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Genetic and environmental dissection of short- and long-term social aggression in pigs

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Declaration

I declare that this thesis is my own composition and that the research described in it is my own work, except where acknowledged. The work described has not been submitted for any other degree or professional qualification.

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Publications

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Abstract

It is common for pigs to engage in physical aggression when mixed into new social groups, in order to establish dominance relationships. Phenotyping aggression is time consuming, however skin lesions resulting from physical aggression are quick to record, are genetically correlated with aggressive behavioural traits, and have low to moderate heritability (0.19 to 0.43). Reducing aggression via selection on skin lesion traits would provide a socially acceptable, long-term solution to the problem. Barriers to implementing selection against skin lesions lie in the lack of understanding regarding the underlying genetic basis of aggression, and its relationship with other behaviour and production traits. This thesis has focused on dissecting the phenotypic and genetic relationship between skin lesions recorded 24 hours after mixing (SL24h), and either 3 or 5 weeks later (SL3wk/SL5wk, respectively), with aggression performed at mixing, and several production traits. Chapter 2 provided evidence of a potential trade-off between involvement in aggression upon first mixing, and receipt of aggressive attacks several weeks after mixing. In particular, animals that avoid aggression at mixing had the highest fresh skin lesion numbers at 3 weeks. This suggests that reciprocal fighting at mixing may be beneficial for long-term group social stability. It also suggests that it may be possible to phenotype the least aggressive individuals in a group using SL3wk. In Chapter 3, I quantified the magnitude of reduction in complex aggressive behavioural traits when using SL24h or SL3wk as selection criteria, to identify the optimum skin lesion trait for selection purposes. The results of Chapter 3 provided evidence that selection against anterior SL24h would result in the greatest genetic and phenotypic reduction in aggressive behaviour recorded at mixing. Although there is evidence that selection for increased SL3wk would reduce aggression at mixing, current understanding of aggressive behaviour

under stable group conditions is insufficient to recommend using this trait for selection purposes. Chapter 4, presented genetic associations between skin lesion traits as a measure of short- and long-term aggression, and commonly used commercial performance measures: growth, feed intake, feed efficiency, and carcass traits. The results suggested that, genetically, animals that receive many lesions show improved performance compared to those with few lesions, except for anterior SL24h, which have been shown to be genetically positively correlated with the initiation of non-reciprocal attacks. The aim of Chapter 5, was to determine whether skin lesion traits are phenotypically or genetically associated with behavioural measures of fearfulness. As found in Chapter 4, there was some evidence of an association between SL5wk and the traits, however this was not the case for anterior SL24h. For the 6th and final Chapter, we used skin lesion data from 1,840 pigs to perform genome wide association studies (GWAS), which detected a single SNP significantly associated with SL5wk on a genome wide level, as well as several SNPs associated with both SL24h and SL5wk on a chromosome wide level.

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List of abbreviations

SL24h	Skin lesions recorded 24h post-mixing
SL3wk	Skin lesions recorded 3 weeks post-mixing
SL5wk	Skin lesions recorded 5 weeks post-mixing
RA	Reciprocal aggression
NRA	Non-reciprocal aggression
SNP	Single nucleotide polymorphism
QTL	Quantitative trait locus
EBV	Estimated breeding value

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Chapter 1 - Introduction

1.1 General introduction

In 2013 the Food and Agriculture Organization of the United Nations (FAO) estimated that 1.5 billion pigs were raised worldwide, either for meat or production purposes (FAO statistical division - FAOSTAT). The majority of industrially-reared pigs are raised indoors. Although specific management practices differ between farms, it is common for animals to be raised under space-limited conditions, in inflexible group sizes. These systems are designed to ease stock flow and management, as well as maximising feed efficiency and growth. Although many systems are designed to minimise the mixing of unfamiliar pigs, in order to ensure equal group sizes and to allow feeding of pigs of a similar weight, mixing is often unavoidable. Physical aggression upon first mixing is common and may be an attempt to drive unfamiliar pigs away, but then serves to establish new dominance relationships, with the majority of aggression occurring within the first hour post-mixing (Meese and Ewbank, 1973).

Social aggression has a number of negative consequences for welfare. Injuries resulting from aggression, such as skin lesions (Francis et al., 1996) and lameness (Rydhmer et al., 2006), can be severe, and are the most obvious visual result of physical aggression. Stress caused by mixing and aggression, is likely to be a greater welfare concern than physical injury, and has received much attention in the literature. Studies have found evidence that sows and growing pigs subjected to mixing have increased salivary cortisol (Otten, 1997; Coutellier at al., 2007; Couret et al., 2009), elevated heart rate (Marchant et al. 1995), and compromised immune function (Morrow-Tesch et al., 1994; Tuchscherer et al., 1998). Aggression arising from mixing may also have an adverse effect on production parameters. At slaughter, mixing may occur at several points: on

farm prior to transportation, on the truck while loading, while unloading at the abattoir, or during lairage. Aggression is likely to occur at this time, and mixing has been recognised as an important factor contributing to decreased carcass value (Faucitano, 2001). Aggression at mixing may deplete muscle glycogen, leading to elevated pH levels, and undesirably dark meat (D'Eath et al., 2010). Stress caused by vigorous aggression or rough handling prior to slaughter may also increase lactate levels, leading to decreased pH post-mortem, and pale meat of degraded quality (D'Eath et al., 2010).

Group-housed sows are also affected by social aggression at mixing, and group housing is now a requirement in the EU since January 2013 (EU Pigs Directive 2008/120/EC). The effects of stress on reproductive parameters have been demonstrated in a range of species, including pigs, and it is possible that social stress arising from mixing may have a detrimental effect on reproduction (Einarsson et al., 2008). Sows with intermediate levels of fight success post mixing were found to have elevated salivary cortisol, and gave birth to lighter piglets compared to sows that had low or high fight successes (Mendl et al., 1992). Additionally, there is evidence that social stress caused by mixing pregnant sows can affect the behaviour and welfare of offspring, and these piglets are more likely to retreat from a fight at weaning; show elevated physiological stress responses and display aggressive maternal behaviour (Jarvis et al., 2006).

A wide range of environmental factors (reviewed by Arey & Edwards, 1998; Greenwood et al., 2014) including prenatal experience (Jarvis et al., 2006), previous life experiences such as mixing prior to weaning (D'Eath, 2005), familiarity with pen mates (Stookey & Gonyou, 1998), prior fighting experience (Ruis et al., 2001), and group size

(Samarakone and Gonyou, 2009) have all been shown to influence the level and severity of aggressive behaviour expressed. Alternative husbandry methods to minimise social aggression have been researched for several decades with mixed success (reviewed by Marchant-Forde & Marchant-Forde, 2005). Methods such as using odour masking agents and maternal pheromones (Barnett et al., 1993 ; Guy et al., 2009), and restricting feed prior to mixing (Arey and Edwards, 1998) have shown some success but were mainly found to simply postpone aggression. Other methods such as providing space and hiding places to escape aggressive interactions (McGlone & Curtis, 1985; Francis et al., 1996), manipulating group size and living space (Turner et al., 2002), grouping animals based on weight (Andersen et al., 2000) and the presence or scent of older boars (McGlone & Morrow, 1988; Grandin & Bruning, 1992; Barnett et al., 1993) have also shown some promise; however none of these methods have proven to be simultaneously effective, practical and cost-effective.

While alternative methods of reducing aggression continue to be explored, genetic selection against aggression could provide a cost-effective and long-term solution to the problem, and is the focus of this thesis. At present, not enough is known about the underlying genetic and environmental factors controlling aggression, how they interact with other important production and behavioural traits, and other traits of economic importance. The remainder of this chapter will give an overview of the literature regarding how aggressive behaviour at mixing interacts with other traits, both on a phenotypic and genetic level. It will go on to discuss the possibility of using selective breeding to reduce aggressive behaviour, by giving an overview of current knowledge of the genetic and genomic basis of social aggression.

1.2 Aggression as a phenotype

Social aggressive behaviour has been shown to be a concern for animal welfare and production. If selection against social aggression is to be successful it is important to demonstrate that each animal has an inherent level of 'aggressiveness' that can be measured and that is consistent across time and contexts. The use of repeated resident-intruder tests over time show that consistent variation in aggressiveness does exist between individuals, with highly aggressive pigs exhibiting a higher probability and shorter latency to attack during the resident-intruder test (D'Eath & Pickup, 2002). Upon mixing, groups containing these highly aggressive individuals are involved in more aggression and receive more skin lesions (Erhard et al., 1997), and the aggressive individuals may persist in fighting for longer (D'Eath, 2002). Terlouw et al. (2005) showed a positive correlation between individual aggression at mixing and aggression initiated during feed and straw competition tests, while Erhard et al. (1997), Ruis et al. (2000), Janczak et al. (2003), D'Eath (2004), D'Eath et al., (2010), and Clark & D'Eath, (2013) all found evidence of repeatability of aggressive behaviour in individual animals over periods of several weeks or months. There is also evidence of neurological differences between individuals, as an up regulation of vasopressin mRNA in certain regions of the brains of highly aggressive pigs has been reported, as well as fewer serotonin receptors compared to low aggression pigs (D'Eath et al., 2005).

1.3 Associations between aggression and other traits

There is much evidence in the literature that aggression is associated with several other traits, which may be of importance to the health, productivity and welfare of the animal. From a production perspective, the effects of mixing and aggression on growth have been the focus of much research, however there is ambiguous/conflicting evidence regarding whether growth rate is affected over the entire fattening period (Rundgren & Löfquist, 1989; Tan et al., 1991; Stookey & Gonyou, 1994; Hyun et al.,

1998). Most studies look at the effects of mixing on growth, rather than aggression *per se*, and very few have focused on associations between growth and individual aggressiveness (Wellock et al. 2003). Velie et al. (2009) found a negative phenotypic correlation between latency to attack in a resident intruder test, average daily gain, body weight, and loin muscle area, suggesting that more aggressive animals grow faster (high latency to attack suggests a less aggressive predisposition). These results were in contrast to those of Cassady (2007), who found a positive correlation between latency to attack and growth rate, although it is unclear why these results contradicted one another.

There is some evidence to suggest that feed intake behaviour is related to aggression in domestic pigs, even in established groups. The majority of aggressive behaviour is seen around the feeder and socially dominant pigs make fewer, longer feeder visits and generally consume more feed than lower ranking individuals (Hoy et al., 2012). Stocking density and feeder space may play a role in feed related behaviour, as feeders with room for multiple animals decreases the level of aggression observed in pigs (Martin and Edwards, 1994). Feeding related aggression may begin at birth, as piglets occupying the anterior teats (which yield higher levels of milk than posterior teats) are involved in more aggression (Scheel et al., 1977) which later affects post-weaning aggressive behaviour. It is likely that feeding and aggressive behaviours are affected by a wide range of factors. Pigs have been found to adjust feeding behaviour when moved from individual to group housing (de Haer & Merks, 1992; Nielsen et al., 1996), however Nielsen et al. (1996) found no specific association between aggression and feeding behaviour. It is likely that a mixture of positive and negative social interactions, together with group dynamics affects feeding behaviour. In summary, there is evidence in the literature to suggest that aggressive behaviour is correlated with many traits,

such as growth and feeding behaviour, although the results of these studies are often contradictory. The remainder of this literature review will outline the current understanding of the genetic and genomic basis of aggression.

1.4 Measuring aggression as a trait for genetic selection

In research, aggressive behaviour in pigs is often quantified either by group observations or by a resident-intruder test. Resident-intruder tests are convenient as they are less time consuming to perform than whole group observations, and are designed to measure the aggressiveness of one pig in a standardised manner, away from the complexity of a group mixing situation. In a resident-intruder test an unfamiliar pig (the intruder) is introduced to the home pen of another, larger animal (the resident). The size and status of the resident pig means that it is highly likely to attack the intruder, and the latency to attack is used as a measure of the resident pig's aggressiveness (D'Eath, 2004). Despite being less time consuming to conduct than group observations, the resident intruder test is still impractical to perform on a commercial level, as it requires dedicated space and time to perform, factors that do not easily fit into the work flow of an active commercial farm. Additionally, environmental factors influencing behavioural patterns may differ on commercial settings compared to small scale research farms, as pigs subjected to resident intruder tests on four different commercial farms were found to have substantially lower attack rates than those previously reported (Turner et al., 2010).

In order to respond to selection a trait must have sufficient heritability and genetic variation. Løvendahl et al. (2005) and Stukenborg et al. (2012) showed that aggressive behaviour in sows and growing pigs is lowly to moderately heritable (h^2 between 0.04 and 0.24), with higher heritabilities reported for the initiation, as opposed to the receipt, of aggression. As directly measuring aggression under a commercial setting is

impractical, a rapid, reliable method of identifying aggressive individuals is required. The measured trait must have sufficient heritability and variance, but also be phenotypically and genetically correlated with aggression. Skin lesions (Figure 1.1) have been used in several studies as an indirect measure of the amount of aggressive behaviour an individual pig has been involved in (Erhard et al., 1997; Francis et al., 1996; Turner et al., 2006). Skin lesions are an attractive candidate to phenotype aggression, as they are easy to identify, do not require specialist training to record, and take approximately 1 minute per animal to count. The duration of time spent in reciprocal fighting, and receiving unreciprocated attacks were shown to be significant determinants of skin lesions on a phenotypic level. It was also found that animals with a high proportion of skin lesions to the anterior region of the body spent more time involved in reciprocal aggression, while animals that received a higher proportion of lesions to the posterior regions of the body received many non-reciprocal attacks (Turner et al., 2006). This is because reciprocal aggression tends to involve opponents attacking each other head-on, while receipt of non-reciprocal aggression typically involves the recipient of an attack fleeing the aggressor.

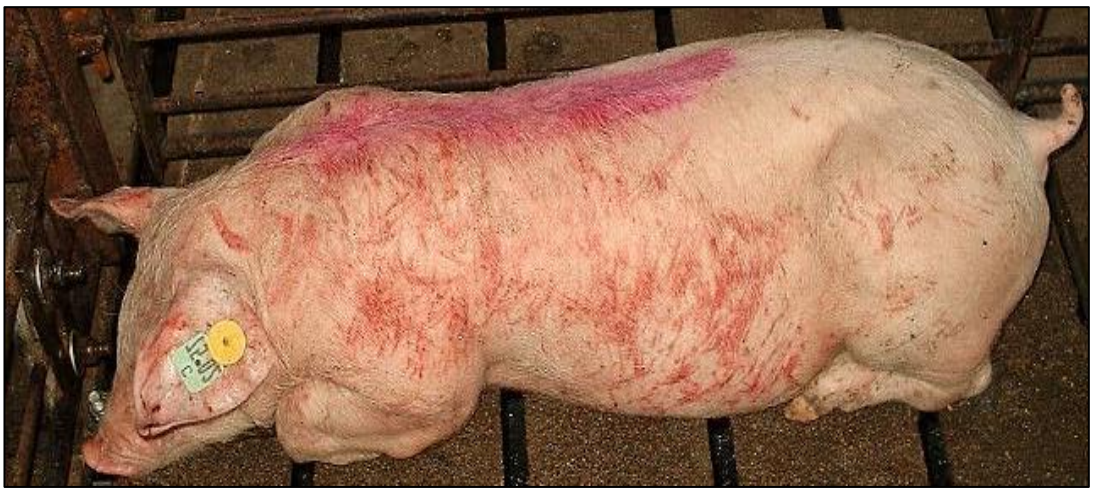

Figure 1.1 Skin lesions caused by aggressive interactions, following the mixing of unfamiliar individuals.

A further study by the same group estimated heritabilities of between 0.17 and 0.46 for skin lesions recorded 24 hours post-mixing, as well as genetic correlations between skin lesions and the initiation of non-reciprocal fighting and reciprocal fighting (r_g = 0.79) (Turner et al., 2008). The same study also found that involvement in, and initiation of reciprocal aggression was moderately heritable (h^2 between 0.37 and 0.46), while receipt of non-reciprocal aggression was lowly heritable (h^2 = 0.17). These results provided evidence that selection on lesions is likely result in responses to reciprocal and delivery of non-reciprocal aggression (Turner et al, 2008). A further study (Turner et al., 2009) reported heritabilites of three behavioural traits (reciprocal, delivery of non-reciprocal and receipt of non-reciprocal aggression), similar to those found in Turner et al. (2008) and Løvendahl et al. (2005). Heritability of skin lesions in this study was also similar to those reported in Turner et al. (2008). Central and posterior skin lesions were shown to be strongly genetically correlated with receipt of non-reciprocal aggression, but not with delivery or receipt of reciprocal aggression. The authors suggest that measuring central and posterior skin lesions would be a less ambiguous method of identifying bullied animals, while anterior skin lesions would be a reliable measure of involvement in reciprocal aggression (Turner et al., 2009). There was a positive correlation between the number of lesions on animals 24 hours and 3 weeks post mixing, indicating that selection using skin lesions will have long term effects. The same study included the pen in which the animals were mixed as a random effect in the statistical analysis, and found that on a group level the severity of lesions was lower in established groups which initially had the most prolonged fighting, suggesting that investment in aggressive behaviour early on may be beneficial in long term effects in forming more stable hierarchies (Turner et al., 2009). Genetic correlations between aggressive behaviour at mixing, and skin lesions recorded 3 weeks later were often in conflict with correlations between skin lesion traits at each

time point. For example, genetic correlations suggest that individuals that were involved with much reciprocal aggression at mixing were likely to have fewer anterior skin lesions 3 weeks post-mixing, while genetic correlations between skin lesions suggested that animals that receive many anterior lesions at mixing are more likely to have many anterior skin lesions under stable social conditions. This suggests that relationships between skin lesions and aggression under newly mixed and stable social conditions may not be straight forward.

1.5 Genetics of aggression

As outlined earlier, it is important to understand whether selection against aggression is likely to affect other important production traits, such as growth, feed efficiency, leanness of meat, meat eating quality and litter size, or positive behavioural traits such maternal behaviour, ease of handling and activity levels. The results of a well-known study initiated by Dmitry Belyaev in the 1950's illustrate how selection against fear and aggression towards humans can affect other, seemingly unrelated traits. Belyaev selected for tame and aggressive behaviour in a population of wild silver foxes (*Vulpes vulpes*), based on behavioural reactions to a human approach. As well as showing a marked change in behaviour, animals selected for tameness displayed physical changes such as coat colour and condition, floppy ears and curled tails, much like domestic dogs (Trut et al., 2009). Tame behaviour was also associated with a down-regulation of the hypothalamic-pituitary-adrenal (HPA) axis, a change in several neurotransmitters, including serotonin, and changes in reproductive behaviour (Trut et al., 2009). Social aggression is only one mode of aggression in pigs. Although the underlying motivation may be different, it is possible that social aggression is related to piglet savaging or tail biting, and that breeding for reduced aggression would also affect these traits, however no studies have investigated correlations between these behaviours. It is also possible that different aggressive behaviours do not share a genetic basis. For example

Naumenko et al., (1989) found that selecting for reduced human-directed aggression in Norway rats resulted in improvements in 'irritable aggression' but not in mouse killing or intermale aggression.

As discussed in more detail in a later section, it has been shown that the biological pathways regulating aggression are also involved in other biological systems, particularly those relating to stress responses. Stress and aggression both involve the HPA axis (Muráni et al., 2010), which can be activated via a wide range of stimuli. It is known that the HPA axis is involved in a wide range of physiological and behavioural responses under tight regulation, therefore it is feasible that selection for a trait controlled by this system could have consequences on other systems (Smith and Vale, 2006). Given that selection in numerous domestic species has shown us how other traits can be inadvertently changed, a more complete understanding of the underlying genetic basis of aggression and the biological pathways involved is required before selecting against the behaviour. There has been some progress made in this area in pigs, as discussed below.

1.6 Genetic correlations of aggression with other behavioural and production traits

Studies in cattle, pigs, and poultry have shown that selection for increased productivity has resulted in unintentional selection for undesirable physiological, immunological and reproductive traits, possibly to the detriment of animal welfare (Rauw et al., 1998). Aggressive and non-aggressive behaviour has been linked to individual coping strategies in other contexts (De Boer et al., 2003), and one argument against selecting against aggression is the concern that it will result in animals that simply have lower activity levels, or are less able to cope with their environment. D'Eath et al. (2009) investigated whether aggressive behaviour in pigs was genetically associated with

home pen activity levels, or reactions to human handling. There were no statistically significant genetic correlations found between home pen activity levels and aggression, however animals that engaged in high levels of aggression were found to have higher activity at weighing (i.e. moving in and out of the crate quickly and becoming restless when held in the crate), while pigs that received more bullying vocalised more while in the weighing crate. These results suggest that other behavioural traits may be affected by selecting against aggression, an idea supported by several genetic studies which show similar effects of aggression in mice (Nelson, et. al., 2006) and drosophila (Zwarts et al., 2011). Løvendahl et al. (2005a) found some evidence that aggressive sows were less responsive to screaming piglets than unaggressive sows, although these correlations were associated with high errors of estimation. This study did not consider infanticide, which is a form of aggression with a known genetic basis (Quilter et al., 2007), that may or may not be correlated with social aggression.

There have been few studies investigating the genetic relationship between aggression in pigs and production traits of interest. Jonsson (1985) estimated a heritability of 0.47 for social rank in boars, as determined by a feed competition test, however the estimated heritability for the same trait was found to be zero for gilts. The same study calculated negative genetic correlations between social rank (low number indicates high rank) and daily gain (r_G = -0.66) and positive genetic correlations between social rank and muscle - fat ratio (r_G = 0.40) in boars, suggesting that higher ranking boars grow faster and have leaner meat. The authors hypothesised that the presence of mature boars adjacent to the gilts may have affected aggressive behaviour in the gilts, therefore masking the genetic effects of aggression in females. It is worth noting that although aggression at mixing is believed to play an important role in forming social relationships, dominance is not always asserted via high levels of aggression, therefore

social rank is not a reliable alternative measure of aggressive behaviour (Francis, 1988). Torrey et al., (2001) found that pigs selected for increased loin area engaged in almost twice as much aggression in the first 5 hours post-mixing than a control line. The same study also found that animals from the selected line were generally more active in the first day post mixing, as they spent less time lying, more time exploring, and more time socially interacting with pen mates. These differences were not observed on the second day. A later study by Turner et al. (2006) found no significant positive relationship between skin lesions, growth rate and backfat depth in a sample of 658 pigs, while Velie et al. (2009) found no genetic correlation between latency to attack in a resident-intruder test, and average daily gain, backfat, or loin muscle area.

1.7 Neuroendocrine mechanisms of aggression

Due to its negative effect on human society, the neuroendocrine basis of aggression in various animal models has received much scientific interest. Serotonin, its receptors and associated pathways have received particular attention as low levels of serotonin have been demonstrated to correspond with heightened aggression in crustaceans (Edwards & Kravitz, 1997), insects (Dierick and Greenspan, 2007) and mammals, including pigs (D'Eath et al., 2005). Increased dietary tryptophan (a precursor of serotonin) has been shown to reduce aggression in a range of species (Poletto et al., 2010; Poeltto et al., 2014). One possible way in which serotonin modulates aggressive behaviour is via the peptide neurotransmitter neuropeptide Y (NPY). Neuropeptide Y is associated with feeding behaviour (Roehe et al., 2003), activity levels (Heilig and Murison, 1987) and aggression (Karl and Herzog, 2007), as well as learning and memory processing (Redrobe et al., 2004). Karl et al., (2004) suggest that the NPY-mediated modulation of serotonergic neurons is an important link between aggression and feeding behaviour, after finding that NPY (Y1) receptor knockout mice were more aggressive in a resident intruder test and show decreased levels of serotonin.

As discussed, various neurotransmitters have been implicated in aggression, however the extent to which they affect aggressive behaviour can also be dependent on gene by environment interactions, as reviewed by Anholt & Mackay (2012). For example, a variant in the monoamine oxidase A (MAOA) gene resulting in low levels of MOAO expression has been previously associated with increased aggression, however in both human and rhesus monkeys aversive early life experience greatly increased the chance of heightened aggression in individuals with the variant (Huang et al., 2004; Karere et al., 2009).

1.8 Present genomic knowledge of aggression

While several biogenic amines, hormones and neurotransmitters such as serotonin, MAOA, nitric oxide, dopamine, androgens and oestrogens (Kuepper et al., 2013; Popova, 2008; Våge et al., 2010; Zwarts et al., 2012) have been identified as playing an important role in aggression, this tells us little about the underlying genetic basis of this behaviour (Edwards et al., 2006). In pigs, mixing-mediated aggression has been shown to result in an up-regulation of adrenal transcripts involved in cholesterol accumulation and a down-regulation for functions associated with cell growth and death (Muráni et al., 2011). D'Eath et al. (2005) showed that aggressive and unaggressive individuals differed in their expression of vasopressin and serotonin 1A receptor mRNA in certain regions of the brain. Muráni et al. (2010) identified two genes within the glucocorticoid and arginine vasopressin receptors (NR3C1 and AVPR1B) that are likely to be involved in the stress response and aggressive behaviour in pigs. Terenina et al. (2012) found associations between aggressive behaviour, as measured via skin lesions, and 9 polymorphisms within genes regulating the serotonergic system. Oster et al. (2014a) used skin lesions recorded 24 hours post mixing (at 10 weeks of age) to assign animals to groups characterised by either high or low psychosocial stress. The relative change

in mRNA abundance was used to show that animals characterised as having high psychosocial stress had increased expression of genes related to tRNA charging, urea cycle, acute phase response, and oestrogen receptor signalling. These 'high stress' individuals also had decreased expression of catechol-O-methyltransferase (COMT), an enzyme previously linked to the regulation of aggressiveness and stress response in mice. In the same population of pigs (Oster et al., 2014b) female pigs with high stress response (characterised by skin lesions numbers, plasma cortisol level, and creatine kinase) had a higher expression of genes involved in immune system responses. The authors suggest that potential infections due to fight injuries may activate signal transduction, which in turn results in the increased expression of these genes. The authors propose that interactions between hormones and stress may explain why castrated males did not show the same response as female animals, as castration has been shown to increase cortisol, which can interrupt the negative feedback within the HPA axis and may affect immune system responses.

While several quantitative trait loci (QTL) have been identified for traits related to meat and carcass quality, health, reproduction, and productivity (Mohrmann et al., 2006; Ramos et al., 2009; Coster et al., 2012; Okamura et al., 2012), none of these QTL have been associated with aggressive behaviour. Several studies have attempted to identify QTL associated with aggression in other species; however, even in specifically selected lines under controlled conditions with limited environmental variation, identifying the underlying genes can be difficult (Albert et al., 2011). Studies using *Drosophila* (Edwards & Mackay, 2009), mice (Brodkin et al. 2002), and rats (Albert et al., 2008) have only uncovered a handful of QTL related to intraspecific and human-directed aggression. Discovering QTL via linkage analysis is best suited for traits under the control of few genes with major effects. They involve either studying the offspring

of an F2 generation, or closely related individuals, in which the broad range of natural variation seen in complex traits is underrepresented (Anholt & Mackay, 2012).

As outlined above, aggression has been associated with a wide range of biological processes, and it is likely that this behaviour is under the genetic control of many genes with small effect. Genome wide association studies compare allele frequencies between large numbers of case controls, and have the power to detect multiple alleles with a lesser effect than those detected by linkage analysis. High density single nucleotide polymorphism (SNP) panels have been developed for several species, allowing genome wide association analysis for SNPs correlated with complex traits. As of yet the 60k porcine chip has been used in very few behaviour studies, however it has been successfully used to identify SNPs associated with other traits such as boar taint (Duijvesteijn et al., 2010) and reproductive traits (Onteru et al., 2011). While genome wide studies alone are unlikely to explain the entire underlying genetic basis of aggression, they do provide an opportunity to identify genomic regions associated with a trait, and in turn search for genes and biological pathways that may be related to aggressive behaviour. To date only one GWAS has been conducted on aggression in a relatively small sample of 552 pigs (Pong-Wong et al., 2010), however no SNPs were found to be associated with either aggressive behaviour or skin lesions.

1.9 Thesis outline and main objectives

Previous research has shown that selection against aggression is possible, and that skin lesions provide a promising method of phenotyping large numbers of aggressive individuals relatively quickly. Although the most intense aggressive behaviour is observed within the first 24 hours post mixing, aggression does persist under socially stable conditions, with variation observed between individuals and groups. Despite the

large number of studies focusing on aggression in the hours and days after pigs are first mixed there are very few studies to date attempting to explain and quantify the behavioural transition from immediately after mixing to stable groups. This is important to consider, as growing pigs and pregnant sows are often socially housed for several weeks or months after mixing, and it is possible that persistent aggression under socially stable conditions may lead to chronically stressed or unproductive animals. It is therefore important to explore the relationship of aggressive behaviour between these two social conditions in more detail, before we can be confident that a particular genetic selection strategy will improve pig production and welfare overall, and not just at mixing. Chapter 2 investigates whether there is evidence that increased aggression at mixing results in greater social stability in the long term on a phenotypic level, by exploring the relationship between aggression at mixing and skin lesions recorded 3 weeks later. While genetic correlations between aggressive behaviour and skin lesions have been shown, these correlations are often associated with a high degree of error, and no studies have attempted to quantify the reduction in aggressive behaviour via selection. Chapter 3 aims to quantify this for the first time. Chapters 2 and 3 each utilised a previously recorded dataset of highly detailed behavioural interactions recorded in the first 24 hours post mixing, as well as skin lesion data recorded 24 hours and 3 weeks post-mixing. For the remaining chapters, data were collected during the course of this PhD.

