mother-young relationship in the two species. In the sheep the ewe's reaction to early suck attempts appears to be one of the most important influences on the developing relationship (e.g. Alexander and Williams 1966a, Nowak et al. 1997, the current study). The mares in this study did not show much variation in their co-operation with their foals' sucking attempts and almost all gave full co-operation. If this is characteristic of all breeds, early sucking interactions are unlikely to have the same potential to influence the development of attachment to the dam in the foal as that seen in the sheep. However the variation in grooming and vocalisation may have consequences for the attachment between the mare and foal.

Tyler (1972) suggested that licking was important for the establishment of foal recognition by the mare. If this is correct the extremely low duration of grooming seen in some of the mares in the current study would be expected to have consequences for the development of the mother-young relationship. This highlights the need for further work investigating the development of attachment between mares and foals.

9.4.7. Potential for the findings of the sheep study to be applied to the horse.

The aim of this chapter was to provide more information about maternal behaviour in the mare, in order to assess the potential for the findings of the sheep study to be applied to the horse. An influence of maternal care in the sheep is likely to be mediated via the ewe's maternal style throughout lactation, for example the consistency of her expression of maternal care or her behaviour in relation to sucking interactions. The results of this study and previous research on maternal behaviour in the horse during later lactation (e.g. Carson and Wood-Gush 1983, Barber and Crowell-Davis 1994) suggest that sheep and horses have different sucking relationships with their young. Mares appear more inclined than ewes to allow their young to suck ad libitum throughout lactation. The nature of the sucking relationship may therefore have less of an influence on the development of the foal, compared to the lamb. However other aspects of maternal style in the mare, such as the amount of initial grooming or the behaviour of the mare when her foal is recumbent, have the potential to influence the mare-foal relationship and there is still potential for the findings of the sheep study to be applied to the horse. Further work is needed to investigate the long term mare-foal relationship and to assess how it influences the development of the foal.

9.5. Summary.

Mares remained recumbent after birth for an average period of 20 minutes. They began vocalising to their foals whilst still recumbent but did not groom their foals until after standing. Both vocalisation and grooming was very infrequent in comparison to the ewe. Aggression towards the foal was rare but the majority of mares squealed when their foal made attempts to find the udder and suck. Maternal experience was not found to influence maternal behaviour but the low number of primiparous mares is likely to have confounded this result.

Differences in the initial behaviour of ewes and mares are likely to affect the way in which their maternal behaviour influences their relationship with their young. However the maternal behaviour and mother-young relationships of the two species' show enough similarities for the findings of the sheep study to have the potential to be applied to the horse.

Chapter 10: General discussion.

10.1. Introduction.

Previous research has shown that the initial maternal behaviour of Blackface ewes is characterised by affiliative and care-giving behaviour with very little rejection. In contrast, the initial maternal behaviour of Suffolk ewes is characterised by less affiliative behaviour and a higher number of animals showing a rejecting maternal style (Dwyer and Lawrence 2000b). The results of the current study show that these differences in maternal style continue throughout lactation. The later maternal behaviour of Blackface ewes is characterised by consistency in their expression of maternal care, continued communication with their lambs and positive interactions with them, in particular a clear control of sucking interactions. In comparison, Suffolk ewes are not as proactive in their ewe-lamb relationship and their maternal behaviour is characterised by very little ewe-initiated interaction with their lambs. In particular, Suffolk ewes do not use distance control of sucking interactions and only indicate whether or not their lambs may suck once they have approached and made an attempt. This results in their lambs receiving frequent rejection from their dams and, as a consequence, the later maternal behaviour of Suffolk ewes can be described as more rejecting than that of the Blackface ewes.

10.2. Reasons for the differences in maternal care between Suffolk and Blackface ewes.

Modern breeds share a common ancestor (Lynch et al. 1992) and as such their behaviour would be expected to be similar. There are many factors which could have contributed to the differences in the expression of maternal behaviour in Suffolk and Blackface ewes, including their physical environment, the farming system in which they are maintained and their previous experience.