This literature review has explored the wide range of interactions reported between aggressive behaviour and other behavioural and production traits, both on a phenotypic and genetic level. If selection against social aggression is to be successful, it is important that this does not come at the expense of other traits that might affect the welfare or productivity of subsequent generations. To investigate this further, skin

lesions were recorded 24 hours and 5 weeks post mixing on almost 2,500 individuals over a 6 month period from a commercial PIC herd. Information on growth, feed intake, and carcass characteristics were provided for these animals, and genetic correlations between these performance traits and skin lesions traits were estimated, and form the basis of Chapter 4. Behavioural tests designed to measure fear responses were performed using a subset of approximately 2,000 animals. Heritabilities of the behavioural traits measured, as well as genetic correlations between these behaviours and skin lesions were calculated, and these results form the basis of Chapter 5. Finally, the literature review highlighted that there is much yet to be understood about the genomic basis of social aggressive behaviour. In order to address this, a subset of 1,840 individuals were SNP genotyped, and a series of genome wide association studies performed, in order to detect SNPs associated with skin lesion traits recorded 24 hours post mixing and 5 weeks post mixing. The results of these studies are presented and discussed in Chapter 6.

Chapter 2 - Analysis of the phenotypic link between behavioural traits at mixing and increased long-term social stability in group-housed pigs

The data used are from a previous project funded by the European Union Sixth Framework Programme and Scottish Government. Statistical analysis and manuscript preparation was carried out by S Desire.

Adapted from: S Desire, S P Turner, R B D'Eath, A B Doeschl-Wilson, C R G Lewis, R Roehe. 2015 Analysis of the phenotypic link between behavioural traits at mixing and increased long-term social stability in group-housed pigs. Appl. Anim. Behav. Sci. 166: 52-62.

2.1 Introduction

In commercial farming, once pigs are mixed for growing they will usually remain in these groups for several months until regrouped again or marketed. As aggression serves to establish dominance hierarchies, it is possible that increased aggression upon first mixing may actually lead to more stable dominance relationships in the long-term. Indeed, there is some evidence that initial increased aggression at mixing results in lower aggression and improved productivity over the entire growing-finishing period (Canario et al., 2012; D'Eath, 2005; Turner et al., 2009). If this is the case, aggressiveness at mixing would be essential to improved long term welfare and production. If reduced aggression in new social groups is found to be detrimental to long-term group stability, then it will be important to quantify any continual welfare or production concerns that arise as a consequence of reducing mixing aggression.

Many pig aggression studies use information taken from small group sizes or staged interactions between individuals. Often they focus on one aspect of aggression, for example the effects of body weight or previous fight success (Andersen et al. , 2000; Francis et al., 1996). This study utilises a dataset comprised of extremely detailed behavioural observations taken from more than 1,100 animals under commercially relevant conditions in the first 24 hours post mixing. This has provided an opportunity to study the behavioural repertoire of the pig when placed in an unstable social environment, with no human interference. These behavioural traits were compared to skin lesions at mixing (SL24h) and in the social stable group, 3 weeks following mixing (SL3wk). The aim of the study was to determine whether there is a phenotypic link between aggression at mixing and increased long-term group stability in the form of reduced skin lesions, and if so, to identify mixing behaviours that improve long-term

social behaviour. In particular it was of interest to identify specific behaviours associated with skin lesions at mixing and three weeks post mixing.

2.2 Methods

2.2.1 Animals and housing

The study comprised 1,166 pigs on a commercial farm in Ransta, Sweden, between October 2005 and January 2007. Information gathered on all individuals included pen identity, sex, breed, litter identity, and unique pig identification (ear tag or notch number). Single sex (intact males, castrated males, and females) and single breed (703 purebred Yorkshire and 463 crossbred Yorkshire x Landrace) groups of 15 were created by mixing 3 pigs from 5 different litters, resulting in 78 groups. Efforts were made to standardise within-pen variation in body weight across groups. Animals were weighed 24 hours post-mixing and had an average live weight of 27.6 kg (SD 5.6) and an average age of 72 days (SD 4.3). Pigs were housed in 4.0 x 3.2 m partially slatted pens (30% slats, 70% lightly bedded solid flooring) with a floor space allowance of 0.85 m^2 per pig. Pigs were fed dry pelleted food *ad libitum* from a single space feeder and had constant access to water via a nipple drinker.

2.2.2 Skin lesion traits

Lesions were counted immediately prior to mixing, and again 24 hours post-mixing by a single observer, and were grouped by location on the body: anterior (head, neck, front legs, and shoulders), central (flanks and back), posterior (rump, hind legs, and tail). The pre-mixing lesion count was subtracted from that taken 24 hours post-mixing for each pig. This served to ensure that only those lesions that occurred as a result of mixing aggression (SL24h) were included in all analyses. Recently received lesions were counted again three weeks post-mixing (90 days (SL3wk), SD 5.2). One uninterrupted scratch was classed as a single lesion, regardless of length or severity. A lesion was considered to be recent if it was vivid red in colour or recently scabbed.

2.2.3 Behavioural traits

Groups were video recorded for 24 hours post-mixing. The time, duration (s), and outcome of reciprocal (RA) and non-reciprocal (NRA) aggression was recorded. Reciprocal aggression was defined as a fight that lasted more than one second where both pigs were involved in pushing, head knocking or biting. Non-reciprocal aggression involved the delivery of these behaviours with no retaliation from the receiver. Non-reciprocal aggression could occur as a unique event independent of a reciprocal fight, as a component of a reciprocal fight, or at the end of a reciprocal fight as the loser retreated. For each fight observers also recorded the duration of time spent engaged in injurious fighting. This is opposed to behaviour such as pushing, head knocking, or chasing, which were not deemed injurious. These basic data were used to derive quantitative aggressive behavioural traits that were used in the statistical analyses in the current study (Table 2.1). Three observers used time-lapse video equipment to extract the duration of each behavioural bout to the nearest second. Analysis of three 1-hour samples of data showed a significant degree of inter-observer agreement ($r = 0.83$, $P < 0.001$) (Turner et al. 2009).

Table 2.1 Definitions of skin lesion traits and behavioural traits used in the analyses

Trait	Description
Skin lesions at 24 hours (SL24h)	Number of skin lesions counted 24 hours post mixing
Skin lesions at three weeks (SL3wk)	Number of skin lesions counted 3 weeks post mixing (stable groups)
Reciprocal aggression (RA)	A fight lasting >1s in which the recipient of the attack retaliated
Non-reciprocal aggression (NRA)	An attack in which the recipient did not retaliate
RA involved with	Total number of reciprocal fights the focal pig was involved with, regardless of which pig initiated the attack
NRA involved with	Total number of non-reciprocal fights the focal pig was involved with, regardless of which pig initiated the attack
Total RA initiated/received	The total number of times an individual initiated or was the recipient of an attack which was reciprocated
Total NRA initiated/received	The total number of times an individual initiated or was the recipient of an attack which was not-reciprocated
Number of pen mates focal pig attacked (RA)	The number of pen mates the focal pig attacked in which the attack was reciprocated
Number of pigs attacked by (RA)	The number of pen mates the focal pig was attacked by which the focal pig retaliated against
Number of pen mates focal pig bullied	The number of pen mates the focal pig attacked which did not reciprocate
Number of pen mates bullied by	The number of pen mates the focal pig was attacked by which it did not reciprocate against
Pen mates involved with	Total number of pen mates with which the focal pig had any aggressive interactions
Average duration RA & NRA involved (s)	Average duration of all aggressive encounters in which the focal pig was involved
Duration of RA initiated (s)	Duration of time spent in RA in which the focal pig was the initiator
Duration of RA received (s)	Duration of time spent in RA in which the focal pig was the recipient of the attack

Trait	Description
Duration of NRA initiated (s)	Duration of time spent in NRA in which the focal pig was the initiator
Duration of NRA received (s)	Duration of time spent in NRA in which the focal pig was the recipient of the attack
Proportion of fights won	Proportion of all reciprocal fights which the focal pig won
Proportion of repeated fights	Proportion of all pen mates fought with which the focal pig had more than one aggressive interaction
Proportion with ambiguous outcome	Proportion of reciprocal fights the focal pig was involved with in which the winner could not be determined
Proportion injurious RA involved with	Proportion of time the focal pig spent in reciprocal fights engaged in what was deemed to be injurious fighting

2.2.4 Characteristics of the data

Skin lesion and behavioural data were available for all 1,166 animals in 78 groups. Mixed animals had an average growth rate of 881 g/day over an 86 day (SD = 4) growth period (Yorkshire: 880 g/day, SD 155; Yorkshire x Landrace: 881 g/day, SD 186). The average weight of pigs at the time of mixing was 27 kg (Yorkshire: 27 kg, SD 5.1]; Yorkshire x Landrace: 29 kg SD 5.4) and the average weight at the end of the finishing period was 104 kg (Yorkshire: 103 kg, SD 11.24; Yorkshire x Landrace: 106 kg, SD 12.49). The characteristics of the skin lesion data for the variables used in the analyses are presented in Table 2.2, while this information is presented in Table 2.3 for behavioural traits. Negative values for skin lesions at 24 hours post mixing are partly due to observer error and partly due to lesions healing between pre and post mixing lesion number counts. Within further analysis of the data, these negative values were set to zero. The lesion numbers and behavioural traits showed skewed distributions (Table 2.2); therefore the data were log transformed (y = LS + 1) and the transformed values were used in all subsequent analyses.

Table 2.2 Characteristics of skin lesion data for individual animals included in the statistical analysis (SK = skewness; K = kurtosis)

	Original scale				Log transformed scale			
Trait	**Min-Max**	**Mean (SD)**	**SK**	**K**	**Mean**	**SD**	**SK**	**K**
Anterior SL24h	-17 to 99	18.84 (17.32)	1.38	2.31	2.56	1.09	-0.88	0.34
Central SL24h	-30 to 100	10.71 (12.02)	1.42	5.99	2.05	1.10	-0.63	-0.55
Posterior SL24h	-42 to 41	3.70 (8.26)	-0.72	4.12	1.36	1.02	-0.11	-1.30
Anterior SL3wk	0 - 63	10.40 (5.63)	1.57	8.67	2.30	-1.13	-1.13	2.60
Central SL3wk	0 - 40	10.35 (5.94)	1.03	1.86	2.28	0.60	-0.93	1.58
Posterior SL3wk	0 - 30	4.51 (3.51)	1.21	3.07	1.48	0.71	-0.51	-0.35

Table 2.3 Characteristics of behavioural data for individual animals included in the statistical analysis (SK = skewness; K = kurtosis)

	Original scale				Log transformed scale			
Trait	**Min-Max**	**Mean (SD)**	**SK**	**K**	**Mean**	**SD**	**SK**	**K**
RA involved with	0 - 56	8.36 (7.14)	1.37	3.05	1.90	0.90	-0.58	-0.38
NRA involved with	0 - 69	7.65 (6.95)	2.86	15.27	1.89	0.75	-0.29	0.23
Total RA initiated	0 - 36	4.19 (4.29)	1.76	4.99	1.32	0.85	-0.13	-0.90
Total RA received	0 - 25	4.17 (3.77)	1.44	3.06	1.36	0.79	-0.31	-0.74
Total NRA initiated	0 - 66	3.84 (5.54)	3.84	25.9	1.14	0.91	0.33	-0.70
Total NRA received	0 - 25	3.81 (3.17)	1.57	3.89	1.36	0.67	-0.26	-0.28
Number of pen mates focal pig attacked (RA)	0 - 11	2.84 (2.32)	0.66	-0.19	1.13	0.69	-0.38	-0.96
Number of pigs attacked by (RA)	0 - 9	2.84 (2.06)	2.06	-0.52	1.17	0.64	-0.57	-0.69
Number of pen mates focal pig bullied	0 - 14	2.56 (2.68)	1.32	1.55	0.99	0.75	0.06	-1.10
Number of pen mates focal pig bullied by	0 - 9	2.56 (1.67)	1.67	0.09	1.15	0.52	-0.56	-0.10
Pen mates involved with	0 - 14	6.67 (3.06)	0.02	-0.67	1.94	0.49	-1.12	1.43
Average duration of NA & NRA involved (s)	1 - 249	42.48 (27.82)	2.04	8.33	3.58	0.64	-0.38	0.45
Duration of RA initiated	0 - 2394	286.26 (364.26)	2.09	5.41	4.27	2.34	-0.87	-0.56
Duration of RA received	0 - 2997	326.45 (351.62)	2.09	6.68	5.08	1.46	-1.09	1.49
Duration of NRA received	0 - 996	41.61 (68.46)	2.87	13.79	3.11	1.34	-0.88	0.32
Duration of NRA initiated	0 - 444	41.29 (46.46)	4.63	40.84	2.52	1.82	-0.19	-1.27
Proportion of fights won	0 - 1	0.30 (0.25)	0.57	-0.22	0.25	0.19	0.22	-0.82
Proportion of repeated fights	0 - 1	0.50 (0.25)	-0.34	-0.35	0.39	0.18	-0.74	0.02
Proportion with ambiguous outcome	0 - 1	0.27 (0.24)	0.87	0.67	0.22	0.18	0.44	-0.37
Proportion injurious	0 - 1	0.59 (0.24)	-1.07	0.76	0.45	0.17	-1.46	1.66

2.2.5 Statistical Analyses

To account for systematic influences on behavioural traits and skin lesions, the effects of breed type (purebred Yorkshire, Yorkshire x Landrace), sex (females, males, and castrates) and experimental batch (pigs were mixed on 14 separate days) were fitted as fixed effects, and body weight as a covariate in the statistical models. The group effect was modelled by including the pen in which the animals were mixed as a random effect. The analysis was carried out using the MIXED procedure of SAS (version 9.1). To predict the individual animal associations and to identify the change in aggression of animals over time, Pearson correlations were obtained between the residuals of all behavioural traits and SL24h with SL3wks. Aggression is often discussed in terms of the individual animal, however pigs are housed in social groups, and the welfare of an individual is likely to be greatly affected by the level of social stability within the group in which it is housed. In order to compare group-level associations between behaviour and lesion numbers, correlations between estimates of pen effects were calculated.

To further explore the relationship between aggression at mixing and skin lesions for individual animals at two time points, a multiple linear regression model was developed that resulted in the best model to predict lesion numbers from a set of behavioural traits. A series of multiple stepwise regression analyses using the REG procedure of SAS (version 9.1) were performed, in which the estimated residuals for lesion and behavioural traits from the initial mixed models for SL24h and SL3wk were set as response variables, and residuals for all behavioural traits were set as predictors. Behaviour traits explaining significant variance in lesion numbers ($P < 0.05$), as predicted by the regression analyses, were included in the final model. Many behavioural traits may be correlated among each other; therefore multicollinearity between behavioural variables included in the final model was estimated using

variance inflation factors (VIF); however no VIF were above 1.38, suggesting that multicollinearity was not a concern. Using residuals of behavioural traits, the final model produced regression coefficients that predicted how various behaviours influenced lesion numbers at both time points, independent of the systematic effects described above.

2.3 Results

There were large variations among pen group means of SL24h, suggesting that groups differed significantly in levels of aggression. There was less variation among pen group means for SL3wk than SL24h. However the averages of all pen group means for SL24h were similar to SL3wk at the centre or posterior of the body but not for those observed at the anterior area (Table 2.4). The distribution of pen group means of skin lesions was approximately normal, as assessed by skewness and kurtosis (Table 2.4).

Table 2.4 Mean group skin lesion numbers for groups with the highest and lowest 10% of skin lesions recorded 24 hours (SL24h) and 3 weeks (SL3wk) post mixing (SK = skewness; K = kurtosis)

Trait	Lowest 10% group means	Average over all group means (*SD*)	Highest 10 % group means	SK	K
SL24h					
Anterior	9.02	19.06 (6.54)	32.08	0.81	2.54
Central	4.84	11.39 (5.11)	21.37	0.79	0.00
Posterior	0.69	5.16 (3.08)	10.93	0.46	-0.66
SL3wk					
Anterior	6.77	10.40 (2.21)	14.34	-0.53	0.02
Central	6.28	10.34 (2.58)	15.37	-0.64	0.86
Posterior	1.79	4.51 (1.62)	7.46	-0.85	0.86

2.3.1 Fixed and random effects on skin lesions

Batch and breed type*sex were included in the mixed models as fixed class effects while body weight at mixing was included as a covariate. Batch effects were statistically significant for almost all lesion traits except for anterior and central SL24h (posterior SL24h: $F = 8.59$; $P < 0.001$; anterior SL3wk: $F = 5.25$, $P < 0.001$; central SL3wk: $F = 7.70$; $P < 0.001$; posterior SL3wk: $F = 5.72$, $P < 0.001$). Breed type*sex affected anterior and central SL3wk (anterior $F = 5.25$; $P < 0.001$; central $F = 3.12$; $P = 0.014$). Crossbreed

females received significantly fewer anterior and central SL3wk ($P < 0.05$) than purebred females. Anterior and posterior SL24h showed significant regression coefficients ($P < 0.001$ and $P = 0.046$, respectively) on body weight at mixing.

2.3.2 Lesion numbers

The proportions of the phenotypic variance attributed to pen effects were significant ($P < 0.05$) in the range from 4 to 12% and 3 to 21% for skin lesions (on the diagonal of Table 2.5) and most behavioural traits, respectively (Table 2.6). On the pen group level, lesions across body regions at the same time point were positively correlated (SL24h: 0.28 to 0.77; $P < 0.01$, SL3wk: 0.65 to 0.75; $P < 0.001$). Between time points, anterior or central pen group SL24h were positively correlated with anterior or central SL3wk (0.24 to 0.36; $P < 0.05$). Lesions to the central region of the body were also positively correlated on a pen group level across time points (0.24; $P < 0.05$) (above diagonal, Table 2.5).

At the individual animal level, lesions across body regions recorded at the same time point were positively correlated for both SL24h (0.38 to 0.54; $P < 0.001$), and SL3wk (0.50 to 0.65; $P < 0.001$). Between these time points, there were significant but small positive correlations between central (0.07; $P < 0.05$) or posterior (0.07; $P < 0.05$) SL24h and anterior SL3wk. In contrast, there was a small negative but significant correlation between anterior SL24h and central SL3wk (-0.06; $P < 0.05$) (below diagonal, Table 2.5)

Table 2.5 Phenotypic proportions of skin lesion number (SL) variance attributable to pen group effects (on diagonal in bold) and the correlation between pen group effects (above diagonal), and individual animal (residual) correlations (below diagonal) between lesion numbers recorded 24 hours post mixing and three weeks post mixing[a]

	SL24h			SL3wk		
Trait	**Anterior**	**Central**	**Posterior**	**Anterior**	**Central**	**Posterior**
SL24h						
Anterior	***0.08 *****	0.45 ***	0.28 **	-0.07	-0.09	0.06
Central	0.53 ***	***0.11 *****	0.77 ***	0.20	0.24 *	0.11
Posterior	0.38 ***	0.54 ***	***0.12 *****	0.36 ***	0.32 **	0.19
SL3wk						
Anterior	-0.00	0.07 *	0.07 *	***0.04 ****	0.65 ***	0.69 ***
Central	-0.06 *	0.00	0.01	0.65 ***	***0.09 *****	0.75 ***
Posterior	-0.02	0.01	0.04	0.50 ***	0.58 ***	***0.07 *****

[a] Residual correlation after accounting for all systematic effects and the group (pen) effects.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2.6 Phenotypic proportions of behavioural variance attributed to pen group effects (column 1), and correlations of estimates of pen group effects between aggressive behaviour and skin lesion numbers recorded 24 hours (SL24h) and 3 weeks (SL3wk) post-mixing.

		SL24h			SL3wk		
Trait	**Proportion of pen variance**	**Anterior**	**Central**	**Posterior**	**Anterior**	**Central**	**Posterior**
RA involved with	0.10 ***	0.61 ***	-0.04	-0.19	-0.21	-0.33 **	-0.10
NRA involved with	0.21 ***	0.09	0.14	0.16	0.03	-0.11	-0.10
Total RA initiated	0.06 **	0.59 ***	-0.01	-0.17	-0.23 *	-0.32 **	-0.10
Total RA received	0.10 ***	0.63 ***	-0.03	-0.18	-0.22	-0.33 **	-0.09
Total NRA initiated	0.04 *	0.21	0.08	0.08	-0.06	-0.20	-0.15
Total NRA received	0.20 ***	0.07	0.22	0.24 *	0.10	-0.01	-0.05
Number of pen mates attacked (RA)	0.07 **	0.58 ***	-0.04	-0.17	-0.21	-0.30 **	-0.08
Number of pigs attacked by (RA)	0.10 ***	0.60 ***	-0.07	-0.18	-0.20	-0.30 **	-0.07
Number of pen mates focal pig bullied	0.03 *	0.23 *	0.08	0.08	-0.10	-0.21	-0.21
Number of pen mates bullied by	0.15 ***	0.12	0.22	0.21	0.02	-0.05	-0.13
Pen mates involved with	0.12 ***	0.41 ***	0.06	0.02	-0.14	-0.26 *	-0.20
Average duration of RA & NRA involved (s)	0.17 ***	0.37 ***	0.03	-0.08	-0.18	-0.14	-0.11
Duration of RA initiated (s)	0.05 **	0.55 ***	0.01	-0.12	-0.18	-0.26 *	-0.06
Duration of RA received (s)	0.08 **	0.57 ***	0.10	0.01	-0.24 *	-0.24 *	-0.14
Duration of NRA initiated (s)	0.03	0.21	0.08	0.07	-0.10	-0.21	-0.23 *
Duration of NRA received (s)	0.13 ***	0.07	0.23 *	0.23 *	0.07	0.01	-0.10
Proportion of fights won	0.03	0.23 *	-0.13	-0.18	-0.24 *	-0.28 **	-0.11
Proportion of repeated fights	0.05 **	0.23 *	-0.01	-0.07	-0.10	-0.15	0.01
Proportion with ambiguous outcome	0.11 ***	0.00	0.01	0.06	0.13	0.08	0.02
Proportion injurious	0.07 **	0.37 ***	-0.14	-0.27 **	-0.12	-0.09	0.10

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

2.3.3 Correlations between behavioural and lesion traits on group (pen) level

Pen group correlations between behavioural and lesion traits are presented in Table 2.6. The aggressive behavioural traits showed mostly significant positive correlations with SL24h (0.23 to 0.61; $P < 0.05$), except for the trait proportion of injurious fights, which was negatively correlated with the posterior region at 24 hours (-0.27; $P < 0.01$). In contrast, significant correlations of behavioural traits with SL3wk were consistently negative (-0.23 to -0.33; $P < 0.05$). Between pen groups, behavioural traits were primarily associated with skin lesions to the anterior regions of the body at 24 hours post-mixing, and to the central region of the body at 3 weeks post mixing. In addition, most significant correlations were found for behavioural traits that were defined as reciprocal aggression, with the exception of total non-reciprocal aggression received (0.24; $P < 0.05$) which positively correlated with the posterior SL24h, and duration of non-reciprocal aggression received, which positively correlated with central (0.23; $P < 0.05$) and posterior (0.23; $P < 0.05$) SL24h (Table 2.6).

2.3.4 Correlations between behavioural and lesion traits on the individual animal level

Under unstable social conditions at mixing, all behavioural traits included in the analysis showed positive correlations with anterior SL24h (0.13 to 0.56; $P < 0.001$) (Table 2.7). Except for the behavioural trait proportion of fights won, all other analysed behavioural traits were positively correlated with central SL24h (0.08 to 0.33; $P < 0.01$) but mostly at a lower magnitude than those of anterior lesions. Even lower correlations were calculated between behavioural traits and posterior SL24h (0.06 to 0.22; $P < 0.05$). The direction of these correlations indicates that individuals that are involved in more aggression at mixing received more SL24h, in particular to the anterior body region.

Many measures of aggressive behaviours at mixing correlated negatively with anterior and central SL3wk but at a lower magnitude than at those found at 24 hours (-0.07 to -0.18; $P < 0.05$). The behavioural traits number of RA involved with, the duration of RA and NRA initiated, and the average fight duration, showed the largest negative correlation with central SL3wk. The behavioural traits total NRA received, number of pen mates bullied by, and the proportion of fights with an ambiguous outcome were not associated with the number of anterior or central SL3wk. The duration of NRA received was negatively associated with central but not anterior or posterior SL3wk (Table 2.7).

Table 2.7 Correlations(a) between estimates of aggressive behaviour and skin lesion numbers recorded 24 hours (SL24h) and 3 weeks (SL3wk) post-mixing at the individual animal level.

	SL24h			SL3wk		
Trait	**Anterior**	**Central**	**Posterior**	**Anterior**	**Central**	**Posterior**
RA involved with	0.56 ***	0.32 ***	0.20 ***	-0.14 ***	-0.18 ***	-0.08 **
NRA involved with	0.34 ***	0.25 ***	0.15 ***	-0.09 **	-0.09 **	-0.05
Total RA initiated	0.43 ***	0.22 ***	0.13 ***	-0.12 ***	-0.14 ***	-0.05
Total RA received	0.48 ***	0.29 ***	0.17 ***	-0.10 ***	-0.16 ***	-0.08 **
Total NRA initiated	0.28 ***	0.17 ***	0.08 **	-0.12 ***	-0.15 ***	-0.07 *
Total NRA received	0.18 ***	0.20 ***	0.18 ***	0.03	0.05	0.00
Number of pigs attacked (RA)	0.50 ***	0.32 ***	0.20 ***	-0.11 ***	-0.15 ***	-0.09 **
Number of pigs attacked by (RA)	0.50 ***	0.32 ***	0.20 ***	-0.11 ***	-0.15 ***	-0.09 **
Number of pen mates focal pig bullied	0.30 ***	0.19 ***	0.09 **	-0.12 ***	-0.16 ***	-0.08 **
Number of pen mates bullied by	0.21 ***	0.20 ***	0.17 ***	0.03	0.04	-0.02
Pen mates involved with	0.48 ***	0.29 ***	0.17 ***	-0.14 ***	-0.15 ***	-0.09 **
Average duration of RA & NRA involved (s)	0.48 ***	0.23 ***	0.18 ***	-0.07 *	-0.12 ***	-0.09 **
Duration of RA initiated (s)	0.49 ***	0.23 ***	0.14 ***	-0.14 ***	-0.17 ***	-0.07 **
Duration of RA received (s)	0.54 ***	0.33 ***	0.22 ***	-0.10 ***	-0.14 ***	-0.10 ***
Duration of NRA initiated (s)	0.29 ***	0.17 ***	0.10 ***	-0.12 ***	-0.17 ***	-0.09 **
Duration of NRA received (s)	0.23 ***	0.22 ***	0.21 ***	0.04	0.08 **	0.00
Proportion of fights won	0.13 ***	-0.05	-0.07 *	-0.12 ***	-0.13 ***	-0.05
Proportion of repeated fights	0.35 ***	0.21 ***	0.16 ***	-0.08 **	-0.08 **	-0.04
Proportion with ambiguous outcome	0.18 ***	0.08 **	0.06 *	0.03	-0.01	0.00
Proportion injurious	0.30 ***	0.12 ***	0.08 **	-0.12 ***	-0.13 ***	-0.03

[a] Residual correlation after accounting for all systematic effects and the group (pen) effects.

*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

2.3.5 Best model for prediction of lesion numbers

Of all skin lesion traits, anterior SL24h showed the highest predictability by aggressive behavioural traits (R^2 = 0.36) (Table 2.8). Central SL24h were affected by the greatest number of behavioural traits. As found with the residual correlations, the regression model predicted a positive association between aggressive behavioural traits at mixing and SL24h, with the exception of the trait proportion of fights won, which was associated with slightly fewer central ($P < 0.001$) and posterior ($P = 0.015$) SL24h. At three weeks, only lesions to the central region of the body could be predicted by aggressive behavioural traits at mixing, however the R^2 value was low. The model predicted a negative association between traits of aggression, with the exception of the duration of NRA received, which was associated with slightly more SL3wk ($P < 0.001$) (Table 2.8).

Almost all behavioural traits included in all prediction models were significantly and positively correlated with each other (0.06 to 0.93; $P < 0.05$). The proportion of fights won was slightly negatively correlated with the total number of NRA received (-0.10; $P < 0.001$) and the duration of NRA received (-0.10; $P < 0.001$). There was no statistically significant correlation between the average duration of RA and NRA involved with and total number of NRA received (Table 2.9). However, highly correlated behavioural traits were not selected for each prediction model by the stepwise regression analysis so that multicollinearity was not a concern.

Table 2.8 Regression model predicting skin lesions recorded 24 hours (SL24h) and 3 weeks (SL3wk) post-mixing from aggressive behavioural traits based on the individual animal information

Skin lesions predicted by	*P* Value	Regression coefficient (SE)	Cumulative R^2 [a]
SL24h			
Anterior			
RA involved with	<0.001	0.47 (0.04)	0.30
Average duration of RA & NRA involved (s)	<0.001	0.53 (0.05)	0.35
Total NRA received	<0.001	0.21 (0.04)	0.36
Central			
Number of pigs attacked by (RA)	<0.001	0.34 (0.07)	0.08
Total NRA received	<0.001	0.30 (0.05)	0.10
Average duration of RA & NRA involved (s)	<0.001	0.32 (0.06)	0.12
Proportion of fights won	0.001	-0.69 (0.17)	0.13
Number of pen mates focal pig bullied	0.002	0.15 (0.05)	0.13
Posterior			
Duration of NRA received (s)	<0.001	0.11 (0.02)	0.04
Average duration of RA & NRA involved (s)	<0.001	0.17 (0.06)	0.05
Proportion of fights won	0.015	-0.51 (0.15)	0.06
RA involved with	<0.001	0.15 (0.04)	0.07
SL3wk			
Central			
Duration of NRA initiated (s)	<0.001	-0.04 (0.01)	0.02
Duration of NRA received (s)	<0.001	0.05 (0.01)	0.03
Average duration of RA & NRA involved (s)	<0.001	-0.11 (0.03)	0.04
Proportion of fights won	0.044	-0.20 (0.10)	0.05

[a] For each body region, cumulative R^2 values represent the proportion of the total phenotypic variance explained by the corresponding predictor in addition to predictors listed in previous rows of the table.