10.2.1. Influence of the environment on maternal behaviour.

The different environments of the two breeds may be responsible for their differences in maternal care (Dwyer and Lawrence 2000b). Blackface sheep are a hill breed, traditionally living in a relatively hostile environment, with little interaction with man. Under these circumstances ewes showing a rejecting maternal style are less likely to successfully raise lambs, compared to more affiliative ewes, and a rejecting style is unlikely to persist in the breeding population. It is therefore possible that environmental pressure has resulted in the affiliative and protective maternal style of Blackface ewes (Dwyer and Lawrence 2000b). In contrast, Suffolk sheep are maintained in a much more hospitable lowland environment, with much greater interaction with the shepherd. As a consequence, behaviours which may have resulted in decreased lamb survival in the hill environment may be compensated for by human intervention, especially during the immediate post-partum period, and consequently persist in the breeding population.

This may be further compounded by the intense selection of the Suffolk breed for increased growth rate and carcass attributes (Simm 1998). During this selection process only the desired attributes are monitored closely and other traits which are relatively less important are unlikely to be taken into account. Unless a ewe shows extreme negative behaviour and completely rejects her lamb, deficits in her maternal care can be tolerated and compensated for by the breeder. This is especially true if the lambs she produces are prime examples of the traits being subject to selection. Suffolk ewes are lambed indoors and delivery is often assisted. The ewe and her lambs are individually penned after parturition and the lambs are assisted to suck. Assistance with early sucking interactions and the individual penning of ewes and lambs is likely to relax the environmental pressure for affiliative and protective maternal behaviour in the Suffolk breed. Whilst a relaxation of environmental pressure will not automatically result in less affiliative maternal behaviour in the ewes it does give the potential for a more rejecting style to develop.

Hansen et al. (2001) have also reported breed differences in maternal behaviour which may be related to intensive management and the selection process. They observed that ewes of heavy breeds selected for traits related to production and economy, Suffolk, Steigar and Dala breeds, did not always seem to know where their lambs were and bleated for them frequently when exposed to a predator. In contrast, lighter breeds, which had not been selected for production traits, appeared to have a stronger ewe-lamb bond and their lambs always stayed close to them (Hansen et al. 2001).

It is also possible that the selection of the Suffolk breed for production traits may have resulted in a reduction in the ewes' general reactivity. In the current study Suffolk ewes as a breed were observed to be less flighty and to react to humans and dogs less overtly than the Blackface ewes. A reduction in general reactivity to the surrounding environment is likely to reduce the ewes' interaction with their lambs throughout lactation and has the potential to result in the maternal behaviour observed in Suffolk ewes, for example sporadic grooming, low vocalisation rate and infrequent communication. Selection for production traits may therefore have an indirect, rather than a direct, influence on maternal behaviour in the Suffolk ewes.

10.2.2. Influence of received maternal care on the ewes' own maternal behaviour.

As discussed in Chapter 8 the maternal behaviour of the dam, in particular her grooming behaviour during the post-partum period, has the potential to influence her offspring's own maternal behaviour. Ewes that received a high frequency of grooming as a neonate also show a high frequency of grooming of their own young, and vice versa. It is not clear whether this influence is mediated by the animal's genetics or through its experience as a neonate. Non-genomic transfer of maternal style has been demonstrated in rats (e.g. Francis et al. 1999a) and a similar situation could account for the differences in maternal style in Suffolk and Blackface ewes. There is even potential for this to have been influenced by human interference during the lambing of Suffolk ewes. Human intervention may disrupt the maternal behaviour of Suffolk ewes, leading to their lambs receiving reduced maternal care and subsequently showing reduced maternal care themselves as mothers. Further work is needed to investigate the ways in which the maternal styles of mother and offspring are linked in the ewe.

10.2.3. Influence of previous experience on maternal behaviour.

One of the main differences between the two breeds is the consistency of their expression of maternal care. Blackface ewes are consistent throughout lactation whereas Suffolk ewes show much more variation in their maternal behaviour. Consistency in the expression of maternal behaviour has previously been studied in primates (Fairbanks 1989, Berman 1990a), where it was found to be due to the mother, rather than the infant. Fairbanks (1989) suggested that maternal consistency may be due to temperamental consistency, particularly with reference to confidence and timidity. Temperamental consistency may be rooted in both the animal's genetics and its experience as an infant (Fairbanks 1989). Berman (1990b) also stated the importance of later experience and a relatively stable social environment during motherhood for consistency in the expression of maternal care.