Table 2.9 Residual correlations between estimates of aggressive behaviours included in final models, as presented in Table 7

	Trait	b	c	d	e	f	g	h
a	RA involved with	0.52 ***	0.19 ***	0.84 ***	0.37 ***	0.65 ***	0.19 ***	0.61 ***
b	Average duration of RA & NRA involved (s)		0.01	0.46 ***	0.20 ***	0.14 ***	0.13 ***	0.16 ***
c	Total NRA received			0.16 ***	-0.10 ***	0.06 *	0.88 ***	0.07 **
d	Number of pigs attacked by (RA)				0.18 ***	0.49 ***	0.17 ***	0.46 ***
e	Proportion of fights won					0.32 ***	-0.10 ***	0.29 ***
f	Number of pen mates focal pig bullied						0.07 *	0.93 ***
g	Duration of NRA received (s)							0.07 *
h	Duration of NRA initiated (s)							

*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

2.4 Discussion

2.4.1 Behaviour and skin lesions on group level

Aggression can be defined at the level of the individual animal or at a group level. Numbers of lesions have been previously validated as a method of measuring the aggressiveness of individual pigs at mixing (Turner et al., 2006[a]) but not as a measure of aggression across entire groups. The current data set was an ideal opportunity to study the group level basis to skin lesions. The direction of the correlations indicates that increased group level involvement in reciprocal aggression, involving more pen mates, resulted in higher average anterior SL24h. Pen level correlations between aggression and lesions at mixing suggest that skin lesions are a useful measure of reciprocal aggression at mixing within a group, but only for anterior regions, which have previously been linked to reciprocal aggression in individuals (Turner et al., 2008).

At the group level, behavioural variables that were positively associated with anterior SL24h were negatively associated with SL3wk; however this relationship was mainly significant for the central body region only. The vigorous, reciprocal aggression that accounts for many anterior lesions at mixing does not often occur in stable groups. Instead, aggression in stable groups is primarily seen in the form of head knocks and bites, often over a resource (Bolhuis et al., 2005), which could explain why a relationship was mainly found for the central region of the body.

Very few traits relating to non-reciprocal aggression were associated with skin lesions on a group level, suggesting that skin lesions are not a useful measure of the amount of non-reciprocal aggression a group has been involved in. The majority of behaviour at

mixing related to anterior SL24h on a group level, whereas lesions from non-reciprocal aggression are more likely to be inflicted to the centre and posterior region of the body as the recipient is often turned away from the attacker, as it attempts to escape. This is reflected in the group level correlations, as the number and duration of non-reciprocal aggression received were positively correlated with central and posterior SL24h.

If skin lesions are a reflection of the amount of aggression within a group, it would be expected that few skin lesions would indicate increased social stability within that group. If increased aggression at mixing leads to stable group structures, it was of interest to determine whether there were any behavioural indicators that predicted increased stability (in the form of reduced skin lesions). With this in mind, a total of 20 behavioural traits were defined for the purposes of this analysis. Several hypothesised scenarios were considered during the design of this study:

1. Number of pen mates fought: if animals have experience of aggression with many group members this might enable effective mutual assessment of fighting ability, and result in reduced aggression in the long term.
2. Repeated fights: It was observed in the data that pairs of animals often repeatedly engaged in aggression with one another. It may be expected that several repeated fights indicates uncertainty over social standing and result in social instability in the long term.
3. Ambiguous fight outcomes: As explained in point 2 above, ambiguous fight outcomes may also be expected to reflect uncertainty over social standing.
4. Injurious fighting: Pigs that receive short, intense attacks may be less likely to challenge social relationships.

There was little evidence that any of these traits affected long term social stability in the current study, as skin lesions at SL3wk did not relate to the proportion of repeated

fights, fight intensity or ambiguous outcomes at mixing on a group level. Groups with a high proportion of successful fights (proportion of fights won) tended to have low SL3wks. Correlations between behavioural traits (results not presented) indicate that groups with a high proportion of fights success also had a large number of unambiguous, intense fights. It is possible that social relationships are influenced by a combination of traits related to fight quality although individual traits do not correlate with skin lesions when considered in isolation.

Negative correlations between reciprocal aggression at mixing and SL3wk offer some support for the hypothesis that increased initial reciprocal aggression on a group level reduces aggression in the long term. Reduced aggression at three weeks could indicate a more stable social hierarchy. If this was the case, it could be that certain fighting experiences, in particular those related to reciprocal aggression, lead to less ambiguity over hierarchy positions, resulting in fewer conflicts over resources.

2.4.2 Behaviour and skin lesions on individual animal level

At the individual animal level, residual correlations between aggressive behavioural variables and SL24h indicate that an increase in almost all measures of aggression at mixing results in more skin lesions across all three body regions. Lesions to the anterior body region have previously been shown to be associated with reciprocal fighting, and the posterior and central regions of the body associated with receipt of non-reciprocal aggression (Turner et al., 2006[a]).

A multiple regression model was developed in the current study in order to further dissect the relationship between various aggressive strategies and the receipt of lesion numbers. As predicted by residual correlations, a general increase in aggression - for example long reciprocal fights - predicted higher lesions across all body regions 24h

after mixing. Fight success (proportion of fights won) predicted fewer SL24h to central and posterior body regions when included in the model. This is likely to be because unsuccessful pigs receive more non-reciprocal aggression, resulting in slightly more lesions than their successful pen mates. Although the receipt of non-reciprocal aggression was weakly negatively correlated with fight success, the number and duration of non-reciprocal aggression received were positively associated with other measures of aggression, including the number of reciprocal interactions involved in and the number of pen mates bullied. Combined, these results suggest that while increased aggression of all descriptions increases the risk of receiving skin lesions, within this more aggressive cohort, the animals with a high fight success rate receive fewer skin lesions than their less successful but aggressive pen mates. Animals that avoid involvement in aggression altogether receive the lowest skin lesions at this time.

Correlations between aggressive behaviour at mixing and skin lesions recorded three weeks post-mixing were lower than those calculated for skin lesions 24 hours post mixing. As described earlier, aggression at mixing and in established groups tends to differ in its form and motivation, lacking the intense reciprocal aggression that constitutes the majority of aggressive behaviour at mixing (Bolhuis et al., 2005; Fraser, 1984). A strong correlation between the two traits was therefore not to be expected. Despite this, many measures of aggression were negatively correlated with SL3wk, indicating that the more aggression an individual is involved in at mixing, the fewer lesions it receives under stable social conditions, particularly to the anterior and central regions of the body. As found for associations with SL24h, behavioural correlations with the posterior region of the body 3 weeks post mixing were lower than those obtained for the anterior and central regions, resulting in extremely low correlations for this body region at that time point. This pattern indicates that posterior

lesions are not as informative as lesions to the anterior body region. This may be because lesions are typically inflicted to the rear of the body during the receipt of aggression, and are therefore not a reflection of an individual's own behaviour, but rather that of its pen mates.

Behavioural traits accounted for very little variation in SL3wk, as predicted by the multiple regression models. The models predicted that the proportion of fights won at mixing accounted for most of the variation in central SL3wk, with the most successful animals receiving the fewest lesions at this time. This implies that skin lesions in stable groups are chiefly related to dominance, as it is likely that the most successful animals at mixing go on to achieve the highest-ranking positions in stable groups. As reflected by the correlations on individual animal level, the model predicted that an increase in the duration of non-reciprocal attacks received at mixing was associated with slightly increased central SL3wk. The duration of non-reciprocal attacks received was positively correlated with number and duration of non-reciprocal attacks initiated, and the duration of reciprocal aggression involved in. Therefore the animals that received much aggression were also actively involved in aggression. This finding reflects those found in a previous study involving a different population (Turner et al., 2006[a]). These results demonstrate that non-reciprocal aggression at mixing is not received by the unaggressive individuals in a group, but rather aggressive but unsuccessful animals, possibly as a means to reinforce a fight outcome.

The results from the correlations and mixed model predictions indicate that while high fight success at mixing results in the lowest stable lesions, involvement in aggression at mixing, even when unsuccessful, leads to fewer lesions in the stable group than animals which avoid aggression at mixing altogether. The simplest explanation is that pigs which avoid aggression are simply the most subordinate individuals; however this does

not explain the observations made on a pen group level. It could be that simply engaging in aggression leads to less ambiguity over social standing, resulting in fewer challenges to hierarchy positions. Alternatively, it is possible that experience in physical aggression is necessary in learning to convey both dominant and submissive behaviours. Studies involving repeated mixing of pigs (Coutellier et al., 2007; Giersing & Andersson, 1998) have shown that the amount of aggression displayed reduces with increased mixing, whereas D'Eath, (2005) found that early socialising of piglets leads to faster hierarchy formation. Frischknecht et al., (1982) demonstrated how mice that had experience of being defeated displayed significantly more submissive behaviours than those that had never experienced agonistic interactions. In the present study, pen group lesions at three weeks were negatively associated with traits related to reciprocal fighting. If important social skills are learned via fighting experience, this may explain why we see more social stability in groups that involved more reciprocal aggression between more group members.

Social instability in the form of long-term aggression may be caused by several factors. It is possible that groups with increased aggression 3 weeks post mixing have a less stable hierarchy than other groups, and therefore frequent physical aggression is required in order to re-establish or maintain dominance relationships. Alternatively, it may be that some individuals fail to recognise dominance relationships, or continue to fight at inappropriate times. As no behavioural data were available three weeks post-mixing, the stability of dominance relationships could not be assessed. As such, it is impossible to deduce whether long-term social instability was the result of unstable dominance relationships or socially dysfunctional individuals within a group.

The results of these analyses confirm that skin lesions are a useful alternative measure of aggressiveness displayed by individual pigs in the first 24 hours post mixing. While increased aggression at mixing leads to more injuries at first, it may be beneficial for the individual in the long term, even if the animal is not successful at fighting.

2.4.3 Lesion correlations

Lesions across body regions at the same time point were positively correlated meaning that animals that received high lesions to one region of the body were likely to receive lesions to other body regions. This is in accordance with the findings from the behavioural data in which animals that engage in a high amount of aggression of any form receive many lesions to all body regions.

Individuals that received high central and posterior SL24h were also somewhat likely to receive high anterior and central SL3wk, although the correlations were of a very low magnitude. These results appear to conflict with the direction of the correlations between aggression at mixing and SL3wk. Although this seems counterintuitive at first, the correlations between skin lesion traits are low, and contradicting correlations can occur due to the various effects that influence the correlations. It can be hypothesised that the contradictory relationship between aggressive behaviour and lesions at different time points may contribute to the reduced correlations between lesions at mixing and the stable group.

Genetic correlations using the same population showed a moderate to strong positive correlation between SL24h and SL3wk (Turner, 2009); however the same study also found negative residual correlations between these traits. This relationship was also observed on a group level, although the correlations were of a higher magnitude than those observed for individuals.

2.4.4 Conclusions

Research into reducing aggression via a combination of genetic and management strategies are on-going. Phenotypic correlations such as those explored in the present study offer some evidence that within groups of mixed aggression levels, increased reciprocal aggression may be beneficial to long term group dynamics. It may prove challenging to identify any single management strategy that will simultaneously reduce both mixing-induced aggression and on-going chronic aggression. In contrast, genetic correlations (Turner et al., 2009) and experiments in which animals were grouped according to aggressive personalities (Erhard et al., 1997) support the theory that reducing the level of aggression displayed by individuals may result in reduced long term aggression. The environment (O'Connell and Beattie, 1999), group size (Andersen et al, 2004; Hemsworth et al., 2014), genetics (Canario et al., 2012; Turner et al., 2006[b]; Turner et al., 2009), early life experience (D'Eath, 2005) and prenatal stress (Jarvis et al., 2006) have all been shown to affect social aggression in pigs. Further work is clearly required to disentangle these factors in order to better predict the possible consequences on aggression. In terms of genetic strategies to control aggression in pigs, this study raises the interesting question that selection for reduced aggression at mixing could result in increased levels of chronic aggression. Further studies should seek to calculate the genetic correlation between metrics of aggression at mixing and then during the stable state to uncover the genetic architecture of these two distinct traits.

The current study cannot address the question of whether skin lesions at three weeks not only relate to aggression, but that increased aggression in stable groups translates to poor welfare. Published studies examining the long term effects of social stress (usually by measuring cortisol or immune responses) have produced conflicting results

(Blanchard et al., 1993; Mendl et al., 1992; Tuchscherer et al., 1998; Ekkel et al., 1997). In the present study, SL3wk were similar in number to SL24h for central and posterior lesions, indicating a comparable level of aggression at the two time points. It could be argued that dominance relationships are a part of the pig's natural behaviour and therefore individuals should be equipped to deal with the stress that arises from these encounters. However in space-restricted pens animals are often unable to adequately avoid persistent attacks (Fraser et al., 1995).

Chapter 3 - Prediction of reduction in aggressive behaviour of growing pigs using skin lesion traits as selection criteria

3.1 Introduction

Previous research, and the results presented and discussed in Chapter 2 have highlighted the complex nature of social aggression, and the relationship between aggressive behaviour and skin lesions. As discussed, skin lesions on one animal can be an indication of the behaviour of other members of the group, as well as the individual in question. For example, an individual may receive skin lesions via willing engagement in reciprocal aggression, or via an unreciprocated attack. Failing to distinguish between the underlying causes of lesions may lead to biased estimates of individual aggression. We have also shown that individuals that are involved in much aggression at mixing tend to have fewer lesions several weeks later, suggesting that avoidance of aggression upon first mixing may be detrimental to individuals in the long term. Finally, genetic variation and heritabilities of skin lesion traits differ between different body regions and time points. In light of the above, it is important to carefully assess the potential impact of selection for reduced aggression via skin lesion traits. Although genetic correlations between skin lesion traits and some aggressive behavioural traits have been previously published (Turner et al., 2009), these correlations do not give an indication of the magnitude of the expected response to selection. In addition, the estimated genetic correlations among skin lesion and behaviour traits are complex, are often in conflict with one another, and are associated with high errors of estimation, therefore predicting the selection response based on genetic correlations can be difficult. Due to time constraints, it is likely that only one skin lesion trait will be recorded under practical conditions, therefore it is necessary to identify the best skin lesion trait for selection. The objective of this study was to identify the optimum skin lesion trait for the purpose of reducing aggressive behaviour, by determining the magnitude of the reduction in aggressive behavioural traits at mixing, when using

lesion numbers on different body regions at mixing and in the stable group as selection criteria.

3.2 Methods

3.2.1 Animals and housing

Management and mixing protocol for this study were identical to those described in Chapter 2. Animals with all skin lesion and behavioural phenotypes were included in the analyses, and the final dataset contained 1,146 individuals (698 purebred Yorkshire and 448 crossbred Yorkshire x Landrace) from 77 social groups. Experimental animals were the progeny of 82 sires and 217 dams, and pedigree information as far back as and including the grandparents was utilized (in total 1,862 animals). Groups mixed on the same day were classed as the same batch.

3.2.2 Skin lesion and behavioural traits

The protocol for recording skin lesions, and aggressive behavioural interactions for this study was identical to those described in Chapter 2. Aggressive behavioural traits used in the present analyses are described in Chapter 2, Table 2.1. The behavioural traits used in the present study were based on data recorded during these behavioural observations.

3.2.3 Characteristics of the data

Only those behavioural traits of aggression shown to be predictive of skin lesion traits on a phenotypic level in the same population in Chapter 2 were chosen for analysis. Characteristics of skin lesion and behavioural traits for the individuals used in these analyses are presented in Table 3.1. Skin lesion and aggressive behavioural traits showed considerably skewed distributions (Table 3.1), therefore a log transformation ($y = \log_e+1$) was used to approach the normal distribution. The transformed values were used to calculate estimated breeding values.

Table 3.1 Characteristics of skin lesion traits recorded 24h post-mixing (SL24h) and 3 weeks post-mixing (SL3wk) and behavioural data for all animals included in the statistical analysis

	Original scale					Transformed scale		
Trait	**N**	**Min-Max**	**Mean (SD)**	**SK**	**K**	**Mean (SD)**	**SK**	**K**
Anterior SL24h	1146	0 - 99	19.07 (17.35)	1.37	2.27	2.58 (1.07)	-0.90	0.43
Central SL24h	1146	0 - 100	10.82 (12.03)	1.43	6.03	2.06 (1.1)	-0.64	-0.52
Posterior SL24h	1146	0 - 41	3.69 (8.30)	-0.73	4.08	1.36 (1.02)	-0.12	-1.30
Anterior SL3wk	1146	0 - 63	10.40 (5.62)	1.59	8.89	2.30 (0.56)	-1.15	2.66
Central SL3wk	1146	0 - 40	10.36 (5.93)	1.03	1.90	2.28 (0.6)	-0.92	1.54
Posterior SL3wk	1146	0 - 30	4.53 (3.49)	1.16	2.92	1.49 (0.71)	-0.52	-0.34
Number of RA involved with	1146	0 - 56	8.43 (7.16)	1.37	3.04	1.91 (0.9)	-0.60	-0.35
Proportion of fights won	1047	0 - 1	0.30 (0.25)	0.54	-0.27	0.25 (0.19)	0.20	-0.85
Average duration NA & NRA involved (s)	1138	1 - 250	42.70 (27.97)	2.03	8.24	3.59 (0.64)	-0.39	0.46
Duration NRA initiated (s)	1146	0 - 996	41.71 (68.81)	4.64	40.72	2.53 (1.82)	-0.19	-1.27
Duration NRA received (s)	1146	0 - 444	41.47 (46.53)	2.88	13.9	3.12 (1.34)	-0.88	0.36
Duration of RA initiated (s)	1146	0 - 2394	289.8 (366.2)	2.07	5.30	4.30 (2.34)	-0.88	-0.53
Duration RA received (s)	1146	0 - 2997	329.6 (353)	2.08	6.62	5.10 (1.45)	-1.08	1.45
Number of pen mates attacked (RA)	1146	0 - 11	2.86 (2.32)	0.65	-0.20	1.14 (0.69)	-0.40	-0.94
Number of pen mates attacked by (RA)	1146	0 - 9	2.86 (2.06)	0.40	-0.52	1.18 (0.63)	-0.59	-0.66
Number of pen mates attacked (NRA)	1146	0 - 14	2.57 (2.69)	1.32	1.54	1.00 (0.75)	0.06	-1.10
Number of pen mates attacked by (NRA)	1146	0 - 9	2.57 (1.67)	0.61	0.1	1.26 (0.45)	-1.64	2.39
Number of attacks initiated (RA)	1146	0 - 36	4.23 (4.30)	1.75	4.97	1.32 (0.85)	-0.14	-0.89
Number of attacked received (RA)	1146	0 - 25	4.20 (3.78)	1.44	3.04	1.37 (0.79)	-0.32	-0.72
Number of pen mates interacted with	1146	0 - 14	6.69 (3.06)	0.02	-0.66	1.94 (0.49)	-1.13	1.48

RA = reciprocal aggression

NRA = non-reciprocal aggression; SK = skewness; K = kurtosis

3.2.4 Statistical Analyses

Univariate analyses were used to estimate genetic components and estimated breeding values of all skin lesion and behavioural traits using the following animal model:

$$\mathbf{y = Xb + Za + Wc + e}$$

where **y** is the vector of records for skin lesions (SL24h and SL3wk) and aggressive behaviour, and **X**, **Z** and **W** are the incidence matrices of fixed effects, genetic and environmental (pen group) effects, respectively. Vectors **b**, **a**, **c** and **e** represent fixed effects, additive direct genetic effects, common environmental effects, and residual error, respectively. Genetic line, sex, and batch (week in the experimental programme that the animals were mixed) were included in all models as fixed categorical effects, while bodyweight at time of mixing was fitted as a covariate. The age at time of mixing was included for SL24h and aggressive behavioural traits. Bivariate analyses were used to estimate genetic and group level correlations between skin lesion traits and aggressive behavioural traits. The same fixed and random effects were fitted for each trait as described for the univariate analyses. Genetic analyses were performed using ASReml (Gilmour et al., 2009).

As skin lesion and behavioural traits are measured on different scales, it is impractical to directly compare genetic and phenotypic values across multiple traits. Estimated breeding values (EBVs) and untransformed phenotypic values were therefore scaled and standardised, and expressed in terms of standard deviations from a population mean of zero. Individuals were chosen for inclusion in each subsequent analysis based on either SL24h EBVs in the lowest 10% of the population, or SL3wk EBVs in the highest 10% of the population. These criteria were chosen based on the evidence

presented in Chapter 2 that SL24h are mostly positively correlated with aggression at mixing, while SL3wk are negatively correlated with aggression at mixing.

3.3 Results

3.3.1 Heritabilities and common pen effects

The variance components of genetic and common environmental effects are presented in Table 3.2. Heritabilities estimated for skin lesion traits ranged from 0.11 to 0.43, with the lowest heritability estimated for posterior SL3wk and the highest estimate for anterior SL3wk. A substantially higher heritability was estimated for anterior SL3wk than anterior SL24h. Heritabilities for behavioural traits ranged from 0.09 to 0.44, with the lowest heritability estimated for the duration of time spent receiving non-reciprocal aggression, and the highest heritability estimated for the number of reciprocal aggressive (RA) interactions an individual was involved in. The proportion of variance attributed to common environmental (pen) effects was generally lower than that attributed to genetic effects for skin lesion and behavioural traits (c^2 = 0.06 to 0.15), except for the traits posterior SL24h, average fight duration, and the duration of time spent receiving non-reciprocal aggression. In terms of labour, behavioural traits are costly to record therefore skin lesions are explored as a time-effect method of phenotyping aggression. The methodology of using the 10% of desired animals based on skin lesions was chosen in this study because, in practice, it is likely that only one skin lesion trait will be used for phenotyping purposes. Furthermore, certain skin lesion trait have been shown to have different associations with aggressive behaviour, for example low anterior lesions at mixing are associated with low involvement in aggression, whereas low anterior lesions 3 weeks post-mixing were associated with high levels of aggression at mixing. This methodology has the additional advantage of predicting the results of selection based on the given data, and not based on complex genetic correlations that have a high level of estimation error, which might affect the accuracy of predicted response using population genetic theory.

Table 3.2 Heritabilities (h^2), phenotypic proportions of pen variances (c^2), phenotypic (σ^2p), and genetic (σ^2a) variances for skin lesion traits recorded 24 hours post mixing (SL24h) and 3 weeks post mixing (SL3wk) and aggressive behavioural traits

	Trait	h^2	c^2	σ^2p	σ^2a
SL24h	Anterior	0.13 (0.05)	0.06 (0.02)	1.14	0.15
	Central	0.21 (0.06)	0.10 (0.03)	1.22	0.25
	Posterior	0.12 (0.05)	0.14 (0.03)	0.87	0.11
SL3wk	Anterior	0.43 (0.08)	0.03 (0.02)	0.31	0.13
	Central	0.39 (0.08)	0.06 (0.02)	0.35	0.13
	Posterior	0.11 (0.05)	0.07 (0.02)	0.44	0.05
Behaviour	Number of RA involved with	0.44 (0.08)	0.07 (0.02)	0.82	0.36
	Proportion won	0.34 (0.08)	0.01 (0.02)	0.04	0.01
	Average duration of NA & NRA involved (s)	0.14 (0.05)	0.15 (0.03)	0.38	0.05
	Duration NRA initiated (s)	0.33 (0.07)	0.03 (0.02)	3.42	1.12
	Duration NRA received (s)	0.09 (0.04)	0.13 (0.03)	1.82	0.16
	Duration of RA initiated (s)	0.35 (0.08)	0.01 (0.02)	5.44	1.92
	Duration RA received (s)	0.28 (0.07)	0.06 (0.02)	2.08	0.58
	Number of pen mates attacked (RA)	0.40 (0.08)	0.04 (0.02)	0.48	0.19
	Number of pen mates attacked by (RA)	0.33 (0.07)	0.07 (0.02)	0.40	0.13
	Number of pen mates attacked (NRA)	0.31 (0.07)	0.02 (0.02)	0.57	0.18
	Number of pen mates attacked by (NRA)	0.11 (0.05)	0.19 (0.04)	0.43	0.05
	Number of attacks initiated (RA)	0.42 (0.08)	0.03 (0.02)	0.72	0.30
	Number of attacked received (RA)	0.32 (0.07)	0.07 (0.02)	0.61	0.20
	Number of pen mates interacted with	0.37 (0.08)	0.09 (0.03)	0.24	0.09

RA = Reciprocal aggression; NRA = Non-reciprocal aggression

3.3.2 Genetic and pen level correlations between skin lesion and behaviour traits

Genetic correlations

There were positive genetic correlations between anterior SL24h and most behavioural traits of aggression (0.56 [SE 0.17] to 0.85 [SE 0.10]; Table 3.3). The lowest correlation was for the duration of non-reciprocal aggression initiated and the greatest was for the number of pigs attacked by (reciprocal aggression). Genetic correlations between anterior SL24h and proportion of fights won, duration of non-reciprocal aggression received, or the number of pen mates non-reciprocal aggression was received from did not significantly differ from zero (Table 3.3). The only behavioural traits genetically correlated with central and posterior SL24h were those that did not significantly differ from zero for anterior SL24h. The proportion of fights won was negatively correlated with central (-0.49, SE 0.18) and posterior (-0.52, SE 0.21) SL24h, while the duration of non-reciprocal aggression received, and the number of pen mates that non-reciprocal aggression was received from were positively correlated (0.54 [SE 0.24] to 0.67 [SE 0.28]) with these traits (Table 3.3).

Table 3.3 Genetic (r_G) and pen level (r_C) correlations[1] between anterior, central and posterior skin lesion traits recorded 24 hours post mixing (SL24h), with aggressive behavioural traits (standard errors presented in parentheses)

	Anterior SL24h		**Central SL24h**		**Posterior SL24h**	
	r_G	r_C	r_G	r_C	r_G	r_C
Number of RA involved with	**0.78 (0.10)**	0.42 (0.21)	-0.02 (0.19)	-0.41 (0.23)	-0.11 (0.22)	**-0.47 (0.21)**
Proportion of fights won	0.24 (0.22)	0.01 (0.56)	**-0.49 (0.18)**	-0.46 (0.56)	**-0.52 (0.21)**	-0.48 (0.48)
Average duration of NA & NRA involved (s)	**0.66 (0.18)**	0.14 (0.22)	-0.17 (0.26)	-0.11 (0.20)	-0.36 (0.27)	-0.15 (0.19)
Duration of NRA initiated (s)	**0.56 (0.17)**	0.10 (0.36)	-0.21 (0.22)	0.01 (0.32)	-0.19 (0.23)	-0.02 (0.30)
Duration of NRA received (s)	0.34 (0.29)	**0.17 (0.03)**	**0.54 (0.24)**	-0.07 (0.04)	**0.67 (0.28)**	**-0.08 (0.03)**
Duration of RA initiated (s)	**0.72 (0.12)**	0.38 (0.39)	-0.10 (0.20)	-0.34 (0.43)	-0.32 (0.22)	-0.47 (0.40)
Duration of RA received (s)	**0.77 (0.12)**	**0.51 (0.20)**	0.10 (0.21)	-0.12 (0.25)	0.06 (0.24)	-0.02 (0.23)
Number of pen mates focal pig attacked (RA)	**0.75 (0.12)**	**0.21 (0.03)**	-0.07 (0.20)	**0.23 (0.03)**	-0.30 (0.22)	**0.22 (0.03)**
Number of pigs attacked by (RA)	**0.84 (0.10)**	**0.50 (0.02)**	0.08 (0.20)	**0.19 (0.03)**	0.16 (0.22)	**0.11 (0.03)**
Number of pen mates attacked (NRA)	**0.59 (0.17)**	0.17 (0.38)	-0.15 (0.22)	0.00 (0.35)	-0.07 (0.23)	0.03 (0.33)
Number of pen mates attacked by (NRA)	0.21 (0.29)	0.18 (0.21)	**0.54 (0.22)**	0.28 (0.18)	**0.67 (0.26)**	0.30 (0.16)
Total RA initiated	**0.73 (0.12)**	0.51 (0.27)	-0.05 (0.20)	-0.35 (0.31)	-0.28 (0.22)	-0.53 (0.28)
Total RA received	**0.85 (0.10)**	**0.58 (0.18)**	0.09 (0.20)	-0.39 (0.23)	0.12 (0.23)	-0.40 (0.21)
Number of pen mates interacted with	**0.69 (0.13)**	0.30 (0.22)	-0.01 (0.20)	-0.16 (0.22)	0.00 (0.22)	-0.05 (0.21)

[1] Bold font signifies correlation significantly different from 0

RA = Reciprocal aggression; NRA = Non-reciprocal aggression

In contrast to SL24h, genetic correlations between anterior SL3wk and most aggressive behaviour traits were generally negative (-0.33 [SE 0.15] to -0.49 [SE 0.14]; Table 3.4). There was a positive correlation between anterior SL3wk and duration of non-reciprocal aggression received (0.51, SE 0.22) and number of pen mates that non-reciprocal aggression was received from (0.47; SE 0.20). Central SL3wk were negatively correlated with the proportion of fights won (-0.45 SE 0.15), duration non-reciprocal aggression initiated (-0.33 SE 0.16), number of pigs that an attack was received from (-0.39 SE 0.15), and total reciprocal aggression received (-0.42 SE 0.15). Positive correlations were found between central SL3wk and duration of non-reciprocal aggression received (0.66 SE 0.19), and number of pen mates that non-reciprocal aggression was received from (0.58 SE 0.18; Table 3.4). No significant genetic correlations were found between posterior SL3wk and aggressive behavioural traits (Table 3.4).

Table 3.4 Genetic (r_G) and pen level (r_C) correlations[1] between anterior, central and posterior skin lesion traits recorded 3 weeks post mixing (SL3wk), with aggressive behavioural traits (standard errors presented in parentheses)

	Anterior SL3wk		**Central SL3wk**		**Posterior SL3wk**	
	r_G	**r_C**	**r_G**	**r_C**	**r_G**	**r_C**
Number of RA involved with	**-0.34 (0.14)**	-0.13 (0.31)	-0.30 (0.15)	-0.39 (0.23)	-0.31 (0.22)	-0.04 (0.26)
Proportion of fights won	**-0.49 (0.14)**	-0.35 (0.80)	**-0.45 (0.15)**	-0.79 (0.63)	-0.30 (0.23)	-0.29 (0.67)
Average duration of NA & NRA involved (s)	-0.19 (0.20)	-0.17 (0.27)	-0.30 (0.20)	-0.13 (0.22)	-0.30 (0.28)	-0.14 (0.22)
Duration of NRA initiated (s)	**-0.38 (0.15)**	-0.22 (0.41)	**-0.33 (0.16)**	-0.34 (0.33)	-0.28 (0.24)	-0.45 (0.34)
Duration of NRA received (s)	**0.51 (0.22)**	**-0.16 (0.04)**	**0.66 (0.19)**	**-0.18 (0.04)**	0.14 (0.34)	-0.06 (0.03)
Duration of RA initiated (s)	**-0.36 (0.15)**	0.05 (0.54)	-0.21 (0.16)	-0.44 (0.43)	-0.35 (0.23)	0.11 (0.45)
Duration of RA received (s)	-0.20 (0.17)	-0.31 (0.31)	-0.25 (0.17)	-0.29 (0.25)	-0.15 (0.25)	-0.17 (0.26)
Number of pen mates focal pig attacked (RA)	**-0.33 (0.15)**	0.05 (0.03)	-0.21 (0.16)	0.08 (0.04)	-0.33 (0.23)	-0.02 (0.03)
Number of pigs attacked by (RA)	**-0.33 (0.15)**	**-0.15 (0.04)**	**-0.39 (0.15)**	**-0.18 (0.04)**	-0.26 (0.24)	**-0.08 (0.03)**
Number of pen mates attacked (NRA)	-0.31 (0.16)	-0.18 (0.45)	-0.30 (0.16)	-0.32 (0.36)	-0.21 (0.25)	-0.41 (0.38)
Number of pen mates attacked by (NRA)	**0.47 (0.20)**	0.14 (0.25)	**0.58 (0.18)**	-0.12 (0.21)	0.22 (0.32)	-0.09 (0.21)
Total RA initiated	**-0.33 (0.15)**	-0.23 (0.39)	-0.20 (0.16)	-0.52 (0.30)	-0.25 (0.23)	-0.05 (0.34)
Total RA received	-0.32 (0.16)	-0.22 (0.31)	**-0.42 (0.15)**	-0.40 (0.23)	-0.32 (0.24)	-0.05 (0.26)
Number of pen mates interacted with	**-0.37 (0.15)**	-0.11 (0.30)	-0.20 (0.16)	-0.32 (0.23)	-0.28 (0.23)	-0.27 (0.24)

[1] Bold font signifies correlation significantly different from 0

RA = Reciprocal aggression; NRA = Non-reciprocal aggression

Group level correlations

Generally, group level correlations between skin lesion traits and aggressive behavioural traits did not significantly differ from zero. Those correlations that did significantly differ from zero were mainly positive (0.11 [SE 0.03] to 0.58 [SE 0.18]). Statistically significant negative group level correlations were found between posterior SL24h and the number of reciprocal fights involved with (-0.47 SE 0.21), or duration of non-reciprocal aggression received (-0.08, SE 0.03; Table 3.3). Negative pen level correlations were found between all SL3wk traits and the number of pigs attacked by (reciprocal aggression) (-0.08 [SE 0.03] to -0.18 [SE 0.04]), and between anterior (-0.16 SE 0.04) and central (-0.18 SE 0.04) SL3wk with duration of non-reciprocal aggression received (Table 3.4).

3.3.3 Low EBVs for SL24h

Associations with SL24h

Individuals with low EBVs for anterior, central or posterior SL24h had EBVs that were between -0.69 (SE 0.09) and -1.89 (SE 0.05) standard deviations below the population mean for all skin lesion traits at mixing. Figure 3.1[a, c, e]). Individuals with low EBVs for anterior, central or posterior SL24h also had low phenotypic values that ranged between -0.38 (SE 0.07) and -0.94 (SE 0.02) standard deviations below the population mean for SL24h (Figure 3.2 [a, c, e]). The untransformed mean lesion numbers for anterior, central, and posterior SL24h for the entire population were 19.0, 10.8 and 3.7, respectively. Individuals with low anterior SL24h EBVs had 15.9, 7.4 and 3.4 fewer lesions compared to the population mean Table 3.5).

Associations with SL3wk

Individuals with EBVs in the lowest EBVs for SL24h had EBVs for SL3wk that ranged between -0.15 (SE 0.09) and -0.41 (SE 0.09) SD below the population mean (Figure 3.1[a, c, e]). Phenotypically, individuals with low EBVs for SL24h did not differ significantly in the number of SL3wk they received in comparison to the population as a whole (-0.05 SD [SE 0.10] to 0.07 SD [SE 0.10]; Figure 3.2 [a, c, e]).

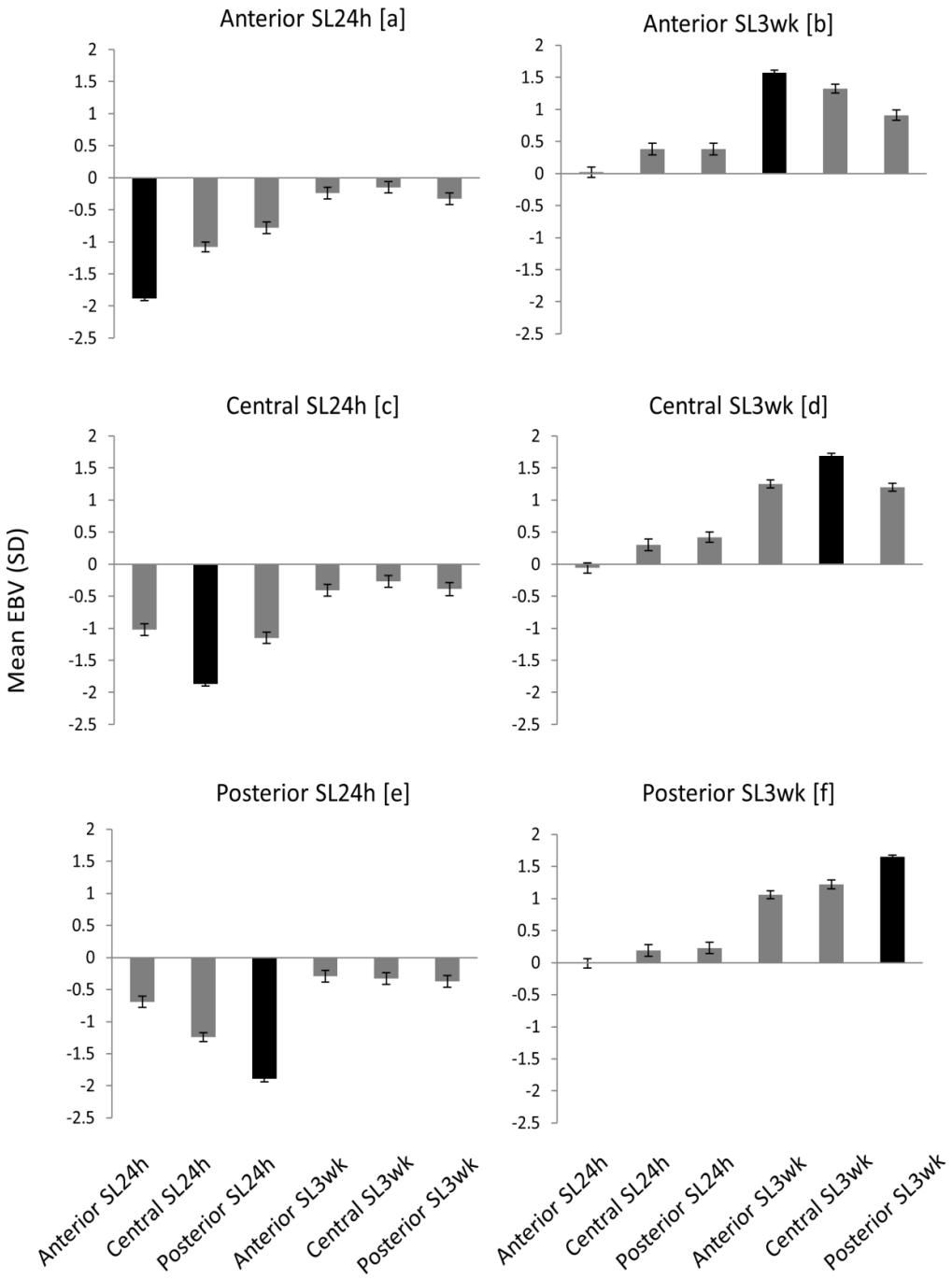

Figure 3.1 Mean estimated breeding values (EBVs) for skin lesion traits of pigs with EBVs in the lowest 10% for either anterior (a), central (c) or posterior (e) skin lesions recorded 24 hours post-mixing (SL24h), or highest 10% EBVs for anterior (b), central (d) or posterior (f) skin lesions recorded 3 weeks post-mixing (SL3wk). Skin lesion trait that selection was based on is indicated above each panel and shaded black.

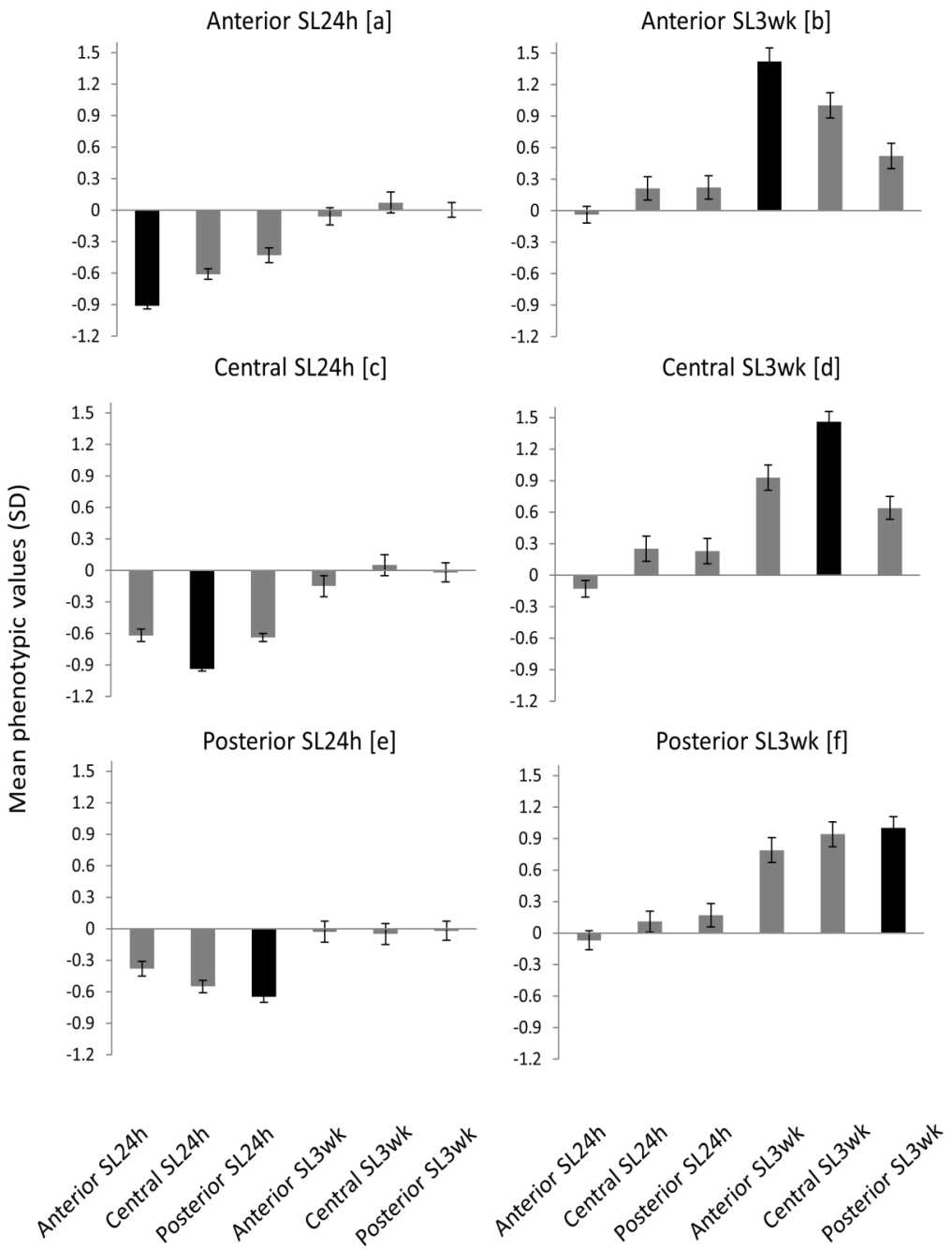

Figure 3.2 Mean phenotypic values for skin lesion traits of pigs with EBVs in the lowest 10% for either anterior (a), central (c) or posterior (e) skin lesions recorded 24 hours post-mixing (SL24h), or highest 10% EBVs for anterior (b), central (d) or posterior (f) skin lesions recorded 3 weeks post-mixing (SL3wk). Skin lesion trait that selection was based on is indicated above each panel and shaded black.

Associations with aggressive behaviour

Individuals with low EBVs for anterior SL24h had low EBVs for all behavioural traits of aggressiveness compared to the population as a whole (-0.21 to -1.17 SD; Figure 3.3 [a]). The greatest difference was observed for the duration of reciprocal aggression received and the lowest for the proportion of fights won. Other than the traits proportion of fights won, duration of non-reciprocal aggression initiated, and the number of pen mates attacked (non-reciprocal aggression), individuals with low central SL24h EBVs had EBVs that were lower than the population mean for aggressive behavioural traits (-0.28 to -0.51 SD).

Estimated breeding values were significantly lower than the population average in individuals with low posterior SL24h for four of the 14 behavioural traits, i.e. duration of non-reciprocal aggression received (-0.74 SD, SE 0.08); duration of reciprocal aggression received (-0.41 SD, SE 0.12); number of pigs attacked by (reciprocal aggression) (-0.27 SD, SE 0.10); total number of reciprocal attacks received (-0.27 SD, SE 0.10). Conversely, individuals with low breeding values for posterior SL24h had significantly higher EBVs for the proportion of fights won (0.45 SD, SE 0.08) and the duration of reciprocal aggression initiated (0.19 SD, SE 0.09), compared to the entire population average (Figure 3.3 [e]).

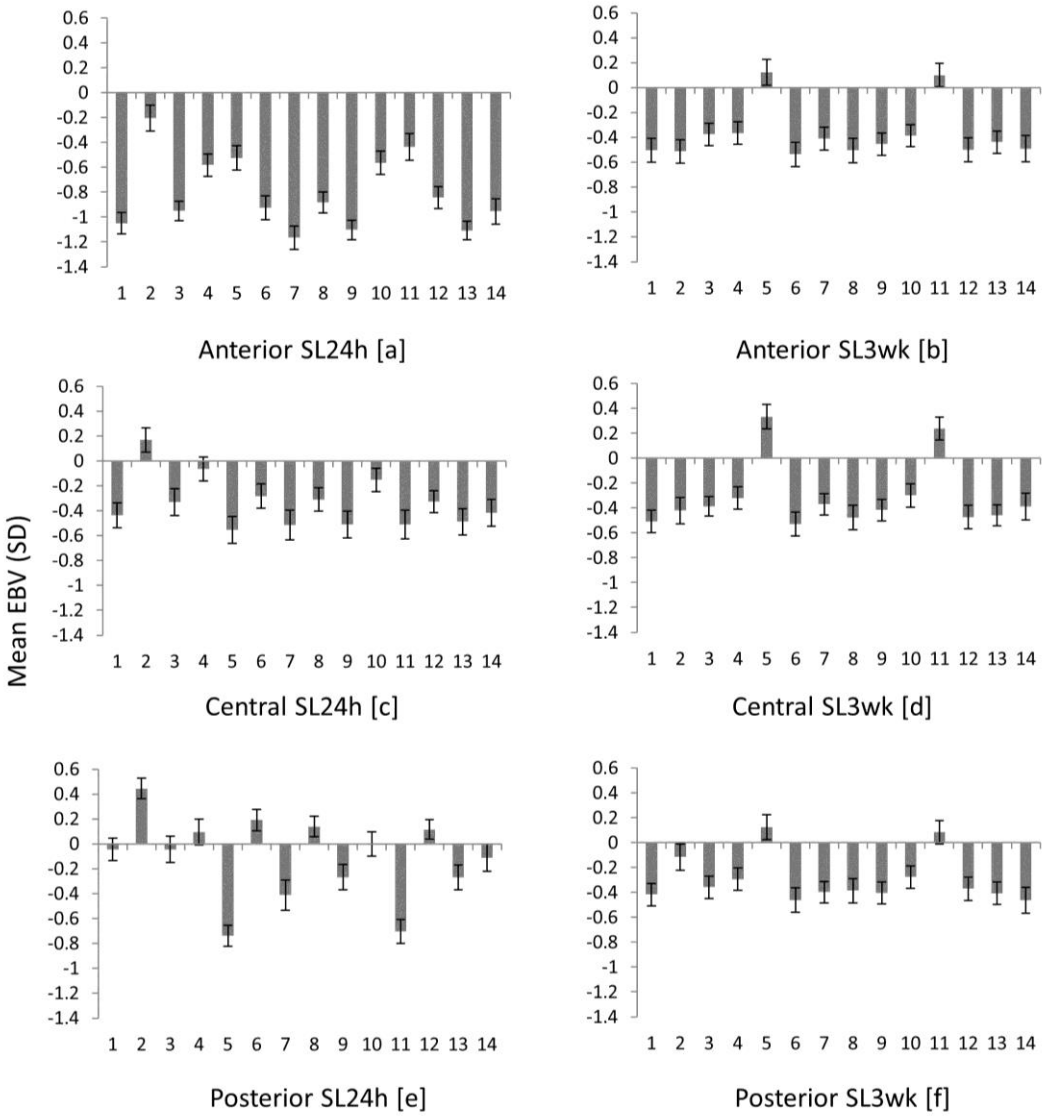

Figure 3.3 Mean estimated breeding values (EBVs) for aggressive behavioural traits of pigs with EBVs in the lowest 10% for either anterior (a), central (c) or posterior (e) skin lesions recorded 24 hours post-mixing (SL24h), or highest 10% EBVs for anterior (b), central (d) or posterior (f) skin lesions recorded 3 weeks post-mixing (SL3wk). Skin lesion trait that selection is based on is indicated beneath each panel. Numbers on horizontal axes correspond with the following behavioural traits: 1 - number of RA involved with; 2 - proportion of fights won; 3 - average duration of RA and NRA involved (s); 4 - duration of NRA initiated (s); 5 - duration of NRA received (s); 6 - duration of RA initiated (s); 7 - duration of RA received (s); 8 - number of pen mates focal pig attacked (RA); 9 - number of pigs attacked by (RA); 10 - number of pen mates attacked (NRA); 11 - number of pen mates attacked by (NRA); 12 - total RA initiated; 13 - total RA received; 14 - number of pen mates interacted with. RA = reciprocal aggression; NRA = non-reciprocal aggression.

With the exception of the proportion of fights won, individuals with low EBVs for anterior SL24h were involved in low levels of aggression on a phenotypic level compared to the population prior to selection (-0.24 to -0.74 SD). The greatest phenotypic difference was seen for the number of pen mates an individual received an attack from, while the least difference was for the duration of non-reciprocal aggression received by an individual (Figure 3.4 [a]). The untransformed mean number of pen mates individuals received a reciprocal attack from for the whole population was 2.9. In comparison, individuals with low anterior SL24h EBVs received an average of 1.5 attacks (Table 3.5).The mean duration for a non-reciprocal attack received by an individual for the whole population was 41.7 seconds. Individuals with low anterior SL24h EBVs received non reciprocal attacks for an average duration of 16.3 seconds Table 3.5. Individuals with low EBVs for central SL24 were also involved with less aggression on a phenotypic level (-0.28 [SE 0.10] to -0.55 [SE 0.11]), with the exception of the traits proportion of fights won, duration of non-reciprocal aggression initiated, and the number of pen mates attacked (non-reciprocal aggression), which did not significantly differ from zero (Figure 3.4 [c]). Phenotypically, individuals with low EBVs for posterior SL24h only significantly differed from the population mean for the duration of non-reciprocal aggression received (-0.19 SD, SE 0.08) and the total number of reciprocal attacks received (-0.19 SD, SE 0.08; Figure 3.4[e]).

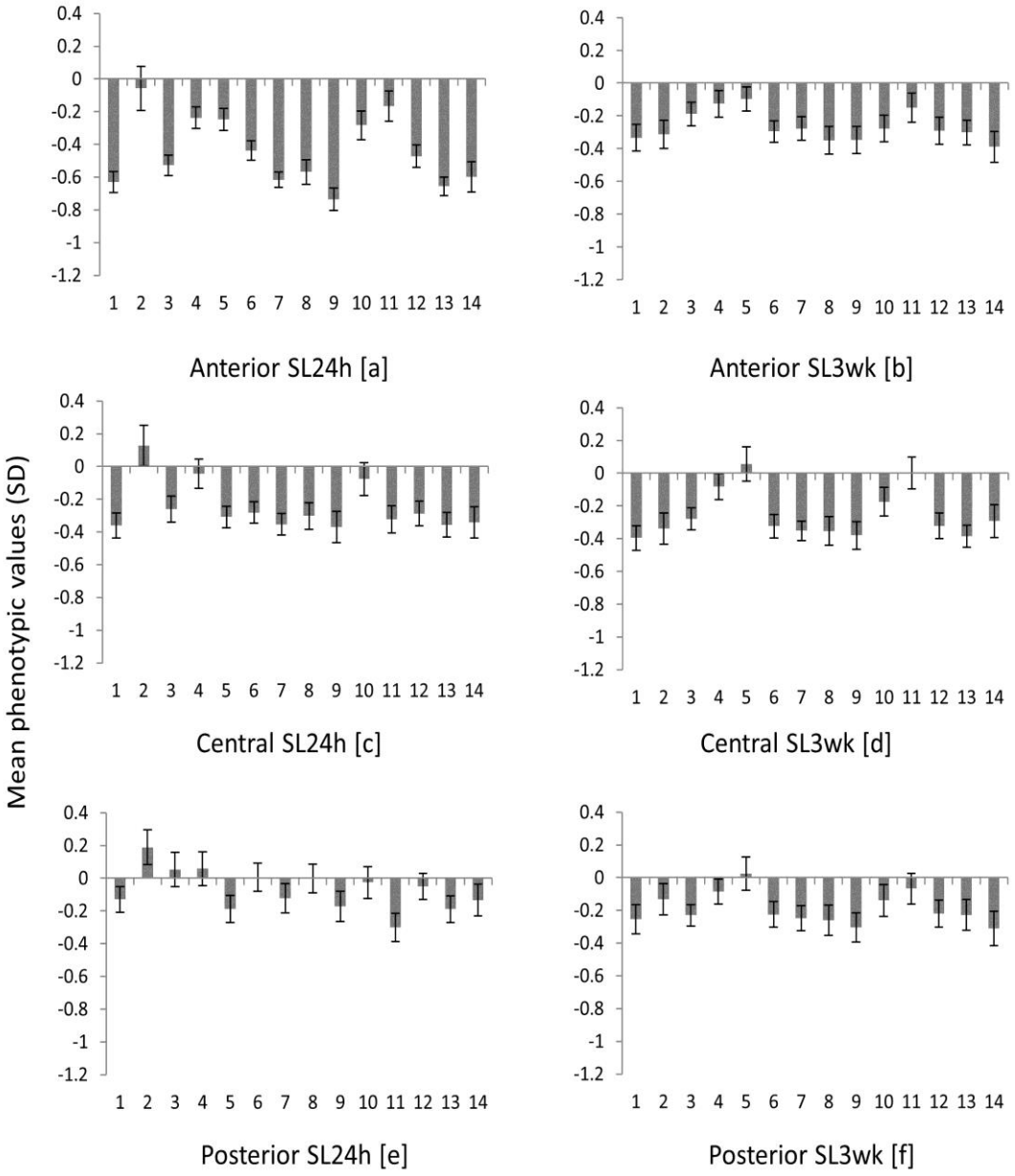

Figure 3.4 Mean phenotypic values for aggressive behavioural traits of pigs with EBVs in the lowest 10% for either anterior (a), central (c) or posterior (e) skin lesions recorded 24 hours post-mixing (SL24h), or highest 10% EBVs for anterior (b), central (d) or posterior (f) skin lesions recorded 3 weeks post-mixing (SL3wk). Skin lesion trait that selection is based on is indicated beneath each panel. Numbers on horizontal axes correspond with the following behavioural traits: 1 - number of RA involved with; 2 - proportion of fights won; 3 - average duration of NA and NRA involved (s); 4 - duration of NRA initiated (s); 5 - duration of NRA received (s); 6 - duration of RA initiated (s); 7 - duration of RA received (s); 8 - number of pen mates focal pig attacked (RA); 9 - number of pigs attacked by (RA); 10 - number of pen mates attacked (NRA); 11 - number of pen mates attacked by (NRA); 12 - total RA initiated; 13 - total RA received; 14 - number of pen mates interacted with. RA = reciprocal aggression; NRA = non-reciprocal aggression.

Table 3.5 Comparison of lesion traits and aggressive behavioural traits for all pigs and pigs in the lowest 10th percentile of EBVs for anterior skin lesions recorded 24 hours post mixing (SL24h). Raw mean values for all pigs, mean phenotypic differences (in SD) between all pigs and selected pigs, and expected mean change (in trait units) after selection for SL24h.

	Trait	Raw mean of all pigs	Mean difference between all and selected pigs in SD (SE)[1]	Expected change after selection[2]
SL24h	Anterior (selection trait)	19.07	**-0.91 (0.03)**	-15.85
	Central	10.82	**-0.61 (0.05)**	-7.35
	Posterior	3.69	**-0.43 (0.07)**	-3.60
SL3wk	Anterior	10.40	-0.06 (0.08)	-0.34
	Central	10.36	0.07 (0.10)	0.43
	Posterior	4.53	0.00 (0.07)	0.01
Behaviour	Number of RA involved with	8.43	**-0.63 (0.06)**	-4.51
	Proportion of fights won	0.30	-0.06 (0.14)	-0.01
	Average duration of NA and NRA involved (s)	42.70	**-0.53 (0.06)**	-14.76
	Duration of NRA initiated (s)	41.71	**-0.24 (0.06)**	-16.29
	Duration of NRA received (s)	41.47	**-0.25 (0.07)**	-11.55
	Duration of RA initiated (s)	289.80	**-0.44 (0.06)**	-160.42
	Duration of RA received (s)	329.60	**-0.62 (0.05)**	-217.42
	Number of pen mates attacked (RA)	2.86	**-0.57 (0.08)**	-1.32
	Number of pigs attacked by (RA)	2.86	**-0.74 (0.07)**	-1.52
	Number of pen mates attacked (NRA)	2.57	**-0.28 (0.09)**	-0.76
	Number of pen mates attacked by (NRA)	2.57	**-0.44 (0.11)**	-0.73
	Total RA initiated	4.23	**-0.47 (0.07)**	-2.03
	Total RA received	4.20	**-0.66 (0.06)**	-2.48
	Number of pen mates interacted with	6.69	**-0.60 (0.09)**	-1.83

RA = Reciprocal aggression; NRA = Non-reciprocal aggression

[1] Untransformed phenotypes were scaled and standardised and the corresponding change in SD after selection based on breeding values was used to calculate the expected change in aggressive behaviour

3.3.4 High EBVs for SL3wk

Associations with SL24h

Individuals with high EBVs for SL3wk did not differ significantly from the population mean for anterior SL24h EBVs but has had higher EBVs for central and posterior SL24h than the population average (0.19 [SE 0.09] to 0.42 [SE 0.08] SD; Figure 3.1[b, d, f]). In individuals with high central SL3wk, only central SL24h significantly differed from the population mean on a phenotypic level (0.25 SD, SE 0.12; Figure 3.2 [d]). No other SL24h trait was predicted to be significantly influenced at the phenotypic level following selecting for SL3wk (Figure 3.2[b, d, f]).

Associations with SL3wk

Individuals with high SL3wk EBVs for each body region had high EBVs for all other skin lesion traits in the stable group (0.91 to 1.69 SD; Figure 3.1[b, d, f]). These individuals also had high skin lesion numbers on a phenotypic level compared to the population average (0.52 [SE 0.12] to 1.45 [SE 0.10] SD; Figure 3.2[b, d, f]). Population mean phenotypic values for SL3wk were 10.4, 10.4 and 4.5, for anterior, central and posterior regions respectively. Individuals with high anterior SL3wk EBVs had 8.0, 5.9 and 1.8 more lesions than the population mean Table 3.6.

Associations with aggressive behaviour

Other than the duration of non-reciprocal aggression received, individuals with high EBVs for anterior SL3wk had significantly lower EBVs for all behavioural traits of aggression compared to the population average (-0.37 [SE 0.09] to -0.54 [SE 0.10] SD; Figure 3.3[b]). The greatest difference was observed for the duration of reciprocal aggression initiated, while the least difference was observed for the duration of non-reciprocal aggression initiated (Figure 3.3 [b]). Mean EBVs of all behavioural traits included in the analysis were predicted to be significantly affected following selection for increased central SL3wk (Figure 3.3[d]). Other than the duration of non-reciprocal aggression received, which increased (0.33 SD, SE 0.10), all behavioural traits were predicted to reduce following selection for this trait (-0.30 [SE 0.09] to -0.53 [SE 0.10] SD). Individuals with high high posterior SL3wk had low mean EBVs for all behavioural traits (-0.28 [SE 0.09] to -0.46 [SE 0.10] SD), other than the proportion of fights won, and the duration of non-reciprocal aggression received, which did not significantly differ from the population mean (Figure 3.3[f]).