Given the differences in maternal behaviour between the two breeds, the ewes in the current study are likely to have had different early life experiences. The Blackface ewes would be expected to have had a more stable relationship with their dam and consequently a more stable infancy. In contrast, Suffolk ewes would have experienced much more variability in maternal care, resulting in a more unstable social environment during their early life. This could account for the differences in the consistency of their expression of maternal care. The maintenance of the ewes as a single flock reduces the possibility of the ewes' experiences during motherhood contributing to differences in behavioural consistency. However it does not completely rule out the possibility of differences in later experience or the social environment of the ewes during motherhood.

10.2.4. Influence of physiology on maternal behaviour.

During the immediate post-partum period physiological factors, in addition to the presence of the offspring, are involved in eliciting maternal attraction to the lamb (reviewed by Poindron and Le Neindre 1980). This may provide some insight into how the breed differences in early maternal behaviour are mediated. The onset of maternal care is mediated via circulating estrogens and central oxytocin (reviewed by Poindron and Le Neindre 1980, Levy et al. 1996). Maternal oestradiol concentration in the ewe is strongly related to affiliative behaviours such as grooming (but not to ewe responsiveness to sucking attempts) and Blackface ewes have been shown to have higher plasma oestradiol-17β levels than Suffolk ewes during the last 6 weeks of pregnancy (Dwyer et al. 1999). Some of the variation in initial maternal behaviour between the two breeds of ewe may therefore result from variation in the ewes' levels of plasma oestradiol. In addition, in rats, females showing high levels of pup licking, grooming and arched-back nursing (desirable maternal traits) have significantly higher levels of oxytocin receptors in brain regions known to mediate the expression of maternal care, compared to females exhibiting low levels of these maternal traits (Francis et al. 2000). This suggests that individual differences in maternal behaviour may be directly related to variations in receptor expression (Francis et al. 2000). However, it is not yet known if these relationships between maternal physiology and maternal behaviour are causal, or just coincidental, and further work is needed to assess the role of maternal physiology in variation in the expression of maternal care.

10.3. Implications of variation in maternal care for the development of offspring.

Regardless of why the two breeds show differences in their maternal care, their different maternal styles have potential implications for the development of their offspring. The results of this study do not allow separation of the genetic and behavioural influences of dam breed but they do indicate a potential for variation in maternal care to influence offspring development. Ewes with a Blackface dam, and therefore having experienced a more stable ewe-lamb relationship and more affiliative maternal care, are quicker to learn a spatial memory task than ewes with a Suffolk dam, and also show an active behavioural response to aversive situations. Suffolk-raised ewes, which have experienced less affiliative care and more rejection, not only show a comparatively reduced cognitive ability but also show a passive behavioural response to aversive situations. These differences in offspring behaviour are likely to have implications for both animal welfare and animal husbandry.

10.3.1. Behavioural strategy in aversive situations.

Active and passive behavioural strategies in challenging situations have also been identified in the pig (Erhard et al. 1999). The implications of the different behavioural strategies for animal husbandry are context dependent: passive animals are easier to handle and hold down during the administration of an injection but active animals are easier to move down a raceway (Erhard et al. 1999). In normal sheep husbandry the administration of injections, or other procedures requiring the same type of restraint, do not happen as frequently as animal movement. Consequently animals employing an active strategy may be more desirable for the shepherd. Movement is also a potentially stressful procedure for the sheep (Hargreaves and Hutson 1997). Animals which can be moved quickly and easily are therefore likely to experience less stress than animals which are moved less efficiently. An active behavioural strategy in the sheep may therefore be desirable for both animal welfare and animal husbandry.

Behavioural strategy during aversive situations may also have implications for sheep welfare in the field, as well as during husbandry procedures. In wild animals an active response to an aversive situation promotes fitness, due to life expectancy being increased if the animal can react to avoid sources of danger such as predators (Boissy et al. 2002). Although domesticated, sheep are still exposed to predation or attack from foxes and dogs. An active response to such a threat may improve welfare as it will reduce the chances of injury or even death.

10.3.2. Cognitive ability.

As discussed in Chapter 8, sheep employ selective behaviour during grazing. Spatial memory aids preferential patch use and selection of a better diet than that achieved by random foraging (Edwards et al. 1996). The greater cognitive ability of animals receiving an affiliative maternal style would therefore give these animals an advantage over animals receiving more rejecting maternal behaviour, as they are more likely to have a better foraging strategy and therefore less likely to experience poor nutrition. Whilst this would be advantageous for the animal in any environment it would be most beneficial and promote good welfare in more hostile environments, for example the hill environment of the Blackface breed. In these environments the vegetation is more heterogeneous than lowland areas and preferential food patches would be expected to be more widely distributed than in lowland fields. In addition, animals maintained in a hill environment receive less contact with the shepherd and are less likely than lowland animals to receive supplementary feed if the grazing is sub-optimal, increasing the need for an efficient foraging strategy.