On a phenotypic level, individuals with high EBVs for anterior SL3wk were involved in less aggression compared to the population average for all traits (-0.19 [SE 0.07] to -0.39 [SE 0.09] SD), with the exception of duration of non-reciprocal aggression initiated and received, and the number pen mates attacked (non-reciprocal aggression), which did not differ statistically from the population mean. Individuals with high anterior SL3wk interacted with 0.39 SD (SE 0.09) fewer pen mates than the population average. This corresponded to a decrease of 1.2 animals from 6.7. Individuals with low anterior SL3wk were involved in 2.4 fewer reciprocal fights than the population average (population mean = 8.4 fights) Table 3.6. Phenotypic values for all behaviour traits

other than those related to involvement in non-reciprocal aggression were predicted to significantly reduce (-0.28 [SE 0.07] to -0.40 [SE0.07] SD) following selection for central SL3wk (Figure 3.4 [d]). Phenotypically, individuals with high EBVs for posterior SL3wk did not significantly differ from zero for traits relating to involvement in non-reciprocal aggression, and the proportion of fights won. All other traits were significantly lower than the population average in this cohort (-0.22 [SE 0.08] to -0.31 [SE 0.10] SD; Figure 3.4[f]).

Table 3.6 Comparison of lesion traits and aggressive behavioural traits for all pigs and pigs in the highest 10th percentile of EBVs for anterior skin lesions recorded 3 weeks post mixing (SL3wk). Raw mean values for all pigs, mean phenotypic differences (in SD) between all pigs and selected pigs, and expected mean change (in trait units) after selection for SL3wk

	Trait	Raw mean of all pigs	Mean difference between all and selected pigs in SD (SE)[1]	Expected change after selection[2]
SL24h	Anterior	19.07	-0.04 (0.08)	-0.76
	Central	10.82	0.21 (0.11)	2.58
	Posterior	3.69	0.22 (0.11)	1.79
SL3wk	Anterior (selection trait)	10.40	**1.42 (0.13)**	7.95
	Central	10.36	**1.00 (0.12)**	5.91
	Posterior	4.53	**0.52 (0.12)**	1.82
Behaviour	Number of RA involved with	8.43	**-0.33 (0.08)**	-2.39
	Proportion of fights won	0.30	**-0.31 (0.09)**	-0.08
	Average duration of NA and NRA involved (s)	42.70	**-0.19 (0.07)**	-5.30
	Duration of NRA initiated (s)	41.71	-0.13 (0.08)	-8.72
	Duration of NRA received (s)	41.47	-0.10 (0.07)	-4.50
	Duration of RA initiated (s)	289.80	**-0.30 (0.07)**	-108.12
	Duration of RA received (s)	329.60	**-0.28 (0.07)**	-98.06
	Number of pen mates focal pig attacked (RA)	2.86	**-0.35 (0.09)**	-0.81
	Number of pigs attacked by (RA)	2.86	**-0.35 (0.08)**	-0.71
	Number of pen mates attacked (NRA)	2.57	**-0.28 (0.08)**	-0.75
	Number of pen mates attacked by (NRA)	2.57	0.1 (0.09)	0.17
	Total RA initiated	4.23	**-0.29 (0.08)**	-1.25
	Total RA received	4.20	**-0.3 (0.07)**	-1.14
	Number of pen mates interacted with	6.69	**-0.39 (0.09)**	-1.19

SL24h = skin lesions recorded 24 hours post-mixing; SL3wk = skin lesions recorded 3 weeks post-mixing; RA = reciprocal aggression; NRA = non-reciprocal aggression

[1] Bold font signifies change significantly different from 0. [2] Untransformed phenotypes were scaled and standardised, and the corresponding change in SD after selection based on breeding values was used to calculate the expected change in aggressive behaviour.

3.4 Discussion

3.4.1 Heritabilities

Heritabilities for skin lesion traits were of a low to moderate magnitude. These estimates differed from those reported by Turner et al (2009) for the same population, as only those animals with behavioural data available were used in the present analysis. When data from all animals with recorded skin lesion traits (regardless of the availability of behavioural information) were included, heritabilities were very similar to those estimated in the aforementioned paper. Higher heritabilities were estimated for anterior SL3wk than anterior SL24h. This is likely to be due to lower environmental variance 3 weeks post mixing compared to 24 hours post-mixing. The lowest heritability estimated for behavioural traits was for the receipt of non-reciprocal aggression. Receipt of non-reciprocal aggression results from the behaviour of other individuals in a group, and not the individual itself, which is likely to be the reason that direct genetic effects account for so little of the genetic variation in this trait. The highest heritabilities were estimated for those traits related to involvement in reciprocal aggression. During engagement in reciprocal aggression, the individual animal is actively involved in the event, choosing to either attack or respond to an attack, which is likely the reason these traits showed the highest heritabilities.

Social genetic effects describe genetic variation due to interactions between pen mates (Bijma & Wade, 2008). It is highly likely that social genetic effects contribute significantly to mixing-related aggression in pigs, particularly with respect to traits such as non-reciprocal aggression received by an individual. Ideally, both direct and social genetic effects would be considered when assessing the genetic basis of aggression in pigs, however these effects are difficult to estimate, optimally requiring

several hundred groups composed of few families (Bijma, 2010). For these reasons it was not possible to include social effects in this study, however common environmental (group) effects were included in the genetic model to approximate those social effects. Common environmental effects generally had a low influence on the number of skin lesions and involvement in aggression. In contrast to heritability estimates, pen group effects had the lowest influence on traits that related to the initiation of aggression by an individual. As expected, traits that related to the behaviour of other group members, for example the receipt of non-reciprocal aggression, tended to have slightly higher common environmental effects.

3.4.2 Expected response following selection for reduced SL24h

Consistent with the strength and direction of genetic correlations published previously in this population (Turner et al., 2009), individuals with SL24h EBVs in the bottom 10% had significantly lower genetic and phenotypic values for SL24h to all body regions when compared to the population as a whole. The least difference was observed on posterior SL24h when selecting for anterior SL24h, and vice versa. There is a tendency for lesions to the anterior of the body to occur as a result of involvement in reciprocal aggression, whereas lesions to the posterior of the body are typically the result of receipt of non-reciprocal aggression (Turner et al., 2006). As these body regions reflect involvement in opposing behaviours, this is likely the reason for the low association between anterior and posterior SL24h. On a genetic level, there was generally a positive association between skin lesions at mixing and in the stable group, in that individuals with low SL24h EBVs had slightly reduced EBVs for SL3wk compared to the population prior to selection, and vice versa. On the whole, however, this relationship was not observed on a phenotypic level. If the aim of using skin lesions for selection purposes was to only reduce lesion numbers, central or anterior SL24h would be

preferable traits to use. The predicted differences calculated on the phenotypic level have to be interpreted with care because the distribution of the traits were highly skewed.

The main goal of any breeding program incorporating skin lesions however, would be to reduce aggression, preferably on both a short and long term basis; therefore the results suggest that selection against anterior SL24h would have the greatest genetic and phenotypic effect on aggressive behaviour. Associations between EBVs in the bottom or top 10% of skin lesion traits with aggressive behavioural traits were generally in accordance with genetic correlations between the same traits. Selecting individuals based on low anterior SL24h was predicted to result in the greatest reduction in mean EBVs for behavioural traits relating to involvement in reciprocal aggression. Reciprocal contests make up the majority of time spent engaged in physical aggression and carry the biggest risk of injury, therefore reducing this behaviour is highly desirable. A slightly greater reduction in receipt of reciprocal attacks was predicted, in comparison to initiation of reciprocal attacks, suggesting that the recipient of an attack may be more likely to become injured than the initiator. This may be because the initiator of an attack is more likely to win a contest, inflicting more damage in the process (Stukenborg et al., 2011). The results suggest that the response of traits related to involvement in non-reciprocal aggression would not be as great as those related to involvement in reciprocal aggression. As the majority of lesions are inflicted towards the rear of the body during receipt of non-reciprocal aggression (Turner et al., 2006), it is to be expected that section against anterior lesions would have a lower influence on this trait. However, as involvement in non-reciprocal aggression is also positively genetically correlated with involvement in reciprocal aggression (results not

presented), the mean values for these traits were also significantly reduced following selection against anterior SL24h.

The possible role of social genetic effects on social aggression was briefly introduced in the previous section. Where there is a negative correlation between direct and social genetic effects, selection based on direct breeding values alone can result in an undesirable result (e.g. selecting for reduced SL24h may result in an increase in aggression; Ellen et al., 2014). Studies in mink and deer mice suggest that there may be a positive correlation between direct and social effects for aggressive traits, meaning that animals that have a low genetic propensity to become involved in aggression also have a low chance of being attacked (Wilson et al., 2011; Alemu et al., 2014). Conversely, negative correlations between social and direct effects have been found for dominance traits (Wilson et al., 2009; Sartori & Mantovani, 2012) however social effects were estimated to account for little of the variation in dominance traits in these studies. If a positive correlation exists between social and direct breeding values for aggressive traits, combined selection for social and direct EBVs of SL24h may reduce aggressive behaviour to an even greater degree than the results of this analysis suggest.

Of all behaviours analysed, mean breeding values for proportion of fights won were predicted to be the least affected by selection for low anterior SL24h EBVs. Genetic correlations calculated on the whole population confirm that there is a low, positive genetic correlation between these traits. On the phenotypic level, previous research has shown that high levels of aggression are associated with high anterior skin lesion counts, however the most successful (and presumably the most dominant) animals receive slightly fewer lesions than other highly aggressive, but unsuccessful animals (Desire et al., 2015). On the genetic level, it is likely that the low correlation between

anterior SL24h and the proportion of fights won is due to the fact that the proportion of fights won is independent from the duration of time spent engaged in aggression. For example, an individual with a very high fight success may have spent very little time engaged in aggression (receiving few lesions in the process), or much time engaged in aggression (receiving many lesions). Likewise, the same can be true for animals with a low fight success rate. Because of this relationship, individuals with low EBVs for anterior SL24h were involved in very low levels of aggression, but contained individuals with both high and low EBVs for proportion of fights won. These results may address the criticism that selection for low lesions may simply result in selection for meek animals, as it would seem that some dominant individuals are able to convey social rank with very little involvement in aggression, either through behavioural cues such as body posture, or short, decisive fights.

Genetic correlations indicate that high fight success and low receipt of non-reciprocal attacks are associated with few lesions to the central and posterior regions of the body. These correlations alone would suggest that selection against either of these traits would select for high dominance genotypes. The results indicated that selection for individuals with EBVs at the extreme end of the distribution would result in a reduction of several other behaviours, including initiation of reciprocal fighting. This suggests that individuals with low genetic merit for central SL24h also have a low propensity to be involved in both reciprocal and non-reciprocal aggression. Traits likely to be related to dominance, such as proportion of fights won, duration of non-reciprocal aggression initiated, and the number of pen mates attacked (non-reciprocal aggression), were not affected by selection for low central SL24h. This conflict seems to suggest that central lesions are an ambiguous proxy measure of aggression, as this trait appears to capture both successful individuals that are also likely to be involved in much aggression, as

well as unaggressive individuals. In contrast, selection for low posterior SL24h was predicted to result in an increase in the proportion of fights won and duration of reciprocal aggression initiated, and a decrease in all traits related to the amount of aggression received, suggesting that selecting for decreased posterior SL24h would result in selection for dominant genotypes. Correlations between central or posterior SL24h and aggressive traits presented in the present study sometimes conflicted with those calculated in a different population using similar phenotyping methods (Turner et al., 2008). The strength and direction of genetic correlations between anterior SL24h and behaviour traits however, were similar between the two populations, providing further evidence that anterior SL24h is the best trait overall for reducing aggression at mixing.

3.4.3 Effects of selection for increased SL3wk

It was known from previous studies that skin lesions recorded under stable social conditions are negatively phenotypically correlated with involvement in aggressive behaviour at mixing (Turner et al., 2009). For this reason, in the present study individuals with high EBVs for lesion numbers under stable social conditions (3 weeks post-mixing) were included in the analyses, in order to assess the effect this might have on behaviour at mixing. Due to lower genetic correlations between aggressive traits at mixing and skin lesions 3 weeks post-mixing, and lower genetic variance of skin lesion traits 3 weeks post mixing, selection for increased SL3wk did not reduce mean levels of aggressive behaviour to the same extent as selection for anterior SL24h. Despite this, there was some evidence that mean aggressive EBVs and phenotypes would also be reduced, following selection for SL3wk. With the exception of the duration of non-reciprocal aggression received, mean EBVs for aggressive behaviour were lower for individuals with high anterior or central SL3wk EBVs , suggesting that selecting for increased lesions under stable social conditions would result in a favourable response

in aggressive behaviour at mixing. The degree to which behavioural traits were affected was very similar for anterior and central lesions.

Individuals with high central SL3wk had significantly higher EBVs compared to the population average for the duration of non-reciprocal aggression received, in accordance with genetic correlations between these traits. The aim of applying selection pressure to skin lesions would be to reduce the level of aggression performed; therefore applying selection to a trait that would appear to increase certain levels of aggression may seem counterintuitive. However, as high stable lesions appear to be indicative of an unaggressive genotype, selection for this trait would result in a higher proportion of unaggressive genotypes in subsequent populations. Current evidence suggests that these lesions are caused by individuals with highly aggressive genotypes. As these genotypes would not be selected for, it would be expected that the duration of non-reciprocal aggression received would actually decrease in subsequent populations, despite the positive genetic correlations between skin lesions and this behavioural trait. Similarly, it is expected that the number of skin lesions that would also reduce under stable conditions, despite selecting for increased lesions at this time, as this would ultimately reduce the amount of aggression experienced by subordinate animals, as hypothesised above. These results draw attention to the fact that skin lesions recorded 3 weeks post mixing are a more accurate measure of the behaviour of other individuals in a group, and not the individual on which the lesions appear. This is discussed in more detail below, when comparing the merits of using SL24h or SL3wk as a method of phenotyping for selection purposes. From a behavioural perspective, the results suggest there would be little difference between using anterior or central SL3wk for selection purposes.

Aggression is most intense upon first mixing and it is behaviour at this time point that has been the focus of most research. It is worth considering the implications of aggression under stable social conditions as, once mixed, animals are often housed for several weeks or months within these groups. With regard to growing pigs, from a practical perspective, counting skin lesions on larger, older animals in a socially stable environment is less time consuming than counting lesions on younger animals, as there are fewer lesions to count, and the animals are more settled and tend to show less avoidance of an observer present in the pen. Furthermore, heritability estimates of skin lesion numbers in stable social groups have generally been found to be of a higher magnitude to those inflicted under newly mixed conditions, possibly due to less environmental noise at this time, (Turner et al., 2009) potentially increasing the response to selection for these traits.

At present, it is still not well understood how lesions three weeks after mixing are related to longer-term aggressive behaviour. No study has yet looked at long-term aggressive behaviour in sufficient detail to allow for a thorough investigation into the genetic and phenotypic relationships between skin lesion traits and aggression performed under stable and unstable social conditions. Although the results of the current study suggest that selection for increased SL3wk would reduce aggression at mixing, the correlations between aggression performed at mixing and skin lesions recorded 3 weeks later were lower than those calculated between aggression and skin lesions at mixing. The weaker relationship between traits across time points suggests that the individuals with the most lesions at three weeks may not always necessarily be the least aggressive individuals in the stable group. Without behavioural information it is unknown what factors contribute to aggression performed under stable group conditions, and under what circumstances individuals engage in aggression. For

example, lesions received under stable social conditions may be the result of attacks by dominant individuals, or reciprocal fighting between subordinate individuals, perhaps partly due to unstable or ambiguous dominance hierarchies. Genetic correlations between SL24h, SL3wk and aggressive behaviour performed at mixing provide a conflicting narrative. Positive genetic correlations between SL24h and SL3wk (Turner et al., 2009) suggest that individuals that receive many lesions at mixing go on to receive many lesions under stable group conditions, whereas negative correlations between most behavioural traits at mixing and SL3wk suggest that, on the whole, animals that are aggressive at mixing go on to have fewer lesions 3 weeks later. Group dynamics and social structure are likely to influence these correlations, however direct behavioural observations on animals in stable groups will be required in order to explore this further. As it stands, until long-term aggressive behaviour is better understood, skin lesions recorded under stable group conditions only provide information on the aggression performed by a group as a whole, and not the individual in question. In contrast, the relationship between skin lesions at mixing and aggressive behaviour is well established. In particular, anterior SL24h are known to be highly correlated with involvement in reciprocal aggression, meaning that skin lesions to this region of the body are the result of the actions of the individual in question. When anterior SL24h are used to phenotype an individual, it is a good proxy measure of the behaviour of that individual, and not of other animals in the group. Moreover, although mixing aggression has been studied for several decades, the damaging effects of long-term aggression are yet to be quantified.

As discussed above, correlations between SL24h and SL3wk, and between aggressive behaviour at mixing and SL3wk appear to contradict one another. While genetic correlations between SL24h and SL3wk are positive between all body regions (Turner

et al., 2009), the lowest correlations were found between anterior SL24h and SL3wk (0.26 to 0.28). The present study suggested that selection for anterior SL24h would have the least effect on SL3wk, compared to central and posterior SL24h, providing further evidence that anterior SL24h would be the optimum skin lesion trait for selection purposes. It should be noted that in this study skin lesion numbers recorded immediately prior to mixing were subtracted from those counted 24 h later, to ensure only those lesions resulting from mixing aggression were included in the analysis. This methodology effectively doubles the amount of labour required to record SL24h, however correlations between raw anterior lesion numbers recorded 24 h post mixing and lesion numbers adjusted for pre-mix counts were very high (0.95; $P < 0.001$) suggesting that recording skin lesions prior to mixing is not necessary for this trait.

The current study has provided evidence that significant reductions in social aggression could be achieved via selection for skin lesions. Much of the variation of skin lesion numbers is attributed to environmental factors, and previous research has demonstrated that variation in management systems can affect the phenotypic expression of aggression (reviewed by Arey & Edwards, 1998), however information regarding how environmental factors affect the genetic expression of these traits is limited. Appel et al. (2013) estimated variance components for aggressive traits performed in the first 30 minutes post-mixing in genetically similar pigs, housed under different management systems. In this study, pigs were housed on separate farms (farm A and farm B) that differed in group size, space allowance, pen design, floor and bedding type, and feeding systems. They found low levels of reciprocal aggression and a low heritability for this trait on farm A ($h^2 = 0.04$) compared to farm B ($h^2 = 0.33$). Residual variances were similar for each farm, therefore the authors hypothesised that the management system on farm A may have masked genetic differences between

individuals. It is possible that the environment on farm A favoured later onset of aggression after mixing (Turner et al., 2010), therefore the observation period (first 30 minutes post mixing) may not have captured aggressive behaviour at its peak. The authors rule out genotype by environment interactions, due to genetic correlations of 1 for the same aggressive traits between farms. In the present study, genetic associations between skin lesions and behavioural traits were looked at in detail. Although previous studies have found phenotypic correlations between skin lesions and aggression (Stukenborg et al., 2011; Tönepöhl et al., 2013; Turner et al., 2006) few studies have estimated genetic correlations across these traits. Present and previous results found in populations housed under different management systems suggest that anterior SL24h is a reliable measure of social aggression in growing pigs (Turner et al., 2008, Turner et al., 2009). In practice, selection for skin lesions would be incorporated into a selection index tailored to a wider set of breeding goals. Therefore, further research is required to estimate the genetic correlation with other traits in the breeding goal and derive the marginal economic and non-economic value of skin lesions to allow these traits to be weighted within a multi-trait commercial index.

3.4.5 Conclusion

The objective of this study was to identify the best skin lesion trait for selection purposes, and to determine the magnitude of reduction in aggressive behaviour when selection was applied to this trait. In order to establish this, univariate analyses of traits were carried out to predict the response to selection independent from any other trait. This serves to identify the unique effect of selecting a specific trait on the response in all other traits. It is likely that only one trait will be recorded in practical breeding programmes and therefore the methodology used in this study would reflect the most likely implementation. Furthermore, to quantify the amount of genetic and phenotypic

response gives a clear overview of how and in what amount selection on one trait affects many other aggressive behavioural traits.

The results of the study suggest that selection against anterior SL24h would have the greatest effect on behaviour at mixing, both on a genetic and phenotypic level. The results also suggest that anterior skin lesions recorded 24 hours post-mixing are a more accurate representation of the behaviour of the individual in question, as opposed to other skin lesion traits, which may be more representative of the behaviour of other individuals in the same pen environment. There is also evidence that selection for increased skin lesions recorded 3 weeks post mixing would have the favourable effect of reducing aggressive behaviour at mixing, although to a substantial lesser degree than section against anterior SL24h due to less genetic variance and lower genetic correlations. Although there are several advantages to using skin lesions recorded under stable social conditions to phenotype individuals for selection purposes, at the present time there is insufficient research into the relationship between aggressive behaviour at mixing and aggression under stable social conditions. In conclusion, with the evidence currently available, anterior SL24h would be the preferable trait to utilise in genetic selection, as it has the potential to significantly reduce levels of aggression observed in the first 24 hours post mixing, and also to reduce the genetic trend in longer-term aggression (three weeks after mixing).

Chapter 4 - Genetic associations of short- and long-term aggressiveness identified by skin lesion with growth, feed efficiency and carcass characteristics in growing pigs

Data collection, statistical analysis, and preparation of the manuscript was carried out by S Desire.

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4.1 Introduction

Social aggression has been shown to temporarily negatively affect growth on a phenotypic level (Tan et al., 1991; Stookey and Gonyou., 1994), however no study has analysed the genetic associations between production traits and skin lesions at different body regions, particularly in stable social groups (> 3 weeks post-mixing). The objective of this study was to further dissect the phenotypic and genetic associations between skin lesion traits with production and carcass traits to ascertain how selection based on skin lesions may affect other commercially important traits. Specifically, it was of interest to explore these associations for lesions recorded across different regions of the body, and in both newly mixed and socially stable groups.

4.2 Materials and methods

4.2.1 Animals and housing

Data were gathered from a total of 2,413 pigs (1,202 females and 1,211 castrates) from a PIC commercial herd between December 2012 and June 2013. Experimental animals were progenies of 116 sires and 391 dams, and pedigree information as far back as and including the grandparents was utilized (in total 4,104 animals). Pigs from 7 different terminal genetic lines were available, which were based on crosses of 1 maternal line and 7 sire lines. Pigs were housed in fully slatted pens and had constant access to water via a nipple drinker. Animals were fed dry pelleted food *ad libitum*. The floor space allowance was 0.65 m^2 per pig.

4.2.2 Mixing and lesion counting

Single sex groups of 18 pigs of mixed genetic line were formed by mixing 9 pigs from 2 non-adjacent weaning pens. Pen groups that were mixed on the same day were regarded as being in the same batch. Eight pen groups were formed per batch and 17 batches were used in total to generate 138 groups in total (batch 1 contained 10 pen groups). On average, animals from 11.6 (SD 2.1) litters were represented in each pen, and the mean number of pigs per litter per pen was 1.5 (SD 0.81). Once mixed, animals remained in these groups until the end of the test period. Animals had a mean age of 69 d (SD 5.2) at time of mixing. Skin lesions took approximately 1 min to be recorded per pig, and all skin lesions were counted by a single observer to eliminate inter-observer variation in counting technique. Lesions were recorded using the same protocol described in chapter 2. Based on evidence that lesions received to different body regions may be indicative of different aggressive behaviours, on both a phenotypic (Turner et al., 2008; Desire et al., 2015) and genetic (Turner et al., 2009) level, lesion

numbers were counted separately on 3 regions of the body: anterior (head, neck, front legs, shoulders), central (flanks and back), and posterior (hind quarters and rear legs).

Lesions were counted in the same way once again 5 weeks post-mixing (mean 34 d post-mixing, SD 9.5) as a measure of aggression in a socially stable group. Additional information gathered on subjects included pen identity, sex, batch (day the animals were mixed), genetic line, and unique pig identification. Animals were weighed without feed or water restriction 24 hours post-mixing and at the end of the growing period (172 d, SD 4.6).

4.2.3 Growth and production traits

Average daily gain on test (test daily gain) was calculated for the test period from 70 to 172 days of age (877 g/d, SD 120.17) and during the entire life (lifetime daily gain) from birth (685 g/d, SD 76.76). Feed intake data were collected at the individual pig level using single-space electronic feed intake recording equipment (**FIRE**; Osborne Industries, Osborne, KS) every alternate 2 weeks throughout the finishing period, as per the usual protocol on the farm. In the intervening weeks, when animals did not have access to FIRE feeders, feed was provided via a multi-spaced trough. Feed efficiency was calculated as test daily gain divided by daily feed intake. Animals were slaughtered at an average age of 178 d (SD 4.6) in a commercial abattoir. Hot carcass weights (kg) were collected, and back fat (mm) and loin depth (mm) were measured on the carcass at the tenth rib using Fat-O-Meter equipment (SFK, Hvidovre, Denmark).

4.2.4 Characteristics of the data

The characteristics of the data used in the analyses are presented in Table 4.1. Skin lesion traits showed skewed distributions; therefore these data were log transformed to approach a normal distribution. Additionally, data from the 7 genetic lines

represented in the population sample were pooled to achieve a sufficient sample size. Animals were weighed at time of mixing (28 kg, SD 4.6) and at the end of the test period, before slaughter (118 kg, SD 12.8).

Table 4.1 Descriptive statistics[1] for skin lesion, production and carcass traits

		Original scale					Transformed scale			
	Trait	**Mean**	**Min-Max**	**SD**	**SK**	**K**	**Mean**	**SD**	**SK**	**K**
SL24h	Anterior	16.65	0 – 94	14.15	1.42	2.62	2.50	1.00	-0.83	0.38
	Central	14.46	0 – 81	13.04	1.54	2.96	2.32	1.04	-0.76	0.04
	Posterior	8.44	0 – 51	7.98	1.64	3.28	1.86	0.95	-0.48	-0.42
SL5wk	Anterior	2.72	0 – 29	3.21	2.27	8.18	0.99	0.81	0.20	-1.00
	Central	2.20	0 – 28	3.00	2.55	9.75	0.84	0.77	0.47	-0.73
	Posterior	1.35	0 – 19	2.17	3.03	13.22	0.59	0.67	0.87	-0.07
Production	Lifetime daily gain, g/d	684.99	435 - 894	76.76	-0.08	-0.25	-	-	-	-
	Test daily gain, g/d	877.36	477 - 1222	120.17	-0.12	-0.11	-	-	-	-
	Daily feed intake, g/d	2248.40	1380 - 3420	318.20	0.15	-0.18	-	-	-	-
	Feed efficiency[3]	0.40	0.28 – 0.62	0.04	0.78	1.87	-	-	-	-
Carcass	Back fat, mm	18.01	7 – 37	4.65	0.60	0.30	-	-	-	-
	Loin depth, mm	62.18	36 – 89	8.97	0.06	-0.22	-	-	-	-
	Hot carcass weight, kg	92.08	62 - 128	9.88	0.03	-0.18	-	-	-	-

[1] SK = skewness; K = kurtosis

[2] SL24h = skin lesions recorded 24 hours post mixing; SL5wk = skin lesions recorded 5 weeks post mixing

[3] Feed efficiency is calculated as test daily gain (g) divided by daily feed intake (g)

4.2.5 Statistical Analyses

Genetic and environmental variance components were estimated via a series of univariate analyses using the following animal model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e},$$

where **y** is the vector of recorded traits, and the vectors **b, a**, **c** and **e** represent the vectors of the fixed effects, additive genetic effects, common environmental effects (the pens the animals were mixed into), and residual error, respectively, and **X**, **Z** and **W** are the incidence matrices of fixed, additive genetic, and common environmental effects. The fixed effect vector **b** contains the genetic line and sex effects for all traits, except for skin lesions recorded 5 weeks post mixing (SL5wk) where the sex effect was not significant. Additional fixed effects were only included in the model when they showed significant ($P < 0.05$) influences on the trait. For skin lesions recorded 24 hours post mixing (SL24h), batch (pen groups that were mixed on the same day), and line*age at mixing were included as additional fixed effects, whereas age and weight at point of mixing were fitted as linear covariates. Eight pen groups were mixed within each batch, so there were time differences between lesion counting of the first and last pen groups, during which aggression could still occur. The time difference in lesion counting was therefore also included as a linear covariate for SL24h. For SL5wk, batch was included as an additional fixed effect in the model whereas age of each animal at time of lesion counting was included as a linear covariate. Weight at mixing had no effect on SL5wk ($P > 0.05$) and therefore was not considered in the model. The production traits test daily gain, daily feed intake and feed efficiency were significantly influenced by batch, weight at time of mixing (start weight), and age at the end of the test period, which were additionally included in the model. The model for lifetime daily gain included batch, and age at time of mixing. For all carcass traits (loin depth, back fat, and hot carcass weight), the date of slaughter was additionally considered in the model. Furthermore,

the carcass traits loin depth and back fat were adjusted for hot carcass weight using a linear covariate. The variance-covariance structure of the fitted model was as follows:

$$\mathbf{V}\begin{bmatrix}\boldsymbol{a}\\ \boldsymbol{c}\\ \boldsymbol{e}\end{bmatrix} = \begin{bmatrix}\boldsymbol{A}\otimes\boldsymbol{G} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \boldsymbol{I}\otimes\boldsymbol{C} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \boldsymbol{I}\otimes\boldsymbol{R}\end{bmatrix},$$

where **G**, **C** and **R** represent the variance-covariance matrices of the genetic effects, common environmental pen effects, and residual environmental effects, respectively. **A** and **I** are the additive genetic relationship matrix and identity matrix, respectively. A series of bivariate analyses were used to estimate genetic and phenotypic correlations between skin lesion traits and production traits. Fixed and random effects fitted for each trait were based on the univariate analyses as described above. All genetic analyses were performed using the program ASReml (Gilmour et al., 2009).

4.3 Results

4.3.1 Heritabilities and common environmental effects

The phenotypic proportion of the variance attributed to genetic and common environmental (pen group) effects are presented in Table 4.2. The heritabilities of skin lesions traits were of a low to moderate magnitude, with the lowest value estimated for anterior SL24h (0.08, SE 0.03) and the greatest for central SL5wk (0.22, SE 0.04). Skin lesion heritabilities were greater under stable social housing conditions (SL5wk) than in newly mixed groups (SL24h). The phenotypic fraction of the variance attributed to pen effects was significant and showed low variation among lesions traits, ranging from 11% to 14%. Phenotypic variation was greater for SL24h than for SL5wk. Heritabilities for production and carcass traits were greater than those found for skin lesions, with the lowest genetic influence estimated for feed efficiency (0.26, SE 0.05) and the greatest for back fat (0.65, SE 0.06). Common pen effects were significant for all production traits but non-significant for the carcass traits loin depth and back fat (Table 4.2).

Table 4.2 Heritabilities (h^2), phenotypic fractions of pen group effects (c^2), and phenotypic variances (σ^2_p) for skin lesion, production and carcass traits (standard errors presented in parentheses)

	Trait	h^2	c^2	σ^2_p
SL24h	Anterior	0.08 (0.03)	0.13 (0.02)	0.78
	Central	0.11 (0.04)	0.12 (0.02)	0.84
	Posterior	0.12 (0.03)	0.14 (0.02)	0.71
SL5wk	Anterior	0.18 (0.04)	0.11 (0.02)	0.49
	Central	0.22 (0.04)	0.13 (0.02)	0.47
	Posterior	0.16 (0.04)	0.13 (0.02)	0.37
Production	Lifetime daily gain, g/d	0.55 (0.06)	0.06 (0.01)	9772
	Test daily gain, g/d	0.48 (0.06)	0.09 (0.02)	3780
	Daily feed intake, g/d	0.40 (0.06)	0.19 (0.03)	67232
	Feed efficiency[2]	0.26 (0.05)	0.23 (0.03)	0.002
Carcass	Back fat, mm	0.65 (0.06)	0.02 (0.01)	53.49
	Loin depth, mm	0.28 (0.05)	0.00 (0.01)	12.96
	Hot carcass weight, kg	0.53 (0.06)	0.06 (0.01)	68.61

[1] SL24h = skin lesions recorded 24 hours post mixing; SL5wk = skin lesions recorded 5 weeks post mixing

[2] Feed efficiency is calculated as test daily gain (g) divided by daily feed intake (g)

4.3.2 Genetic and phenotypic correlations

Within each time point (24 hours or 5 weeks post mixing) genetic correlations for skin lesions across body regions were positive and high (Table 4.3). Generally, genetic correlations between lesion traits 5 weeks post mixing were greater than 24 hours after mixing. Anterior and central SL5wk showed the strongest correlation (0.99, SE 0.06), and anterior and posterior SL24 showed the weakest (0.66, SE 0.16). Phenotypic correlations for skin lesions between body regions were lower than genetic correlations, although still significant, with the greatest value for central and posterior SL24h (0.56, SE 0.02) and lowest for anterior and posterior SL5wk (0.39, SE 0.02) Table 4.3). Pen level correlations between skin lesion traits across body regions at the same time point were significant for all traits (0.66 to 0.97), and of a greater magnitude than phenotypic correlations between these traits (Table 4.3).

Table 4.3 Estimated genetic, phenotypic and group pen level correlations[1] among skin lesion (SL) traits across 3 body regions recorded at 24 hours (SL24h) or 5 weeks (SL5wk) post mixing, with standard errors presented in parentheses

	SL trait	SL24h		SL5wk	
		Central	Posterior	Central	Posterior
Genetic correlations	Anterior	**0.92 (0.12)**	**0.66 (0.16)**	**0.99 (0.06)**	**0.86 (0.10)**
	Central	-	**0.83 (0.11)**	-	**0.81 (0.10)**
Phenotypic correlations	Anterior	**0.55 (0.02)**	**0.40 (0.02)**	**0.49 (0.02)**	**0.39 (0.02)**
	Central	-	**0.56 (0.02)**	-	**0.52 (0.02)**
Pen group correlations	Anterior	**0.75 (0.07)**	**0.66 (0.09)**	**0.89 (0.05)**	**0.88 (0.06)**
	Central	**-**	**0.95 (0.04)**	**-**	**0.97 (0.03)**

[1] Bold font signifies correlation significantly different from 0

Between time points, genetic correlations for skin lesions were positive and of moderate to high magnitude (0.46, [SE 0.20] to 0.81 [SE 0.17]). The lowest estimated genetic correlation between time points was between posterior skin lesions, and the greatest was between central skin lesions (Table 4.4). Phenotypically, skin lesions from different time points were also positively correlated (0.09 [SE 0.03] to 0.13 [SE 0.03]), although the correlations were considerably lower than those estimated on the genetic level. No significant pen group level correlations were found between SL24h and SL5wk (data not presented). Other than between anterior SL24h and anterior SL5wk (0.07, SE 0.03), no significant residual correlations were found between skin lesion traits from different time points (Table 4.4).

Table 4.4 Genetic (r_G), phenotypic (r_P) and residual (r_R) correlations[1] between skin lesion traits recorded on the same body region 24 hours and 5 weeks post-mixing (standard errors presented in parentheses)

Body region	r_G	r_P	r_R
Anterior	**0.76 (0.21)**	**0.12 (0.03)**	**0.07 (0.03)**
Central	**0.81 (0.17)**	**0.13 (0.03)**	0.04 (0.04)
Posterior	**0.46 (0.20)**	**0.09 (0.03)**	0.06 (0.04)

[1] Bold font signifies correlation significantly different from 0

Genetic and phenotypic correlations between test daily gain and lifetime daily gain were 1; therefore correlations between skin lesion traits and test daily gain have not been presented. Central and anterior SL24h and all 3 body regions for SL5wk were positively genetically correlated with lifetime daily gain (Table 4.5). Phenotypic correlations between skin lesion traits and lifetime daily gain were non-significant ($P > 0.05$), with the exception of posterior SL5wk for which a small positive correlation was observed (0.10, SE 0.03). Negative residual correlations were found between lifetime daily gain and the skin lesion traits central SL24h (-0.15, SE 0.06), posterior SL24h (-0.14, SE 0.06), and posterior SL5wk (-0.14, SE 0.06).

Genetically, central SL24h, anterior and posterior SL5wk were correlated positively with feed efficiency (0.39 [SE 0.17] to 0.50 [SE 0.14]), indicating that the animals converting food more efficiently received more skin lesions. None of the skin lesion traits were genetically correlated with daily feed intake. On the phenotypic level, there was a low positive correlation between posterior SL5wk and daily feed intake (0.08, SE 0.03) (Table 4.5).

Table 4.5 Genetic (r_G), phenotypic (r_P) and residual (r_R) correlations[1] between skin lesion traits[2], and lifetime daily gain, daily feed intake or feed efficiency[3] (standard errors presented in parentheses)

Trait	Lifetime daily gain (g/d)			Daily feed intake (g/d)			Feed efficiency		
	r_G	r_P	r_R	r_G	r_P	r_R	r_G	r_P	r_R
SL24h									
Anterior	0.12 (0.18)	0.06 (0.03)	0.04 (0.05)	0.14 (0.19)	0.06 (0.03)	-0.02 (0.05)	-0.10 (0.21)	0.00 (0.03)	0.07 (0.04)
Central	**0.36 (0.15)**	0.00 (0.03)	**-0.15 (0.06)**	0.06 (0.17)	0.00 (0.03)	-0.05 (0.05)	**0.39 (0.17)**	-0.01 (0.03)	-0.07 (0.04)
Posterior	**0.36 (0.14)**	0.02 (0.03)	**-0.14 (0.06)**	0.13 (0.16)	0.03 (0.03)	-0.04 (0.05)	0.24 (0.17)	-0.01 (0.03)	-0.06 (0.04)
SL5wk									
Anterior	**0.31 (0.13)**	0.05 (0.03)	-0.09 (0.06)	0.11 (0.13)	0.03 (0.03)	-0.03 (0.05)	**0.39 (0.13)**	0.00 (0.03)	-0.10 (0.05)
Central	**0.29 (0.12)**	0.06 (0.03)	-0.08 (0.06)	0.13 (0.12)	0.05 (0.03)	0.01 (0.06)	0.25 (0.14)	0.00 (0.03)	-0.07 (0.05)
Posterior	**0.52 (0.11)**	**0.10 (0.03)**	**-0.14 (0.06)**	0.20 (0.14)	**0.08 (0.03)**	-0.01 (0.05)	**0.50 (0.14)**	-0.01 (0.03)	-0.08 (0.04)

[1] Bold font signifies correlation significantly different from 0

[2] SL24h = skin lesions recorded 24 hours post mixing; SL5wk = skin lesions recorded 5 weeks post mixing

[3] Feed efficiency is calculated as test daily gain (g) divided by daily feed intake (g)

Non-significant genetic and phenotypic correlations ($P > 0.05$) were estimated between all skin lesion traits and the carcass traits loin depth or back fat (Table 4.6). However, positive genetic correlations ($P < 0.05$) were estimated between hot carcass weight and all skin lesion traits other than anterior SL24h (0.29 [SE 0.12] to 0.54 [SE 0.11]). In addition, positive phenotypic correlations were found between hot carcass weight and anterior SL24 as well as all body regions for SL5wk (0.07 [SE 0.03] to 0.10 [SE 0.03]). Residual correlations between these traits were non-significant (Table 4.6).

Common environmental (pen group) correlations between skin lesion traits and production or carcass traits were generally non-significant, other than between anterior SL24h and daily feed intake and between posterior SL5wk and feed efficiency (Table 4.7).

Table 4.6 Genetic (r_G), phenotypic (r_P) and residual (r_R) correlations[1] between skin lesion traits[2], and loin depth, back fat or hot carcass weight (standard errors presented in parentheses)

Trait	Loin depth (mm)			Back fat (mm)			Hot carcass weight (kg)		
	r_G	r_P	r_R	r_G	r_P	r_R	r_G	r_P	r_R
SL24h									
Anterior	0.11 (0.20)	0.04 (0.02)	0.04 (0.04)	0.09 (0.18)	-0.02 (0.03)	-0.07 (0.06)	0.11 (0.19)	**0.07 (0.03)**	0.10 (0.05)
Central	0.30 (0.17)	0.02 (0.02)	-0.02 (0.04)	0.07 (0.16)	-0.01 (0.03)	-0.05 (0.06)	**0.33 (0.16)**	0.01 (0.03)	0.03 (0.05)
Posterior	-0.16 (0.18)	0.02 (0.02)	0.08 (0.04)	0.16 (0.15)	-0.01 (0.03)	-0.08 (0.06)	**0.34 (0.15)**	0.02 (0.03)	0.10 (0.05)
SL5wk									
Anterior	0.18 (0.14)	0.00 (0.02)	-0.04 (0.04)	-0.09 (0.12)	-0.03 (0.03)	-0.01 (0.06)	**0.38 (0.12)**	**0.07 (0.03)**	-0.01 (0.06)
Central	0.02 (0.14)	0.02 (0.02)	0.03 (0.04)	0.02 (0.12)	0.02 (0.03)	0.02 (0.07)	**0.29 (0.12)**	**0.07 (0.03)**	-0.01 (0.06)
Posterior	0.08 (0.15)	0.01 (0.02)	0.00 (0.04)	-0.08 (0.13)	-0.03 (0.03)	-0.02 (0.06)	**0.54 (0.11)**	**0.10 (0.03)**	0.01 (0.06)

[1] Bold font signifies correlation significantly different from 0

[2] SL24h = skin lesions recorded 24 hours post mixing; SL5wk = skin lesions recorded 5 weeks post mixing

Table 4.7 Common environmental (pen group) correlations[1] between skin lesions[2], lifetime daily gain, daily feed intake, feed efficiency[3], loin depth, back fat or hot carcass weight (standard errors presented in parentheses)

Trait	Lifetime daily gain (g/d)	Daily feed intake (g/d)	Feed efficiency	Loin depth (mm)	Back fat (mm)	Hot carcass weight (kg)
SL24h						
Anterior	0.10 (0.16)	**0.30 (0.12)**	0.22 (0.12)	-0.17 (0.52)	-0.14 (0.23)	0.19 (0.16)
Central	-0.12 (0.16)	0.13 (0.13)	0.17 (0.13)	-0.73 (0.85)	-0.02 (0.23)	-0.15 (0.17)
Posterior	0.01 (0.16)	0.16 (0.13)	0.09 (0.12)	-0.48 (0.70)	-0.24 (0.23)	-0.14 (0.16)
SL5wk						
Anterior	-0.02 (0.16)	0.10 (0.13)	0.24 (0.12)	-0.80 (0.98)	0.10 (0.22)	0.06 (0.16)
Central	0.00 (0.15)	0.03 (0.12)	0.13 (0.12)	-0.10 (0.45)	0.07 (0.21)	0.08 (0.15)
Posterior	-0.01 (0.16)	0.20 (0.12)	**0.38 (0.11)**	-0.07 (0.46)	0.08 (0.21)	0.08 (0.16)

[1] Bold font signifies correlation significantly different from 0

[2] SL24h = skin lesions recorded 24 hours post mixing; SL5wk = skin lesions recorded 5 weeks post mixing

[3] Feed efficiency is calculated as test daily gain (g) divided by daily feed intake (g)

4.4 Discussion

4.4.1 Heritability and correlations of skin lesion traits

The heritabilities of skin lesion traits were in a range that suggests that skin lesions and the associated aggressive behaviour can be reduced by selection. The heritability of posterior SL24h was identical to that estimated by Turner et al. (2008), and the heritability for posterior SL5wk was similar to that reported in a later study by the same group (Turner et al., 2009). Heritabilities for anterior and central SL24h and anterior SL5wk were less than half of those estimated in the aforementioned studies, despite similar lesion numbers and trait variance found in all 3 studies (Turner et al., 2008 only estimated h^2 for SL24h). Variance attributed to pen group effects was slightly greater in the current study, but could only partly explain the lower magnitude of skin lesion heritabilities. Although the overall sample size was greater in the present study, data from 7 genetic lines were utilized, a factor that was included as a fixed effect in all models. Genetic line alone accounted for between 4% and 8% of the variation in skin lesions numbers, and line was found to be a highly significant factor in all skin lesion models. In contrast, Turner et al. (2008 & 2009) pooled data from purebred Yorkshire and Yorkshire x Landrace pigs, and found no significant differences between the 2 lines, most likely because these lines were more similar than those in the present study. Repeating the univariate analyses for skin lesion traits without controlling for line on the present data produced heritability estimates that were very similar to those estimated by Turner et al. (2009). Heritabilities of skin lesions were greater at 5 weeks post mixing than at 24 h, a result that was also found previously (Turner et al., 2009). Genetic correlations between skin lesions on different body regions at the same time point were high and similar to those found previously (Turner et al. 2009). Although the genetic correlations suggest that anterior and central lesions are very similar traits,

these body regions were analysed separately because the results presented in the previous chapter, as well as previously published research (Turner et al., 2009) showed that they are due to different underlying behavioural traits, as discussed in more detail in a later section. Moreover, from a practical perspective, the recording of lesions on a single body region would substantially reduce the amount of time required to record these phenotypes.

Phenotypically, there was a weaker relationship between skin lesions across body regions, although the relationship was still positive. Under stable social conditions, aggression is less likely to be influenced by environmental factors such as physical disruption, fatigue or environmental novelty. Additionally, lesions at this time point reflect stable levels of aggression experienced by animals in a long-term social environment. Between lesions at mixing and in the stable group, strong genetic correlations were found for anterior and central lesions but only moderate correlations were found for posterior lesions. While phenotypic correlations of lesion traits between these time points were also positive, these correlations were of a much lower magnitude than the genetic correlations. This suggests that skin lesions are a genetically stable trait over time, whereas the environmental effects at mixing and in the stable group seem to be large, resulting in low phenotypic correlations.

4.4.2 Common group environment

Common environmental effects (pen group) significantly contributed to variance for all skin lesion traits. Pen group effects were of a greater magnitude than the estimated genetic components for SL24h, indicating that common environmental effects played a more important role in aggression at mixing than genetic effects. Group level variations in skin lesion numbers will depend on differences - such as the temperamental

disposition - between the individuals within a group; therefore group level variance is unsurprising for these traits.

Pen level correlations between body regions at the same time point were of a high magnitude, however pen group correlations between SL24h and SL5wk were close to zero. In a population of a different breeding organization, Turner et al. (2009) found low pen group level correlations between corresponding traits of SL24h and SL3wk in the range of -0.09 to 0.22, suggesting that aggression experienced at the pen group level is less stable over time. The proportion of the variance attributed to pen effects, and pen level correlations within each time point were similar in both studies, however there was no evidence of stability in pen level lesion numbers across time points in the current study.

Pen group effects also significantly contributed to the phenotypic variance for all production traits except for the carcass traits loin depth and back fat. Variance attributed to common environmental effects was greatest for daily feed intake and feed efficiency. There were no pen level associations between skin lesion traits and production traits, other than anterior SL24h and daily feed intake, and posterior SL5wk and feed efficiency. Social interactions are likely to affect group level observations. It is known that social interactions affect feeding behaviour and intake on a phenotypic level. For example the majority of aggressive behaviour in socially stable groups has been found to occur in and around the feeding area, with socially dominant pigs making fewer and longer feeder visits (Chapinal et al., 2008; Hoy et al., 2012). Social housing also increases variation in feeding behaviour between individuals, compared to individually housed pigs (de Haer and Merks, 1992; Nielsen et al., 1996), suggesting that interactions between individuals affects behaviour on a group wide level. In the

current study, even though a significant proportion of the variance could be attributed to pen group effects, there were few significant correlations ($P < 0.05$) between skin lesion traits with growth, feed, or carcass traits due to the pen effect. Nielsen et al. (1996) found no phenotypic relationship between dominance rank or aggression and feeding behaviour. It is likely that each individual animal affects the phenotypes of others in the group via both positive and negative social interactions (Camerlink et al., 2012). This might explain why correlations at pen level were low between skin lesions – a measure of negative social interactions – and production traits.

4.4.3 Phenotypic correlations

In the current study, there was little to no phenotypic relationship between skin lesions and growth, feed intake or carcass traits. One likely reason is that lesions are influenced by the environment over 24h whereas production traits are affected by the environment over the entire growing period. Several phenotypic studies have shown that social aggression can negatively affect growth and feeding efficiency upon first mixing, however these effects are usually only temporary (Tan et al., 1991; Rundgren and Löfquist, 2010). By weighing the animals each day, Tan et al. (1991) were able to dissect the effects of social mixing on average daily gain over a period of 3 weeks. Although growth was depressed in mixed groups during the first week of the experiment, by the end of the 3 weeks there was no difference in average daily gain between mixed and unmixed groups. It is therefore possible that growth was temporarily affected after mixing in this population however the effects could not be detected over the duration of the test period.

As demonstrated in the above study, it is advantageous to use unmixed control groups as a comparison when studying the effects of social aggression on growth. In addition to mixed and unmixed control treatments, Stookey and Gonyou (1994) added a third

treatment, in which animals were mixed for 24 hours and then reunited with familiar pen mates. Over a 2 week period, the groups that only experienced temporary mixing gained significantly less weight than the unmixed control groups, despite aggression almost returning to levels observed in the unmixed groups once animals were returned to their original pens. It could be that the experience of mixing, and not necessarily aggression itself, contributes to observed phenotypic reductions in growth. For example, Hyun et al. (1998) found that mixing temporarily altered feeding patterns, which they suggest partially depressed growth rate during this period. All animals in the current study experienced mixing therefore there was no control group with which to compare growth.

4.4.4 Genetic correlations

Very few studies have incorporated genetic effects when examining interactions between social aggression and production traits. Turner et al. (2006) investigated the genetic relationship between total numbers of skin lesions at mixing and daily weight gain or back fat depth, but found no significant correlations. In that study, total skin lesion numbers were found to correlate highly with anterior lesions, therefore it was assumed that animals that engage in much reciprocal aggression also receive many lesions to other body areas. For this reason, skin lesions from the entire body were totalled for all genetic analyses. The decision to pool body regions may have affected the outcome of the analysis, as the majority of lesions at mixing are received on the anterior region of the body; however anterior SL24h did not correlate with any production traits in the present study. Velie et al. (2009) studied the genetic and phenotypic relationships between aggression, as measured by a resident intruder test, and the following production and carcass traits: body weight (1 and 21 d of age), average daily gain, back fat and loin muscle area in 486 growing pigs. No genetic

relationship was found between attack latency or number of attacks given and any of the performance traits, which may have been due to the small sample size.

To date there are no publications that have investigated the associations between aggression in socially stable groups and of production and carcass traits, as presented in this study. The direction of the genetic correlations of skin lesion traits central and posterior SL24 and SL5wk with the production traits lifetime daily gain and hot carcass weight suggest that, genetically, animals that receive many lesions grow faster and have an increased hot carcass weight. In addition, animals that receive many lesions to the central region of the body 24 hours post mixing, or anterior and posterior regions of the body under stable social conditions were found to have increased feed efficiency. On a phenotypic level, these correlations are very small, when present at all. The direction of the residual correlations suggests that genetic rather than environmental factors influenced the phenotypic correlations, where significant.

Positive genetic relationships between skin lesions and production traits could suggest that aggression may be beneficial from a production standpoint. However, anterior SL24h is the skin lesion trait with the strongest positive genetic and phenotypic association with involvement in high levels of aggression (Turner et al., 2009; Desire et al., 2015), but was the only skin lesion trait in the present study not genetically correlated with any production or carcass trait. To dissect the association between aggression and production further, the problem must be considered from a behavioural perspective. Although several studies have found a relationship between skin lesion traits and aggression (Barnett et al., 1996; Spoolder et al., 2000), the only dataset that allows us to look at the relationship between aggression and skin lesions across time on a genetic level is Turner et al. (2009). This study found that individuals which

initiated many one-sided attacks (bullying behaviour) had many anterior lesions at mixing, but had few lesions to the centre and posterior regions of the body, the areas that receive the brunt of non-reciprocal attacks. It is likely that these individuals are dominant animals, as non-reciprocal attacks are often directed towards the loser of a fight as a means to reinforce a fight outcome. This theory is supported by the fact that these animals also had few lesions under stable social conditions, as would be expected for dominant animals. The study also found that animals that are genetically predisposed to engage in high levels of reciprocal aggression at mixing initially had many lesions to the anterior of the body at mixing, but had few lesions under stable conditions. Conversely, animals that avoided aggression at mixing had few anterior lesions at mixing, and had many lesions across all body regions in stable groups. Tönepöhl et al. (2013) also investigated the relationship between aggression at mixing and skin lesions several weeks later. The results of that study differed to those described above, in that they did not find a relationship between the number of initiated attacks at mixing and skin lesions recorded several weeks later, and very weak tendency for animals that received much aggression at mixing to have fewer lesions after several weeks. The methodology used by Tönepöhl et al. (2013) differed considerably from that used in Turner et al. (2009), in that aggressive behaviour was observed in dynamic groups of differing sizes (between 10 and 20 sows), however skin lesion numbers were recorded after sows had been mixed into larger pen groups (up to 52 sows). As sows are very likely to have fought once mixed into the larger group, and are unlikely to have gained the same position in the social hierarchy, it is understandable that few associations were found between skin lesions recorded under these conditions and behaviour recorded in smaller groups.

If the behavioural results reported in Turner et al. (2009) can be extrapolated to the population represented in the present study, it is possible that the animals that receive the most lesions to the centre and posterior regions of the body at 24 hours after mixing and all body regions in stable groups are actually the least aggressive individuals. Rauw et al. (1998) argue that selection for increased production in farm animals may limit the internal resources available for processes other than growth and reproduction, for example movement or reaction to stress, resulting in inadvertent selection for unfavourable traits. With this in mind, it could be that the animals that avoid aggression have more resources left for growth, which may explain the direction of the genetic correlations estimated in the present study.

If increased lesions in stable groups are indicative of animals that are less aggressive at mixing, then these results suggest that selection for increased lesions within stable groups would not only reduce mixing aggression, but improve performance traits. This strategy seems contradictory to the aim to reduce lesions, however, based on the genetic correlations, it is expected that selection for increased lesion scores should reduce the amount of aggression experienced by subordinate animals, and in turn the average skin lesion scores of the population in the next generation. Lesions recorded in stable groups have greater heritabilities and therefore have a greater potential for selection response. In addition, there are fewer lesions at this time point, which would decrease the time required for recording.

Selection for reduced aggression based on increased stable lesions is based on the theory that animals that have more lesions at this time are unaggressive. This assumption has been made based on correlations between behaviour at mixing and skin lesions 5 weeks post mixing. Skin lesions at 5 weeks are an indication of which

animals have received aggression, however cannot be used to infer which animals initiate the aggression. It is possible that animals that receive lesions under socially stable conditions have not learned socially acceptable behaviours, or are unable to convey submissive behaviours, and receive attacks as a result of this. In this case selecting for increased lesions in stable groups may select for unwanted phenotypes.

Although skin lesion traits show greater heritabilities under stable social conditions, receipt of aggression at mixing has shown consistently lower heritabilities, compared to involvement in reciprocal aggression. Løvendahl et al. (2005) found heritabilities of between 0.04 and 0.06 for received aggression in newly mixed sows, compared to heritabilities of between 0.17 and 0.24 for initiated aggression. Turner et al. (2009) reported heritabilities of 0.08 for receipt of non-reciprocal aggression, and 0.43 for involvement in reciprocal aggression. Similarly, Stukenborg et al. (2012) calculated heritabilities of between 0.06 and 0.07 for received aggression, and 0.06 and 0.20 for initiated aggression for newly weaned pigs, however heritabilities for receipt of aggression in growing pigs (40 d post weaning) were greater (between 0.15 and 0.22). A clearer understanding of the relationship between aggression and skin lesions both in newly mixed and stable groups would be beneficial.

The ethical implications of selecting for increased skin lesions in stable groups are unclear. Mixing and aggression related stress tend to be quantified via physiological responses, which can themselves be influenced be several factors (Salak-Johnson and McGlone, 2007). Mendl et al. (1992) measured basal cortisol in gilts 5 weeks after mixing, at which point it would be expected that social relationships would be stable. This study found that those individuals of intermediate social rank (determined by their ability to displace others in agonistic interactions) had higher cortisol levels than

low or high ranking individuals. In contrast, Otten et al. (1997) measured cortisol levels within 24 hours of mixing and found that high ranking animals engaged in high levels of aggression at this time, and have higher levels of cortisol as a result. Couret et al., (2009) found that mixed animals had higher levels of salivary cortisol within 24 hours of mixing compared to unmixed controls, however found no difference between high, medium or low success individuals. Several other studies have demonstrated an increase in salivary cortisol following regrouping events (Tsuma et al., 1996; Olsson and Svendsen, 1997; Jarvis et al., 2006; Coutellier et al., 2007). Studies investigating the relationship between social rank and immunological responses to stress were reviewed by Salak-Johnson and McGlone (2007), who concluded that social stress may supress or enhance immune responses, depending on age, genetics, social status, or the state of the immune system prior to the stressful event. Aggression aside, it is likely that the act of regrouping itself is stressful. Regrouping can be chaotic and usually involves moving individuals from a familiar environment with an established social structure to an unfamiliar environment with uncertain social relationships. Given the difficulties posed when attempting to disentangle the effects of regrouping, involvement in aggression, and social status, and the fact that aggression is always studied in relation to mixing events or staged resident-intruder interactions, it is impossible to determine whether subordination and stress are linked in a manner that is unrelated to physical involvement in aggression, i.e. whether subordinate animals are always more fearful or stressed, even in the absence of aggressive individuals. If this was the case, then it deserves further attention, regardless of whether aggression is reduced via selection for reduced skin lesions at mixing, or increased skin lesions in socially stable groups, as the goal in either case would be to reduce aggressive genotypes within the population. In the current population, animals with greater stable skin lesions showed improved

performance on a genetic level; therefore low social rank does not appear to be genetically linked to poor performance.

4.4.5 Conclusions

The results presented in the current study suggest that lesions at mixing have a lower heritability compared to lesions obtained in the stable group. This may be due to the fact that lesion in stable groups are a measure of baseline aggression and are less affected by environmental noise, or by the specific combination of certain unfamiliar pigs present in a mixed group. With the exception of anterior lesions at mixing, increased lesions scores in the stable groups and at mixing showed consistently positive genetic correlations with growth rate and feed efficiency. Anterior SL24h have previously been shown to have a strong genetic correlation with aggressive behaviour at mixing, but the low genetic correlations between anterior lesions and performance traits found here suggest that selection for reduced anterior lesions at mixing would not influence the analysed performance traits. Anterior lesions recorded 24 hours post mixing have previously been positively associated with non-reciprocal (bullying) aggression against other individuals within a group. This result contrasts with the genetic correlations between non-reciprocal aggression and skin lesions on other regions of the body, which were all negative. This suggests that anterior SL24h would be the only viable trait for use in selection against both reciprocal and non-reciprocal aggression. Individuals that receive many lesions under socially stable conditions are likely to exhibit low levels of aggression at mixing. It may therefore be possible to reduce aggression via selection for increased skin lesions within socially stable groups. While selecting for increased stable lesions may technically reduce aggression, it cannot be recommended without a clearer understanding of the relationships between skin lesions and behaviour both at mixing and in stable groups, as well as the affective state of unaggressive individuals. In reality, the most efficient selection strategy would

likely be a multiple trait index with the goal of reducing anterior lesions at mixing, assuming no behaviour traits are recorded in the population. The mean of 39.6 lesions (sum of all body areas) found at mixing was almost 7 times greater than the stable group mean of 5.82 lesions. From an animal welfare perspective, it thus seems sensible to focus on reducing this much more damaging mixing aggression, particularly because mixing can occur at several times in the production cycle for both sows and weaner-grower-finisher pigs.

Chapter 5 - Genetic associations between human-directed behaviour and intraspecific social aggression in growing pigs

5.1 Introduction

This thesis has focused on skin lesions as a predictor of aggressive behaviour, both immediately after mixing, and in socially stable groups. It is important that selection against aggression does not result in inadvertent selection for traits that might adversely affect welfare, such as an increased fear response. Behaviours thought to measure fearfulness and the ability cope in stressful situations have been previously shown to have low to moderate heritabilities in several species (Haskell et al., 2014; D'Eath et al., 2009) and it is likely that these behaviours are genetically associated with social aggression. For example, D'Eath et al. (2009) found a genetic correlation between movement and vocalisations during weighing and aggressive behaviour at mixing, suggesting a shared genetic basis between reaction to human handling and intraspecific aggression. The objective of this study was to determine whether skin lesions recorded 24 hours and 5 weeks post-mixing are genetically or phenotypically correlated with fear related behavioural reactions to: a) a human observer in the home pen environment, b) approach by a human while isolated from pen mates, and c) while restrained in isolation in a weighing crate.