Cognitive ability also has the potential to influence the behaviour of animals during routine husbandry practice. An animal's previous experience affects its subsequent responses to handling through memory and learning and there is scope to use the learning ability of sheep to improve the ease and efficiency of handling (Kratzer 1971, Hargreaves and Hutson 1997). Grandin (1989) showed that sheep could be trained to accept handling, thereby reducing the time and labour involved in the handling process. A reduction in handling time is also likely to benefit the animals' welfare as the potential stress caused by handling would also be reduced (Grandin 1989).

The exploitation of learning ability in improving handling efficiency does not have to involve direct training of the animal. Training involves the repetition of a procedure until an animal performs the procedure fluently and without prompting or, in the case of handling, accepts the procedure without overt behavioural reaction. During routine husbandry practice sheep are exposed to a number of repeated procedures, for example moving down a race, being gathered in a pen, or restraint for examination. Naturally occurring repetition in farming practice therefore has the potential to act as inadvertent training. Repeated exposure to restraint has been shown to reduce the time spent struggling by ewes (Mateo et al 1991) and familiarisation with yard and race configurations in frequently used facilities has been shown to benefit the movement of sheep (Hutson 1980).

In the present study affiliative maternal care was found to be linked to a better spatial memory in the offspring. If this reflects an increase in the general cognitive ability of the animal, an affiliative maternal style could benefit animal welfare and animal husbandry via the animal's cognitive ability as well as via its behavioural strategy.

10.3.3. The importance of maternal care throughout life.

The implications of maternal style for the lamb whilst it is still with its dam were discussed in Chapters 3, 4, 5 and 8. In summary, a more affiliative maternal style is likely to be advantageous for the lamb as lambs receiving affiliative care have a much closer bond with their dams. Although there was no lamb mortality in the current study a closer ewe-lamb bond is known to increase lamb survival chances (Alexander 1988). The above discussion shows that the maternal style of ewes continues to influence the welfare of their offspring after they have been weaned. A more affiliative style is linked with behavioural traits which have the potential to improve the animal's welfare. Consequently the maternal care an animal receives is important throughout its life, not just whilst it is dependent on its dam.

10.4. Future work.

Examination of the influence of maternal care on offspring development in the present study was preliminary but has shown that there is much potential for future work in this area. Sheep are subject to a range of experiences during normal husbandry practice which could be aversive, resulting in the experience of stress and consequently reducing welfare. In addition to the restraint and social separation (visual contact with other sheep maintained) investigated in the current study, normal husbandry practice also includes social isolation (no visual contact), mixing with unfamiliar animals, vehicular transport, having to acclimatise to unfamiliar environments, human contact, the use of sheepdogs during the movement of animals and shearing.

Flocking and following are pervasive characteristics of the sheep and any handling that involves separating individual animals or disrupting groups is stressful for the animals involved (Hargreaves and Hutson 1997). Sheep which have no visual contact with their flockmates show extreme behavioural disturbance and a marked physiological stress response, even when they still have auditory contact with other sheep (Price and Thos 1980, Cockram et al. 1994). Yarding or gathering in a pen can also cause social disruption within a group. Crowding compounds the disruption and the experience of stress is exacerbated if the animals are unfamiliar with each other

(Hargreaves and Hutson 1997). Mixing with unfamiliar animals has been shown to result in behavioural and cardiac disturbance (Baldock and Sibly 1990) and a rise in salivary cortisol (Hall et al. 1998a).

Routine husbandry practice may also necessitate the transport of sheep in a trailer or transporter, for example between the lambing shed and field or during the transfer to market. Vehicular transport has been shown to be stressful for sheep, especially at loose stocking densities (Hall et al. 1998b). The most stressful events associated with transport are those associated with novelty and loading (Hall et al. 1998b) and the introduction of sheep to a novel environment has been shown to result in the experience of stress (Fordham et al. 1991, Cockram et al. 1994). The movement of sheep from the field to the farmyard for routine practices such as lambing therefore also has the potential to be stressful as the animals will have to acclimatise to an unfamiliar environment.