5.2 Methods

5.2.1 Animals and housing

Experimental animals were those used in Chapter 4, therefore husbandry and mixing procedures, as well as the protocol for recording of skin lesions were are as outlined previously. On the day animals were mixed into new groups, pigs were weighed by farm staff using the same weighing crate used during the behavioural experiments described in this study. Due to the time consuming nature of the behavioural experiments, it was not possible to collect behavioural data on all of the individuals represented in Chapter 4.

5.2.2 Crate response and individual human test

A single observer handled and tested the pigs throughout all of the described procedures. Figure 5.1 illustrates the experimental arena for the following experiment. Either 1 or 2 days after mixing (due to time constraints not all animals could be tested on the same day), each pen of pigs was transferred from the home pen by a single handler using a plastic stock board into a holding pen located adjacent to a weighing crate (A in Figure 5.1). Each pig was then individually moved into the weighing crate using a plastic stock board (B in Figure 5.1), where they were scored for signs of tail and ear biting (ear and tail scores not presented). While isolated in the crate each pig was given a single score from 1 to 4 based on restlessness while in the crate. Table 5.1 shows the scoring system used for this portion of the test. This test will be referred to as the 'crate response' in the remainder of this chapter.

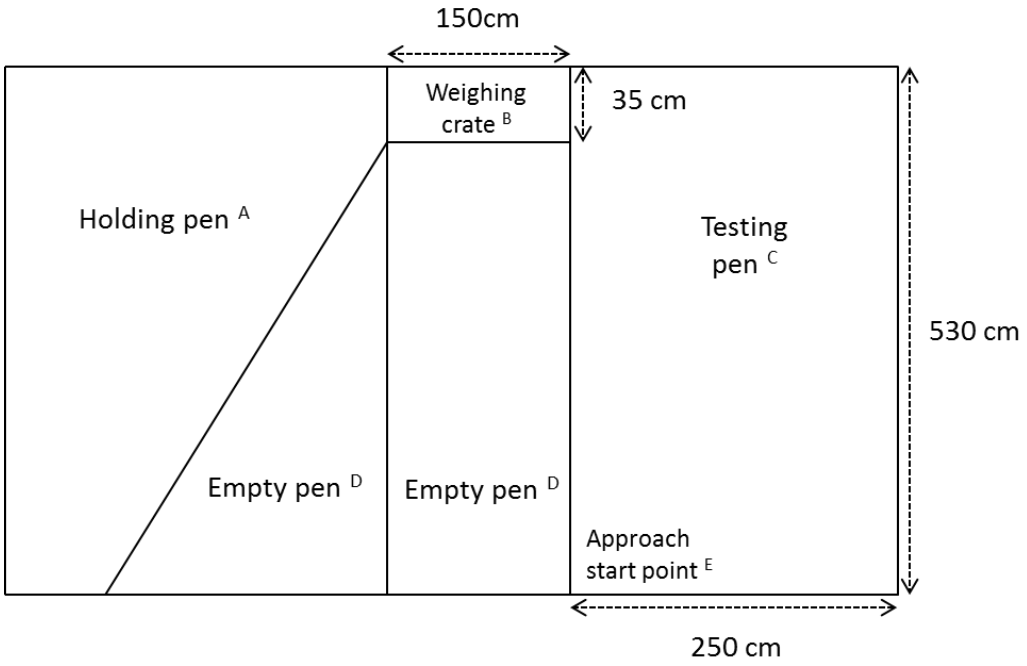


Figure 5.1 Diagram illustrating the layout of the testing area for the individual human test (not to scale). The entire group of pigs were held in pen A. Each pig was individually moved to the weighing crate and their behavioural response recorded. After approximately 1 minute each pig was then moved to the testing pen (C) and the behavioural response to a human walking towards them from area E was recorded. Pigs were returned to the holding pen with the rest of the group after testing.

Table 5.1 Description of scoring system for behaviour of pigs while isolated in a weigh crate

Score	Description
1	Exploratory behaviour including sniffing and rooting of the crate floor and walls
2	Shifting from side to side, attempts to turn.
3	Vigorous movements, attempts to escape by, turning or running backwards and forwards
4	Serious, persistent attempts to escape by jumping over crate wall

After approximately 1 minute the pig was released into an empty testing pen (C in Figure 5.1). While in the testing pen individuals were able to see pen mates, they were not able to physically touch them due to 2 empty pens (D in Figure 5.1) between the holding and isolation pens. Approximately 30 seconds after the pig entered the pen, starting in the same corner of the pen each time (E in Figure 5.1), the observer walked towards the pig at a steady pace, and recorded the animal's reaction to their approach.

Three separate scores were given for each individual based on the severity of movement, vocalisations, and vigilance. Table 5.2 describes the scoring system for each behaviour.

Table 5.2 Description of scoring system for behavioural reactions of pigs isolated in a pen to a human approach

Reaction to human approach	Score			
	0	**1**	**2**	**3**
Movement	None	Walk	Trot	Run
Vocalisation	None	Quiet grunts	Loud grunts/squeals	-
Vigilance	None	Medium (occasional glances at human)	High (completely focused on human)	-

After each animal had been tested it was marked with spray paint and moved back into the holding pen (A in Figure 5.1). This test will be referred to as the 'individual human test' in the text.

5.2.3 Group human approach test

Pigs were tested for behavioural responses to the presence of a single human observer in the home pen (approximately 2.1 x 5.6m) at two time points during the growth period (on average 6 days (SD 4.9) and 25 (SD 15.9) days post-mixing). To begin the test the observer entered the pen by climbing over the gate, and walked once around the perimeter of the pen at a normal speed to ensure all animals were alert and aware of the human presence. The observer then walked around the pen a second time, and noted the identity (via ear tags) of each pig that followed the observer for more than 0.5 laps of the pen. At the end of the second lap the observer paused for 1 minute and noted individuals that 1) nosed or rooted at the observer's boots or legs, or 2) bit at the observers legs. This resulted in a binary trait for each behaviour, and for brevity these

behaviours are referred to in the text as 'follow', 'nose', and 'bite', while the test itself is referred to as 'group human approach test'.

5.2.4 Characteristics of the data

Descriptive statistics for behavioural responses are presented in Table 5.3 and Table 5.4.

Table 5.3 Descriptive statistics for behavioural responses. Response to isolation in a crate (crate response) was scored from 1 to 5 based on severity of movement while isolated in a crate. Severity of behavioural reaction to a human approaching the pig (individual human test) was recorded for speed of movement (scored 0-3), vocalisation pitch (scored 0–2), and vigilance towards the human (scored 0-2)

Behaviour	N	Mean	SD	Skewness	Kurtosis
Crate response	1863	2.18	0.83	0.33	-0.41
Individual human test					
Movement	2035	1.95	0.72	-0.30	-0.13
Vocalisation	2035	0.76	0.76	0.43	-1.17
Vigilance	2035	0.78	0.67	0.28	-0.79

Table 5.4 Proportion of animals performing each behaviour during the group human approach test. Tests were taken at 2 time points (denoted by 1 and 2).

Trait	N	Proportion*
Bite [1]	2023	0.13
Bite [2]	2407	0.29
Follow [1]	2023	0.08
Follow [2]	2407	0.14
Nose [1]	2023	0.13
Nose [2]	2407	0.12

* Proportion of animals performing each behaviour (i.e. scored as 1)

Bite = bit observer on the leg; Follow = followed observer for >0.5 laps of the pen; Nose = persistent nosing or rooting at observers legs or boots, without biting.

5.2.5 Statistical analysis

Skin lesions

Although a smaller subset of animals was used in the present experiment, variance components for skin lesions were not re-calculated in this cohort. The methodology for calculating variance components for skin lesions is detailed in Chapter 3.

Behavioural tests

Crate response and individual human test

All genetic analyses were performed using the program ASReml (Gilmour et al., 2009). Although the crate response, and individual human tests were scored on an ordinal scale, the skewness and kurtosis of the data indicated that the traits followed an approximately normal distribution. Genetic and environmental variance components were therefore estimated via a series of univariate analyses using the following animal model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e},$$

where **y** is the vector of recorded traits, and the vectors **b, a**, **c** and **e** represent the vectors of the fixed effects, additive genetic effects, common environmental effects (the pens the animals were mixed into), and residual error, respectively, and **X**, **Z** and **W** are the incidence matrices of fixed, additive genetic, and common environmental effects. The fixed effect vector **b** contains the genetic line, sex, the order the animals were tested, and batch effects for all traits. Body weight at mixing was fitted as a linear covariate. A series of bivariate analyses were used to estimate genetic and phenotypic correlations between skin lesion traits and behavioural traits. Fixed and random effects fitted for behavioural traits were based on the univariate analyses as described above, while effects for skin lesions were fitted based on univariate analyses described in Chapter 4.

Group human approach test

A sire model was applied using the logit link function for binary behavioural traits. Fixed effects were fitted as described above, and tested using the Wald test (Gilmour et al., 2009). In the bivariate analyses, the logit link function was only applied to the binary trait. The pens the animals were mixed into have been included as an additional random effect in mixed models throughout this thesis. Pen did not account for a significant proportion of the variance for group human approach responses, however pen effects are known to account for variation in skin lesion traits (see chapter 3). Bivariate analyses between skin lesions and group human approach responses failed to converge when pen was included as a random effect. Attempts were made to fit pen as a fixed effect for skin lesion traits in order include variation due to this effect in the model, however the model repeatedly failed. Due to time constraints the decision was made to exclude pen effects from the final models for these traits. The implications of this are discussed later in the chapter.

Group human approach responses were performed at two time points. To test for repeatability across time points, phi coefficients (Φ) were calculated for each trait using animals with behavioural scores at both time points (not all animals were tested at time point 1 therefore these animals could not be included in this calculation).

Table 5.5 Data structure used to test for repeatability (Φ coefficients) of binary behavioural responses to a group human approach test across two time points. The letters a, b, c, and d represent the summed combination of responses for each test (e.g. a = total number of animals that scored 1 for a given behaviour across both tests).

		Test 2 response	
		1	**0**
Test 1 response	**1**	a	b
	0	c	d

Table 5.5 illustrates how each behavioural response across tests was summed. This information was used to calculate phi coefficients for each behaviour as follows:

$$\boldsymbol{\Phi} = \frac{\boldsymbol{ad} - \boldsymbol{bc}}{\sqrt{[(\boldsymbol{a}+\boldsymbol{b})(\boldsymbol{c}+\boldsymbol{d})(\boldsymbol{a}+\boldsymbol{c})(\boldsymbol{b}+\boldsymbol{d})]}}$$

The significance of Φ was determined via a chi-square (χ^2) test calculated as:

$$\chi^2 = \mathrm{N}\Phi^2$$

where N is the sum of *a*, *b*, c and d (Table 5.5). Significance was determined using a chi-square distribution table with 1 degree of freedom.

5.3 Results

5.3.1 Systematic effects

For crate responses and individual human tests batch effects accounted for between 2 and 6% of the variation, while genetic line accounted for between 2 and 3% of the variation. Age had a significant effect on the speed of movement away from the human observer, however it only accounted for approximately 1% of the variation in this trait. Neither body weight nor the order the animals were tested in accounted for a significant proportion of the variance in any of the behaviour traits tested. Batch (week the animals were mixed) was the only fixed effect found to have a significant ($P < 0.05$) effect on group human approach responses.

5.3.2 Repeatability of responses to group human approach

Behavioural responses to a group human approach showed repeatability across tests (Φ between 0.11 and 0.27). Most animals did not perform any of the behaviours at either time point (Table 5.6). If an animal bit the observer during test one it was more likely to bite than not bite the observer at test two. The number of animals following the observer increased between tests one and two.

Table 5.6 Count of behavioural reactions to a human presence in the home pen for individuals with records at both time points. Individuals were scored 1 if they followed an observer for > 0.5 laps of the pen, nosed at the observer's feet or legs, or bit the observer.

		Test 2				
		1	**0**	**Trait**	**Φ**	**X^2**
Test 1	**1**	159	102	Bite	0.27	152.68 *
	0	420	1340			
	1	78	80	Follow	0.27	150.31 *
	0	235	1628			
	1	63	201	Nose	0.11	25.82 *
	0	216	1541			

Φ = phi coefficient (analogous to Pearson correlation coefficient)

χ^2 = Chi-square test statistic (* indicates P < 0.001)

5.3.2 Heritabilities and common environmental effects

Estimated heritabilities for the crate response and individual human test were low to moderate (0.14 to 0.30), with the highest heritability estimated for speed of movement away from the approaching observer (Table 5.7). While variance due to pen effects significantly differed from zero for all behaviours, pen effects accounted for little of the variation in these traits. Pen effects accounted for the most variation for vocalisation in response to the approach of a human observer (0.07, SE 0.02).

Table 5.7 Heritabilities (h^2) and phenotypic fractions of pen group effects (c^2) for behavioural responses to isolation in a weigh crate (crate score) and response to a human approach (movement, vocalisation, vigilance). Standard errors are presented in parentheses and all heritabilities and pen effects significantly differed from zero for these traits

Behavioural trait	h^2	c^2
Crate score	0.18 (0.05)	0.03 (0.01)
Movement	0.30 (0.05)	0.02 (0.01)
Vocalisation	0.18 (0.04)	0.07 (0.02)
Vigilance	0.14 (0.04)	0.03 (0.01)

Heritabilities for group response to a human approach were associated with high standard errors and were mainly non-significant (Table 5.8). Low but significant heritabilities for biting and following the observer during the second test were estimated (Table 5.8).

Table 5.8 Heritabilities for binary behavioural responses towards a human in the home pen at 2 separate time points (1: ~6 days after mixing and 2: ~25 days after mixing; numbers in superscript denote the time point the behaviour was measured). Standard errors are presented in parentheses, while a bold font signifies correlation significantly different from 0.

Trait	h^2
Bite [1]	0.19 (0.14)
Bite [2]	**0.35 (0.09)**
Follow [1]	0.15 (0.18)
Follow [2]	**0.30 (0.12)**
Nose [1]	0.06 (0.12)
Nose [2]	0.11 (0.13)

5.3.3 Correlations between behavioural traits and skin lesions

Genetic and phenotypic correlations of skin lesion traits recorded 24 hours post-mixing (SL24h) and 5 weeks post mixing (SL5wk) with crate response and individual human test responses are presented in Table 5.9. Genetic correlations were associated with high standard errors and were generally not significantly different from zero. Significant negative genetic correlations were calculated between central SL5wk and crate response, movement and vigilance in response to a human approach (-0.38 to -0.63). A negative genetic correlation was also found between posterior SL5wk and crate response (-0.56, SE 0.18). This means that individuals that showed the most active escape response during these tests were those that had few lesions 5 weeks post mixing. Genetic and phenotypic correlations between SL24h and SL5wk with traits recorded during the group response to human approach are presented in Table 10. Standard errors for all genetic correlations were very high, therefore correlations did not differ from zero for any of the traits tested. A very low phenotypic correlation was calculated between central SL24h and nose response recorded at the second time point Table 5.10. Pen level correlations between SL24h or SL5wk and behaviour responses to

crate isolation or approach by a human did not significantly differ from zero (Table 5.11).

Table 5.9 Genetic (r_G) and phenotypic (r_P) correlations between skin lesion traits recorded 24 hours post-mixing (SL24h) and 5 weeks post mixing (SL5wk), with behavioural responses to isolation in a weight crate (crate score) and response to a human approach (movement, vocalisation, vigilance). Standard errors are presented in parentheses and a bold font signifies correlation significantly different from 0.

		Movement		**Vocalisation**		**Vigilance**		**Crate**	
Trait		**r_G**	**r_P**	**r_G**	**r_P**	**r_G**	**r_P**	**r_G**	**r_P**
SL24h	Anterior	0.13 (0.21)	0.04 (0.03)	0.10 (0.22)	0.02 (0.03)	-0.09 (0.25)	-0.01 (0.02)	0.00 (0.26)	0.03 (0.03)
	Central	-0.14 (0.19)	0.04 (0.03)	-0.12 (0.20)	0.02 (0.03)	-0.33 (0.21)	-0.02 (0.02)	-0.19 (0.23)	0.01 (0.03)
	Posterior	-0.07 (0.17)	0.00 (0.03)	-0.03 (0.18)	0.00 (0.03)	-0.26 (0.19)	-0.04 (0.02)	-0.20 (0.21)	0.03 (0.03)
SL5wk	Anterior	-0.25 (0.15)	-0.02 (0.03)	-0.18 (0.17)	0.00 (0.03)	-0.31 (0.18)	-0.01 (0.02)	-0.33 (0.18)	-0.04 (0.03)
	Central	**-0.38 (0.14)**	-0.03 (0.03)	-0.19 (0.16)	-0.02 (0.03)	**-0.38 (0.17)**	0.00 (0.03)	**-0.63 (0.14)**	-0.06 (0.03)
	Posterior	-0.17 (0.17)	0.02 (0.03)	-0.01 (0.19)	0.01 (0.03)	-0.18 (0.20)	-0.02 (0.02)	**-0.56 (0.18)**	-0.03 (0.03)

Table 5.10 Genetic (r_G) and phenotypic (r_P) correlations between skin lesion traits recorded 24 hours post-mixing (SL24h) and 5 weeks post mixing (SL5wk), with binary behavioural responses towards a human in the home pen recorded at two time points (denoted by numbers in superscripts). Standard errors are presented in parentheses and a bold font signifies correlation significantly different from 0.

	Trait	**Bite [1]**		**Bite [2]**	
		r_G	**r_P**	**r_G**	**r_P**
SL24h	Anterior	0.96 (0.64)	0.03 (0.02)	0.41 (0.33)	0.01 (0.02)
	Central	0.20 (0.49)	-0.01 (0.02)	0.14 (0.28)	0.01 (0.02)
	Posterior	0.28 (0.43)	0.00 (0.02)	0.00 (0.24)	0.01 (0.02)
SL5wk	Anterior	0.42 (0.36)	0.01 (0.02)	0.23 (0.20)	0.01 (0.02)
	Central	0.04 (0.31)	-0.01 (0.02)	0.15 (0.18)	0.00 (0.02)
	Posterior	-0.39 (0.41)	-0.03 (0.02)	0.15 (0.23)	0.02 (0.02)
		Follow [1]		**Follow [2]**	
SL24h	Anterior	0.88 (0.79)	0.01 (0.02)	0.34 (0.39)	0.03 (0.02)
	Central	-0.10 (0.66)	-0.01 (0.02)	-0.25 (0.34)	0.01 (0.02)
	Posterior	-0.44 (0.54)	0.00 (0.02)	-0.26 (0.28)	0.01 (0.02)
SL5wk	Anterior	1.00 (0.65)	0.00 (0.03)	0.35 (0.25)	0.01 (0.02)
	Central	1.00 (0.63)	0.03 (0.03)	0.08 (0.22)	-0.01 (0.02)
	Posterior	0.72 (0.69)	-0.01 (0.02)	0.07 (0.28)	-0.01 (0.02)
		Nose [1]		**Nose [2]**	
SL24h	Anterior	0.97 (1.33)	0.04 (0.02)	0.75 (0.74)	0.04 (0.02)
	Central	0.82 (1.17)	0.00 (0.02)	0.69 (0.66)	**0.05 (0.02)**
	Posterior	0.99 (1.36)	0.01 (0.02)	0.90 (0.74)	0.04 (0.02)
SL5wk	Anterior	0.20 (0.64)	0.01 (0.02)	0.67 (0.65)	0.03 (0.02)
	Central	0.32 (0.61)	0.03 (0.02)	0.57 (0.57)	0.03 (0.02)
	Posterior	0.13 (0.69)	0.01 (0.02)	0.73 (0.74)	0.02 (0.02)

Table 5.11 Pen level correlations between skin lesion traits recorded 24 hours post-mixing (SL24h) and 5 weeks post mixing (SL5wk), with behavioural responses to isolation in a weight crate (crate score) and response to a human approach (movement, vocalisation, vigilance). Standard errors are presented in parentheses. No correlations significantly differed from 0.

	Trait	Movement	Vocalisation	Vigilance	Crate
SL24h	Anterior	-0.32 (0.24)	-0.21 (0.17)	-0.47 (0.41)	-0.26 (0.22)
	Central	0.05 (0.23)	0.16 (0.17)	-0.36 (0.41)	-0.17 (0.22)
	Posterior	0.08 (0.23)	0.17 (0.17)	-0.19 (0.37)	-0.10 (0.22)
SL5wk	Anterior	0.25 (0.22)	0.20 (0.16)	-0.07 (0.22)	0.18 (0.24)
	Central	0.28 (0.21)	0.12 (0.16)	0.14 (0.21)	0.24 (0.24)
	Posterior	0.27 (0.21)	0.11 (0.16)	0.02 (0.22)	0.09 (0.23)

5.4 Discussion

5.4.1 Heritabilities

Methods of measuring fear or coping responses to a novel or adverse situation in pigs include latency to approach a human while in the home pen (Velie et al., 2009) ; novel object/arena tests (Friel et al., 2015); isolation (Reimert et al., 2014); restraint in a weighing crate (D'Eath et al., 2009), startle tests (Lawrence et al., 1990), elevated plus maze (Rutherford et al., 2012), and struggling behaviour while restrained (back test; Ruis et al., 2000). The current study used variations of some of these tests to measure fearful/bold behavioural responses to a human presence while both in the home pen and in isolation in a pen, and behaviour while isolated in a weighing crate. Where significant, heritabilities calculated for behavioural traits in the current study were low to moderate. D'Eath et al. (2009) estimated a heritability of 0.17 (SE 0.03) for behaviour while in the weigh crate, which is very similar to the heritability estimated for the same trait in the present study. Rohrer et al. (2013) calculated heritabilities for activity at weighing using a 5 point scoring system (0.19, SE 0.03), and three behavioural reactions to a back test: latency to start struggling (0.16, SE 0.08); number of struggle events (0.16, SE 0.08); time spent struggling (0.15, SE 0.08). The estimated heritability of behaviour while in a weigh crate reported by Rohrer et al. (2013) was very similar to that estimated by D'Eath et al. (2009) and in the present study. These results suggest that the heritability of behavioural reactions to confinement in a weight crate is consistent across a range of populations and environments.

Hemsworth et al., (1990) estimated heritabilities for behaviours performed during an experiment the authors refer to as the 'human test'. In that study gilts aged between 25-30 weeks were isolated from pen mates and the behaviour towards a human

observer within a 3 minute period was recorded. In contrast to the current study, the observer stood still in the pen, and the time taken to approach the observer (TA), time spent near the observer (TN), and the latency to first physical interaction with the observer (LP) was recorded. Heritabilities for TA and TN were very low (0.02), while heritability for LP was estimated to be 0.38, however the standard error associated with this estimation was also high (0.19). Higher heritabilities were estimated for behaviours performed while in isolation in the current study. This may be because the methodology used in this study forced a reaction from every pig as the observer approached them. In Hemsworth et al. (1990), a pig that did not approach the observer may have reacted that way out of indifference or shyness, two behavioural reactions with very different motivations.

Scheffler et al. (2014) performed a similar experiment to that described above in Hemsworth et al. (1990) and estimated moderate heritabilities for weaned pigs (0.33), whereas heritabilities for gilts were much lower (0.03). As the sample size of gilts used by Scheffler et al. (2014) was relatively small, the authors could not say whether the low heritability estimated for gilts was the result of age or insufficient sample size. Heritabilities for fear responses have been shown to decline with age in beef cattle, possibly due to habituation to handling or through repeated testing (Haskell et al., 2014). In contrast, in the current study the group human approach test was repeated after approximately 3 weeks, and estimated heritabilities increased for all three of the traits recorded. If this test simply measured fear we might expect the heritability to reduce over time, as the expression of fear responses over time is likely to be masked as animals become habituated to a human presence. It is likely that the first and second test measure separate behaviours, and that the second test partly measures exploratory behaviour, which may have been inhibited by fearfulness during the first

test. At the time of the second test pigs had been housed without enrichment for approximately three weeks, and the human presented a novel rooting and chewing opportunity, which may explain why more pigs were motivated to interact with the observer at this time point.

In the present study the highest heritability was estimated for speed of movement away from the human observer. This test was less subjective and prone to observer error, as the scoring system was not open to interpretation (i.e., movement was zero, walk, trot, or run). Although measures were chosen to be as objective as possible perceptions of behaviour while in the weighing crate, and vocalisation and vigilance during a human approach were more subjective, which may have resulted in greater variability over time in how the scale was used. For example, the behaviour of any given animal may seem more or less extreme in context of the animal tested previously and result in the observer adjusting subsequent scores. In experiments such as these it would be preferable for more than one observer to record behaviour, and the inter observer reliability measured; however this was not possible in this case. The observer in the current experiment was not aware of the genetic background of any animal tested, and therefore did not have preconceptions or biases over how a given genetic line may behave, and observer biases were highly unlikely.

The proportion of the variance due to pen effects was very small for the behavioural traits relating to crate isolation and the individual human test. This is in contrast to skin lesions, where pen effects were been found to account for between 11 and 13% of the observed variation (Chapter 4). As physical aggression is the result of interactions between animals it is understandable that pen effects count for more of the variation in this behaviour. During the crate and isolation tests in the present study, pigs were

tested individually and the behaviour of each pig was unlikely to be affected by its pen mates. Variance attributed to pen effects was highest for vocalisation in response to a human approach. Although pigs were physically isolated from pen mates during this test, they were in audible range of one another, and this might explain why pen effects accounted for more of the variation in this trait, as vocalisations from the rest of the group may have provoked a response from the animal being tested.

Pen effects were not found to explain a significant proportion of the variation in the group human approach tests. This was surprising given that behaviour of pen mates is likely to influence the behaviour of a pig. For example a shy pig might feel more confident approaching a human after observing a pen mate approaching. It is possible that within each pen the behaviour of pen mates influenced the individual behavioural reactions observed, however between pen responses did not differ sufficiently to account for a significant proportion of the variation observed across the population. This could happen if, within each pen, there are bold and shy animals, however one pen does not significantly differ from another in the range of behavioural reactions represented.

5.4.2 Correlations between behavioural responses and skin lesions

There is mixed evidence regarding whether aggressive behaviour is expected to correlate with behavioural traits related to fearfulness/boldness, or coping behaviour on a phenotypic level. Many studies investigating the relationship between aggression and animal personality use the back test as a measure of coping strategy. Animals that struggle a lot during the back test tend to be classed as highly reactive. Some studies have found an association between highly reactive behaviour during the back test and aggressive behaviour. Hessing et al. (1994), Ruis et al. (2000), Bolhuis et al. (2005), and Melotti et al. (2011) found that pigs that are highly reactive during the back test show

higher levels of aggression, however Forkman et al. (1995), Janczak et al. (2003), and Spake et al. (2012) found no association between these traits. Friel et al., (2015) found that individuals that were quick to touch a novel object while in isolation showed more variation in fight duration behaviour post mixing, while animals that showed a high latency to touch a novel object performed low levels of aggression. In the present study there was little to no evidence of a phenotypic link between any of the behavioural measures, and social aggression, as measured via skin lesions.

On a genetic level, central SL5wk were found to significantly negatively correlate with movement, vigilance, and activity while restrained in a weighing crate, while posterior SL5wk were negatively correlated with behaviour in the weighing crate. This means that animals that receive many lesions in the long term are less active while restrained in a weight crate, and are less likely to run away from, or focus their attention on an approaching human while in isolation. Although not statistically significant, the direction and magnitude of the correlations calculated for anterior SL5wk were similar to central SL5wk for the traits movement, vocalisation, and vigilance, suggesting that the significant correlations calculated were not simply found by chance, as might be expected when performing multiple statistical tests.

Genetic correlations calculated using pigs from a different population (see Chapter 3) suggest that individuals that receive many anterior and central lesions under stable social conditions tend to win fewer fights, initiate less one sided aggression, and become involved in less reciprocal aggression at mixing. These animals were also more likely to have received a lot of one-sided aggression at this time point. These behaviours combined suggest that the individuals that receive many lesions under stable social conditions are unaggressive, subordinate animals. If these results hold

true for the population in this study, animals that are less reactive during isolation are less aggressive at mixing, but receive many lesions in the long term, possibly due to a lower social position within the group. Genetic correlations calculated in Chapter 4 indicate that animals that receive many lesions in stable groups are also genetically predisposed to grow faster and have heavier carcass weights. It may be that these animals are less reactive in general, and use less energy engaging in aggression. Alternatively, perhaps these animals find the social dynamics following mixing to be more stressful, and were therefore relatively less stressed when separated from the group.

There are few studies specifically exploring the relationship between fear related behaviours and non-fear related aggressive behaviour. As mentioned earlier in the discussion, reactivity during the back test has been found by some to be related to aggression during resident intruder tests, however the meaning behind behaviour performed during the back test is open to interpretation, and may not be a reliable measure of a specific 'coping style' (Zebunke et al., 2015). D'Eath et al. (2009) calculated genetic correlations between behaviour at mixing with behaviour performed during weighing at the beginning and end of the growing period. Behavioural traits recorded included the speed at which animals entered and exited the crate, vocalisations while in the crate, and movement while in the crate. A negative genetic correlation was found between involvement in reciprocal aggression at mixing and the speed at which animals entered the weigh crate at mixing (r_G = -0.14, SE 0.07), meaning that animals that are involved in little reciprocal aggression at mixing entered the weigh crate more quickly. While an animal that enters a weigh crate quickly is easier to handle, it is difficult to interpret the underlying motivation behind this behaviour

without other behavioural cues. Speed alone could be affected by how stressed, excited, confident, or fearful the animal is.

Reactions to a human observer while in a group situation did not significantly correlate with aggressive behaviour. Behaviour while in isolation may be affected by the novelty of the environment or the stress of isolation, therefore behaviour under these conditions is likely to differ to behaviour while in the home pen with pen mates. In addition, the nature of the traits measured differed between the two human approach tests used in this study. During the individual human test, a reaction was forced from each individual as the human approached it. During the group human approach test, although the observer walked around the perimeter of the pen, no pigs were singled out and the behaviour ultimately measured was a pig's willingness to approach and interact with the observer. In this situation, a pig that did not approach the observer may have done so out of fear or indifference, therefore a score of zero for the recorded traits is likely to have captured opposing reactionary behaviours. This may explain why none of the results measured in the group situation correlated with skin lesion traits.