Although interaction with humans is a frequent occurrence throughout the life of a sheep, human contact is generally aversive and results in alarm, the magnitude of the stress response being dependent on the behaviour of the person (Harlow et al. 1987). The presence of dogs also causes an alarm response (Hargreaves 1988, cited in Hargreaves and Hutson 1997, Baldock and Sibly 1990) and can increase the disturbance caused by the approach of humans (Harlow et al. 1987). Physical interaction with humans, for example capture and restraint or shearing, also results in a stress response (Fell et al. 1985, Hargreaves and Hutson 1990a, 1990b, Chapter 7).

As discussed above, the amount of stress experienced during these situations is likely to be influenced by the animal's behaviour and their ability to learn about their environment. Consequently the maternal care received as a lamb has the potential to have a wide-ranging influence throughout the life of the animal. Future work could therefore be aimed at investigating the influence of maternal care on the experience and behaviour of sheep during various aspects of standard husbandry practice.

A positive influence of maternal care on the welfare of sheep during standard husbandry practice, via their behavioural strategy or cognitive ability, has much potential for improving sheep welfare as a whole. Many of the current recommendations for improving the welfare of farm animals involve major changes to housing or the methods of handling or moving the animals. This is usually impractical or undesirable for the shepherd due to the extra costs and/or time involved. In addition, it is hard to maintain a standard across the farming industry, especially in handling methods. Selection for affiliative maternal behaviour could therefore have a greater potential for improving sheep welfare than recommendations for changing individual aspects of husbandry practice, such as transport or handling. As the initial maternal style of both breeds in the current study was predictive of their maternal style throughout lactation, selection for maternal care could be based on observations during lambing, a time when the shepherd would be interacting with the animals anyway. Any ewes showing rejecting behaviour at this time could be excluded from the breeding flock in subsequent years. Consequently this method of influencing sheep welfare would not involve a large amount of extra work for the shepherd. However the economic implications of removing young healthy animals from the breeding population, and the subsequent need to replace them, is likely to influence the potential for using maternal care to improve sheep welfare.

10.5. Implications for other farm species.

Due to similarities in the maternal behaviour and mother-young relationship of sheep and horses, the results of the sheep study have potential for direct application to the horse. However the general concept of the importance of maternal care for offspring development has implications for the whole of the farming industry. In addition to the variation in maternal behaviour occurring naturally in farm animals there are widespread examples of animals being raised with disrupted, or even an absence of, maternal care.

For example, in pigs the use of farrowing crates limits the ability of the sow to perform her full range of maternal behaviour and weaning occurs much earlier than would be seen under natural conditions, between 3 and 5 weeks rather than 12 weeks. This results in disruption to the maternal behaviour received by young pigs. In addition, dairy calves are removed from their dams within hours of birth and subsequently raised in individual stalls or groups of peers. As cattle are a Hider species (Lent 1974), calves would naturally spend long periods away from their dam, either alone or in close contact with other calves. As such the influence of maternal care may be expected to be reduced in comparison to a Follower species such as the sheep. However, previous studies have shown that restricted access to the dam (2 hours daily) results in an increased stress response to restraint, compared to calves with ad libitum access to their dam (Lay et al. 1998). Consequently maternal care has much potential to influence the development of calves and the absence of maternal care in dairy calves is likely to have a major influence on their development.

An influence of maternal care is not restricted to mammalian species. Domestic pullets which have been brooded by a maternal hen show differences in their later behaviour, compared to pullets raised without a hen (Perre et al. 2002). Laying hens, broiler chicks, turkey poults and ducklings are all routinely incubated and hatched artificially and as a consequence do not experience maternal care. The combination of the potential influence of maternal care on the development and behaviour of offspring and the widespread disruption to maternal care within the farming industry has implications for farm animal welfare. It is therefore important that the maternal care received by an animal is included in the assessment of its welfare at any stage of development, not just during infancy.

10.6. Conclusions.

The influence of maternal care on the development of offspring has received extensive investigation in the laboratory rat but very little attention in the sheep. The results of this study show that this is an area of research with much potential for future investigation. The maternal care received as a lamb has the potential to have a significant influence on the animal's welfare throughout its life and, as such, should be taken into account in any assessment of sheep welfare.

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