In the present study, biting behaviour directed towards a human handler was recorded. The original aim of this experiment was to determine whether there is a phenotypic or genetic association between social aggression in pigs, and human directed aggression. In a review of 7 serious injuries caused by pigs, (Barnham, 1988) found that only 2 of the injuries were caused by biting, while four injuries were caused by a boar tusks, and one occurred while clipping piglet's teeth. That study noted that farmers usually report playful or indifferent behaviour from pigs, and that serious injuries by pigs are very rare. Given the size and strength of pigs, combined with the number of pigs farmed, we would expect to see many more reports of serious injury if growing pigs were very

prone to aggressive behaviour towards humans. Maternal aggression directed towards a stockperson is more common (Grandinson et al., 2003), however this tends to be characterised by distinctive aggressive behavioural characteristics such as tense, sudden movements, focusing all attention on the stock person, vocalisation, and aggressive biting (Marchant Forde, 2002). While conducting the experiment, it became apparent that biting behaviour in this population of growing pigs was not motivated by aggression. In the present study growing gilts and castrated boars were used and behaviour such as that described in Marchant Forde (2002) was never witnessed. When pigs bit the observer in the present study it appeared to be driven by curiosity and playfulness, rather than frustration or dominance, as vocalisations, aggressive biting and charging behaviours were absent.

There were several aspects of the experimental procedures used in the present study that may have affected the observed results. Ideally, crate response and responses recorded in the individual human tests would be carried out in a completely novel environment by an unfamiliar handler. Both the weighing crate and isolation pen were familiar to the animals, as they had been weighed in the same crate and held in the same pens by farm staff 1 or 2 days prior to the tests. Testing pigs within the same time point is also not ideal, as the pigs behavioural state at that moment in time (e.g. calm, agitated) would be likely to carry over, meaning that the tests were not independent. In addition, these pigs were already familiar with the observer carrying out the experiments, as the same observer had previously recorded skin lesions, moved the animals to and from the home pen, as well as moved them into the weighing crate. How aversive the pigs found these events may have affected their behaviour in these tests. For group responses to a human approach, more detailed behavioural observations may have been more informative than simple binary responses. For example, some pigs

immediately followed the observer around both laps of the pen, and persistently bit at the observer for the whole test period, while some hesitantly approached and eventually bit at the observer. These behaviours are probably indicative of different levels of fearfulness/boldness, however both pigs would have simply been recorded as having displayed biting behaviour. Unfortunately, as there were 18 pigs in a pen and only one observer it was impossible to record more detailed behavioural interactions. Moreover, this test was designed to be used as a practical on-farm measure of pig-human interactions, therefore it was of interest to develop a quick and accurate method of measuring these behaviours. As heritability increased between tests, it would be of interest to repeat this test several time across regular time points. Finally, as maternal aggression is more commonly observed, it would also be of interest to investigate whether maternal aggression is correlated with inter pig aggression.

5.4.3 Conclusion

This study found no evidence of phenotypic associations between social aggression (as measured by skin lesions at 24 hours post mixing and 5 weeks post mixing) and reactions to human approach while in isolation in a pen or weigh crate, or behaviours directed towards a human present in the home pen. There was some evidence of a genetic association between skin lesions received under stable social conditions and behaviour while isolated in a weigh crate, speed of movement away from, and level of vigilance towards a human observer. If results from previous studies can be applied to the present study, it may be that individuals that are less aggressive at mixing are less reactive when isolated from pen mates. Evidence from other studies included in this thesis suggest that anterior skin lesion are the best trait for selection against aggression, given the current lack of understanding of the relationship between stable lesions and aggressive behaviour. As no correlations were found between anterior SL24h, and any of the behavioural traits measured in the current study, it suggests that

selection against anterior SL24h should not affect other behaviours, as concluded by D'Eath et al. (2009).

Chapter 6 - Genome-wide association study to identify quantitative trait loci associated with aggressiveness in growing pigs

6.1 Introduction

Genome wide association studies (GWAS) utilise high throughput SNP genotyping to identify quantitative trait loci (QTL) associated with major genes that control a given trait. Identifying QTL strongly correlated to aggressive behaviour could potentially be used in marker-assisted selection and to improve the knowledge of the genetic regulation of aggressive behaviour. Additionally, knowledge of the underlying genomic control of aggressive traits could help identify novel biological pathways for further study. GWAS has been successfully applied to identify QTL regions associated with complex production traits in pigs, such as carcass quality and feeding behaviour (Do et al., 2013; Sanchez et al., 2014), however few studies have applied this methodology to study behavioural traits in pigs. Genome wide association studies have previously been used to search for SNPs associated with skin lesion and aggressive behavioural traits in a separate population, however no significant variants were detected, possibly due to the small population size (Pong-Wong et al., 2010). The main objective of this chapter was to use GWAS to identify SNPs that explain a significant proportion of the genetic variance in skin lesion traits recorded 24 hours post-mixing and 5 weeks post-mixing in a population of 1,840 commercially housed pigs.

6.2 Materials and methods

6.2.1 Animals and housing

Analyses were performed using data recorded on 1,840 pigs (922 females, 918 castrates) from a commercial herd in the United States. Pigs were from crosses of 1 maternal line with 5 differing sire lines (genetic lines have been recoded in the text). Pigs were housed in fully slatted pens and had constant access to water via a nipple drinker. Animals were fed dry pelleted food *ad libitum* either via single-space electronic feeder (FIRE feeder; Osborne Industries, Osborne, KS) or via a multi-spaced trough. Feeder type was changed every alternate 2 wk throughout the finishing period. On average, animals from 11.6 (SD 2.1) litters were represented in each pen, and the mean number of pigs per litter per pen was 1.5 (SD 0.81).

6.2.2 Mixing and lesion counting

At an average age of 69 days (SD 5.2) pigs were mixed into single sex, mixed breed groups of 18. Pigs were mixed into groups of an even number of pigs taken from 2 non-adjacent, single sex, mixed litter weaning pens. Groups mixed on the same day were considered to belong to the same batch, and 8 groups were formed per batch (except for 1 batch which contained 10 groups). Animals remained in these social groups for the remainder of the growing period. Immediately prior to mixing, fresh skin lesions were counted on 3 separate regions of the body: anterior (head, neck, shoulder and forelegs), central (back and flanks), posterior (haunches and hind legs). Lesions were considered fresh if they were bright red or bleeding. Lesions were counted again in the same way 24 hours post-mixing, and the pre-mixing lesion count was subtracted from the post mixing count to ensure only those lesions caused by aggression at mixing were included in the analyses. Fresh skin lesions were counted a third and final time approximately 5 weeks post-mixing (mean 34 days post-mixing, SD 9.5), as a measure

of aggression performed under stable social conditions. In addition to skin lesion numbers, the sex, pen number, and genetic line of each animal were recorded. Animals were weighed (without any feed or water restriction) 24 hours post-mixing (27.7 kg, SD 4.7) and at the end of the growing period (119.6 kg, SD 12.6; aged 172 days, SD 4.6).

6.2.3 Characteristics of the data

The characteristics of the skin lesion phenotypes used in the analyses are presented in Table 6.1. Skin lesion traits showed skewed distributions; therefore these data were $\log_e$ (1 + observation) transformed prior to any analyses to approach a normal distribution. Following quality control procedures, phenotypes from 1,839 animals, and 44,936 autosomal SNPs were available for analysis.

Table 6.1 Characteristics of the raw untransformed values for anterior, central, and posterior skin lesions recorded 24 hours post mixing (SL24h) and 5 weeks post mixing (SL5wk)

		Original scale					Transformed scale			
Trait		**Mean**	**Min-Max**	**SD**	**SK**	**K**	**Mean**	**SD**	**SK**	**K**
SL24h	Anterior	16.57	13.84	0 - 94	1.4	2.69	1.08	0.43	-0.87	0.48
	Central	14.15	12.83	0 - 74	1.57	3.02	1	0.45	-0.76	0.08
	Posterior	8.45	8.01	0 - 51	1.67	3.41	0.81	0.41	-0.47	-0.38
SL5wk	Anterior	2.64	3.14	0 - 27	2.13	7.38	0.42	0.35	0.18	-1.04
	Central	2.13	2.93	0 - 26	2.49	8.86	0.36	0.33	0.5	-0.71
	Posterior	1.33	2.1	0 - 19	2.9	12.27	0.26	0.29	0.85	-0.13

6.2.4 Genotyping and quality control

Tissue samples were obtained from tails, which were docked at birth, as per usual farm protocol. Animals were genotyped using a customized Illumina single-nucleotide polymorphism (SNP) chip. The genotyped animals originated from 85 sires and 319 dams. Data from the 5 crossbred lines represented in the population sample were pooled to achieve a sufficient sample size, and lines were recoded as a, b, c, d, and e. Individuals were removed from further analysis if there was a sex discrepancy, or if the

call rate for that individual over all SNPs was < 0.95. SNPs were excluded from further analysis if they were not in Hardy-Weinberg equilibrium, had a minor allele frequency of < 0.05, were monomorphic, or had a call rate of < 0.95.

6.2.5 Linkage disequilibrium

Linkage disequilibrium (LD) was measured as r^2, and calculated for all SNP pairs on a chromosome as the squared correlation of the alleles at two loci. Average LD was calculated as a mean of r^2 for SNP pairs located a) 1 marker distance away, b) within a 1Mb window. To assess the effect of genetic line on LD, r^2 between SNPs located within a 1 Mb window was also calculated for a single genetic line (line B), and compared to the population as a whole.

6.2.6 Principal components analysis

Following quality control procedures, a principal components analysis (PCA) was performed using allele frequencies after quality control to assess population stratification in this population.

6.2.7 Genome-wide association analysis

A series of genome wide association analyses (GWAS) were performed using the multi locus mixed model (MLMM) in SNP & Variation Suite (SVS) v7.7.8 (Golden Helix Inc., Bozeman, MT). The following model was fitted

$$\mathbf{y} = \boldsymbol{X_1}\mathbf{b} + \boldsymbol{X_2}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{e}$$

where $\mathbf{y}$ is the vector of recorded skin lesion traits, and the vectors $\mathbf{b}$, $\boldsymbol{\beta}$, $\mathbf{a}$ and $\mathbf{e}$ represent the vectors of fixed, vector of coefficients for SNP effects, random additive genetic, and residual effects, respectively. $\mathbf{X_1}$, $\mathbf{X_2}$ and $\mathbf{Z}$ represent the incident matrices relating fixed and random effects with response variables. The vector b contains the sex, batch (day the animals were mixed), pen identity, weight at time of mixing, genetic

line, and the first 5 principal components, which accounts for stratification of the population. The distribution of the additive genetic effects are assumed to be normal with zero means and the following covariance structure:

$$var\begin{bmatrix}\boldsymbol{a}\\ \boldsymbol{e}\end{bmatrix} = \begin{bmatrix}\boldsymbol{G}\sigma_a^2 & 0\\ 0 & \boldsymbol{I}\sigma_e^2\end{bmatrix},$$

where **G** is the genomic relationship matrix (VanRaden, 2008) calculated as:

$$\boldsymbol{G} = \frac{\boldsymbol{SS'}}{2\Sigma_{i=1}^{N} p_{i(1-P_i)}}$$

where S is a centered incidence matrix of SNP genotypes, N is the number of SNPs and p_i is the allele frequency of marker *i*.

Several tools were used to determine the location of SNPs in relation to known genes and QTL previously reported in the literature. SNP positions were mapped to the porcine genome assembly 10.2 (version 74) using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and the GenomeBrowse® tool in SVS was used to establish the location of a SNP within a gene, or the relative distance (base pairs) of SNPs from the nearest porcine genes. The positions of known QTL of interest were taken from PigQTLdb (http://www.animalgenome.org/QTLdb/pig.html; Hu et al., 2007). Both the Ensemble genome browser (http://www.ensembl.org/index.html) and NCBI gene search tool (http://www.ncbi.nlm.nih.gov/gene/) were used to determine the predicted functions of uncharacterized genes.

6.2.8 Statistical inference

In order to account for multiple comparisons in the genome-wide association study (GWAS), *P*-values were adjusted by Bonferroni correction. A SNP was significant at the genome or chromosome level if –log10(p value) was greater than -log10(0.05/n),

where n represents the number of markers across the genome or on a given chromosome.

6.2.9 Quantile-quantile plots

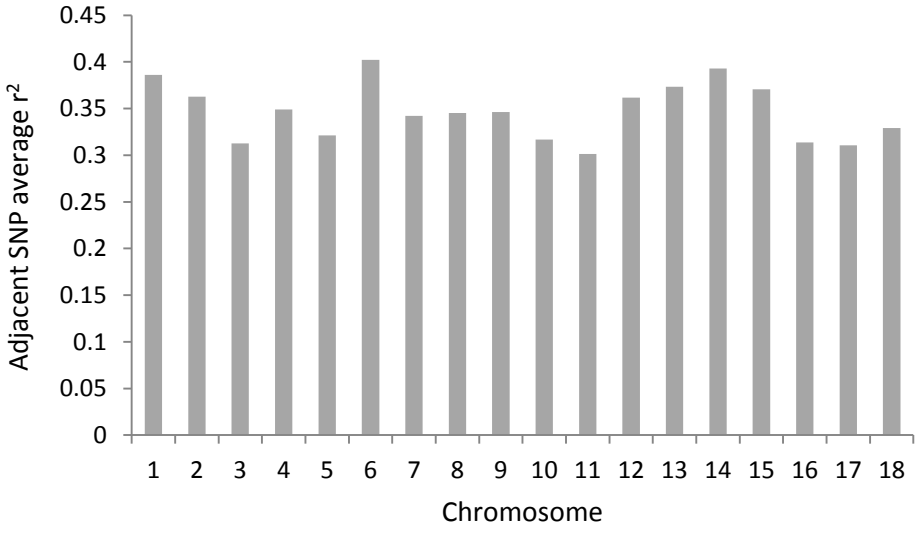
Quantile-quantile plots were created using the results of GWAS to visually assess the deviation of the observed probability distribution from the expected distribution, in order to further assess the population for signs of stratification that were not adjusted for in the model. Manhattan and Q-Q plots were created using the qqman package in R (Turner).

6.3 Results

6.3.1 Linkage disequilibrium

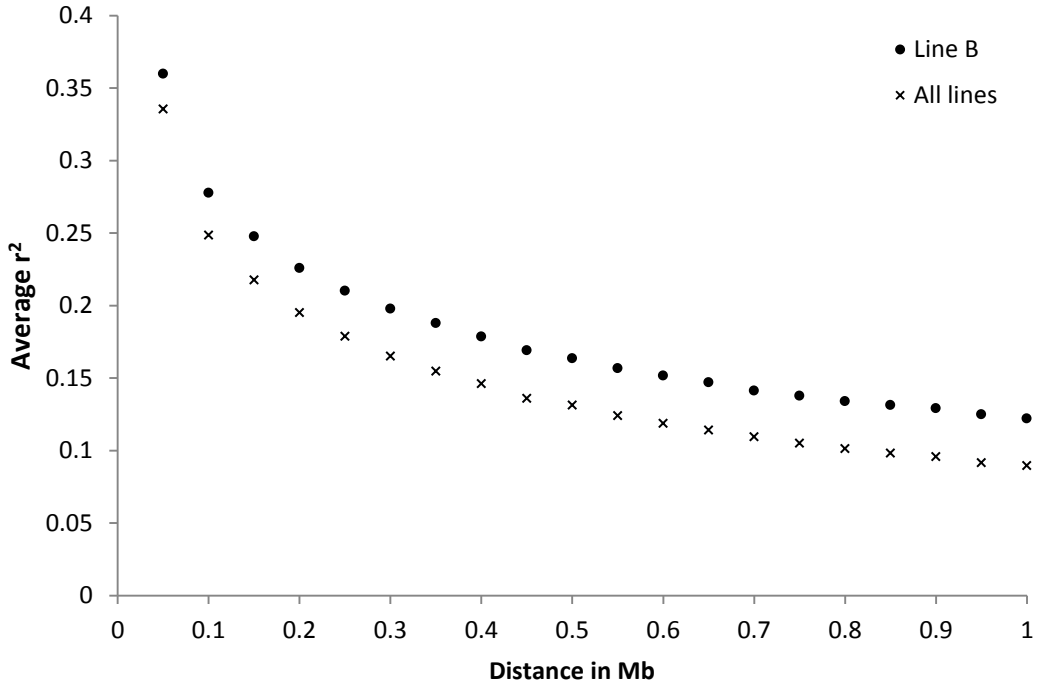
The average LD between adjacent SNPs was 0.35 (SD 0.33) based on 55,049 SNP pairs. Per chromosome, LD ranged from 0.30 to 0.40. Figure 6.1 shows the average LD per chromosome.

Figure 6.1 Average linkage disequilibrium (r^2) between adjacent SNPs per chromosome.



Within a 1 Mb window, average LD between SNPs was 0.15 (SD 0.21) for the population as a whole. The decay of average LD over distance is illustrated in Figure 6.2 for SNPs located within 0.05 Mb windows, across a maximum distance of 1 Mb. The two markers indicate average LD for all genetic lines combined (x symbol), and line B only (• symbol).

Figure 6.2 Average linkage disequilibrium for SNPs located within 0.05 Mb windows, across a maximum distance of 1 Mb, for all genetic lines combined (x symbol), and line B only (• symbol).



6.3.2 Population stratification

The proportion of variance explained by the first 10 principal components is shown in Figure 6.3. Principal components (PC) 1 and 2 accounted for 48% of the variance in SNPs and clustered into 5 distinct groups, which largely corresponded with the 5 genetic lines represented in the population (Figure 2). Lines a, b, c, and d clustered closely together based on PC 1 but are distinct based on PC 2. Based on PC 1, line e was found to be very distinct from the other 4 lines (Figure 6.4). After inclusion of genetic lines and the first 5 principal components into the genetic model, quantile-quantile plots (Figure 6.5) show little evidence of population stratification.

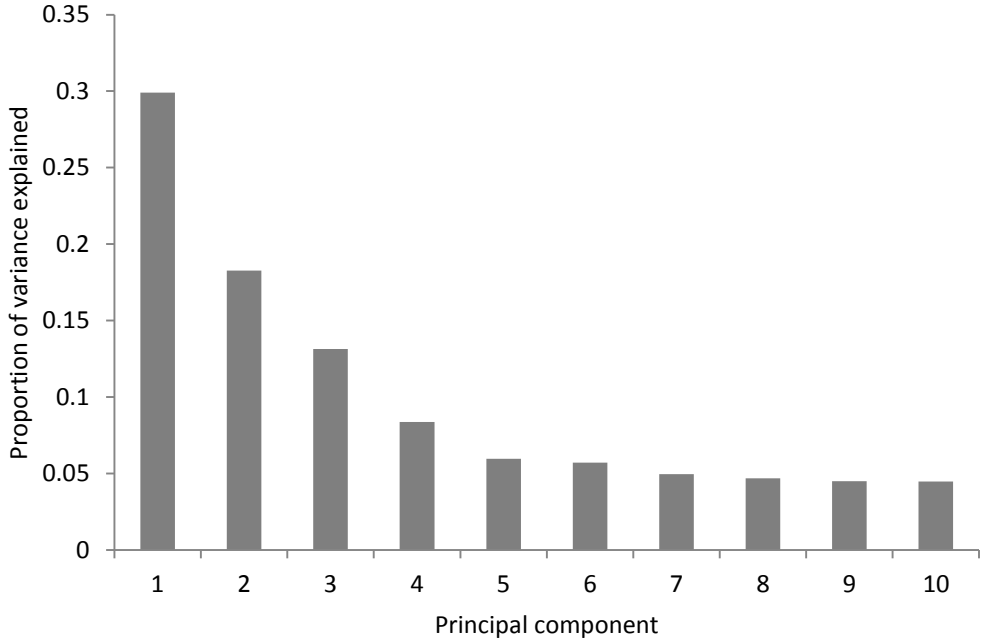


Figure 6.3 Proportion of the variance of allele frequencies explained by principal components 1-10

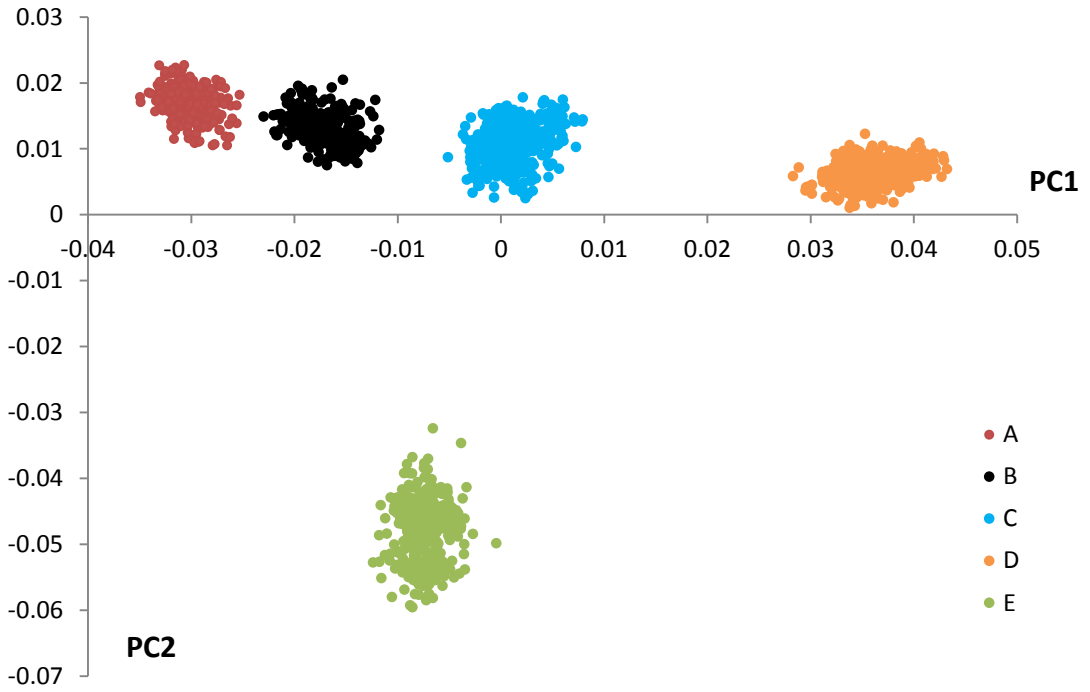


Figure 6.4 Principal components analysis for population stratification in 5 commercial pig breeds (breeds recoded). The genetic line that each colour corresponds with is indicated in the legend in the lower right hand corner

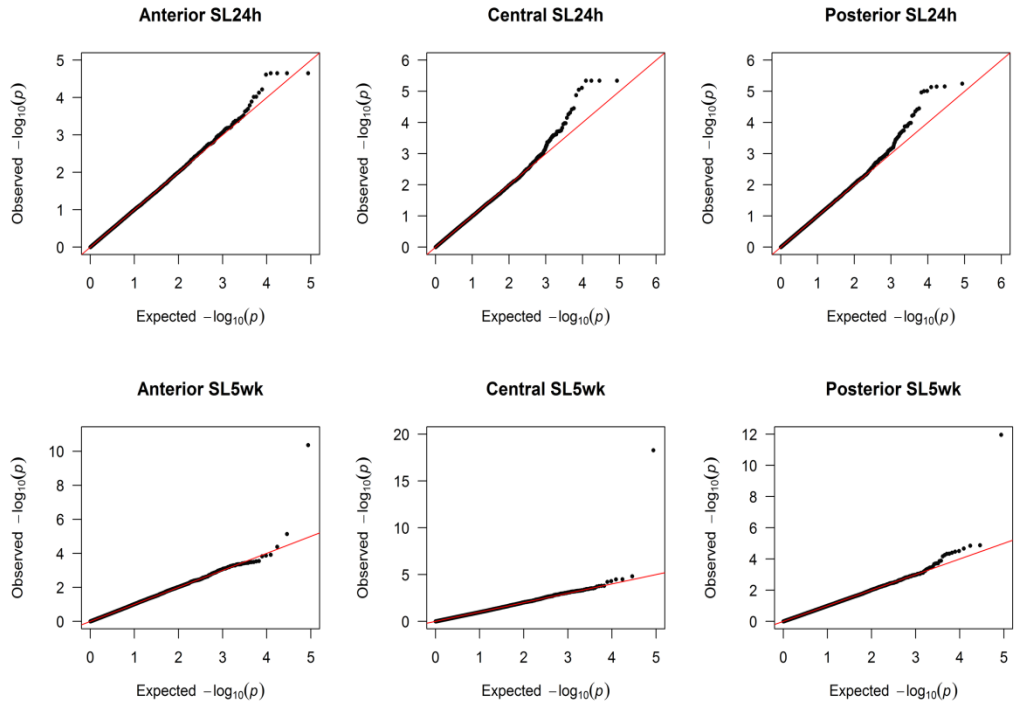


Figure 6.5 Quantile-quantile plots showing the expected –log10 p value (y axis) distribution against the observed –log10 p value (x axis) for genome wide association studies of skin lesion traits recorded 24 hours (SL24h) and 5 weeks (SL5wk) post-mixing. The trait tested for SNP association is indicated above each panel.

6.3.3 GWAS results

Chromosome wide significance

Markers found to be significantly associated with skin lesion traits on a chromosome level are shown in Table 6.2. Six markers located on chromosomes 15 and 13 were found to be significantly associated with central SL24h on a chromosome wide level. Two of these SNPs were located within introns of genes coding for proteins with a known function (HLCS and CASP10 – see section 6.4.2 for further details). Two of the SNPs associated with central SL24h were located within introns of genes of uncharacterised proteins, while the final two SNPs were located 9,312 and 9,750 base pairs (bp) away from the nearest genes. Six SNPs located on chromosomes 13 and 18 were found to be significantly associated with posterior SL24h on a chromosome wide

level. Four of these SNPs were located within introns of genes of a known function (SIM2 and HLCS), while one SNP was located within an intron of a gene of an uncertain function. One SNP associated with posterior SL24h was located within the exon of a gene, of which the function is currently uncertain. A single SNP located on chromosome 16 was found to be significantly associated with central SL5wk, which was located 84,849 bp downstream from the nearest gene. Finally, 3 SNPs on chromosomes 7 and 17 were significantly associated with posterior SL5wk, which were located between 1,830 and 57,588 bp away from the nearest genes (Table 6.2). Chromosome-wide significant SNPs each accounted for 1% of the observed variance. No significant chromosome wide associations were found for anterior SL24h or anterior SL5wk.

Table 6.2 Chromosome wide SNPs associated with skin lesion traits recorded 24 hours (SL24h) and 5 weeks (SL5wk) post mixing.

						Nearest Gene	
Trait	**SNP**	**Chr**	**Position (bp)**	**-log10 P value**	**Variance explained**	**Name**	**Distance from SNP (bp)[1]**
Central SL24h	rs81318091	13	210564479	4.93	0.01	HLCS	Between exons 5 & 6
Central SL24h	rs80959715	15	115816120	5.57	0.01	ENSSSCG00000016093	Between exons 25 & 26
Central SL24h	rs80876421	15	116096311	5.30	0.01	C-FLIP	9,750
Central SL24h	rs339418487	15	116203466	5.57	0.01	CASP10	Between exons 9 & 10
Central SL24h	rs81338625	15	116216948	5.57	0.01	CASP10	-9312
Central SL24h	rs323143577	15	116260971	5.57	0.01	LOC100624904	Between exons 3 & 4
Posterior SL24h	rs81342664	13	210457463	4.96	0.01	SIM2	Between exons 5 & 6
Posterior SL24h	rs81288544	13	210504370	4.96	0.01	HLCS	Between exons 5 & 6
Posterior SL24h	rs81233074	13	210516458	4.80	0.01	HLCS	Between exons 5 & 6
Posterior SL24h	rs341497884	13	210549570	4.78	0.01	HLCS	Between exons 5 & 6
Posterior SL24h	rs81297198	18	30604536	4.59	0.01	LOC100516477	Within exon 7 [2]
Posterior SL24h	rs81468409	18	30638064	4.85	0.01	LOC100516477	Between exons 2 & 3
Central SL5wk	rs81479182	16	71292574	4.82	0.01	CLINT1	84,849
Posterior SL5wk	rs342754370	7	3213405	5.28	0.01	PPP1R3G	-57,588
Posterior SL5wk	rs80895909	7	5822223	5.00	0.01	SLC35B3	11,922
Posterior SL5wk	rs80897407	17	19689679	4.52	0.01	LOC100152679	1,830

[1] A negative value indicates that the SNP is located downstream from the gene.
[2] Missense mutation

Genome wide significance

Figures 4 to 9 show genome plots profiling the -log10 P values of SNPs included in the analyses for anterior, central and posterior SL24h and SL5wk. No SNPs were significantly associated with skin lesions recorded 24 hours post-mixing (Figures Figure 6.6, Figure 6.7, and Figure 6.8). A single SNP explaining between 2% (anterior SL24h) and 4% (central SL24h) of the variance observed was found to be associated with all skin lesion traits recorded 5 weeks post mixing on a genome wide level (Figure 6.9, Figure 6.10 Figure 6.11). This SNP was located on chromosome 15 between exons 25 and 26 of an uncharacterised protein (Table 6.3).

Table 6.3 Genome wide significant (p < 0.05) SNP associated with anterior, central and posterior skin lesion traits recorded 5 weeks post mixing (SL5wk).

						Nearest Gene	
Trait	**SNP**	**Chr**	**position (bp)**	**-log10 P value**	**Variance explained**	**Name**	**Distance from SNP (bp)**
Anterior SL5wk				10.27	0.02		
Central SL5wk	rs80959715	15	115816120	18.29	0.04	ENSSSCG00000016093	Between exons 25 & 26
Posterior SL5wk				11.74	0.03		

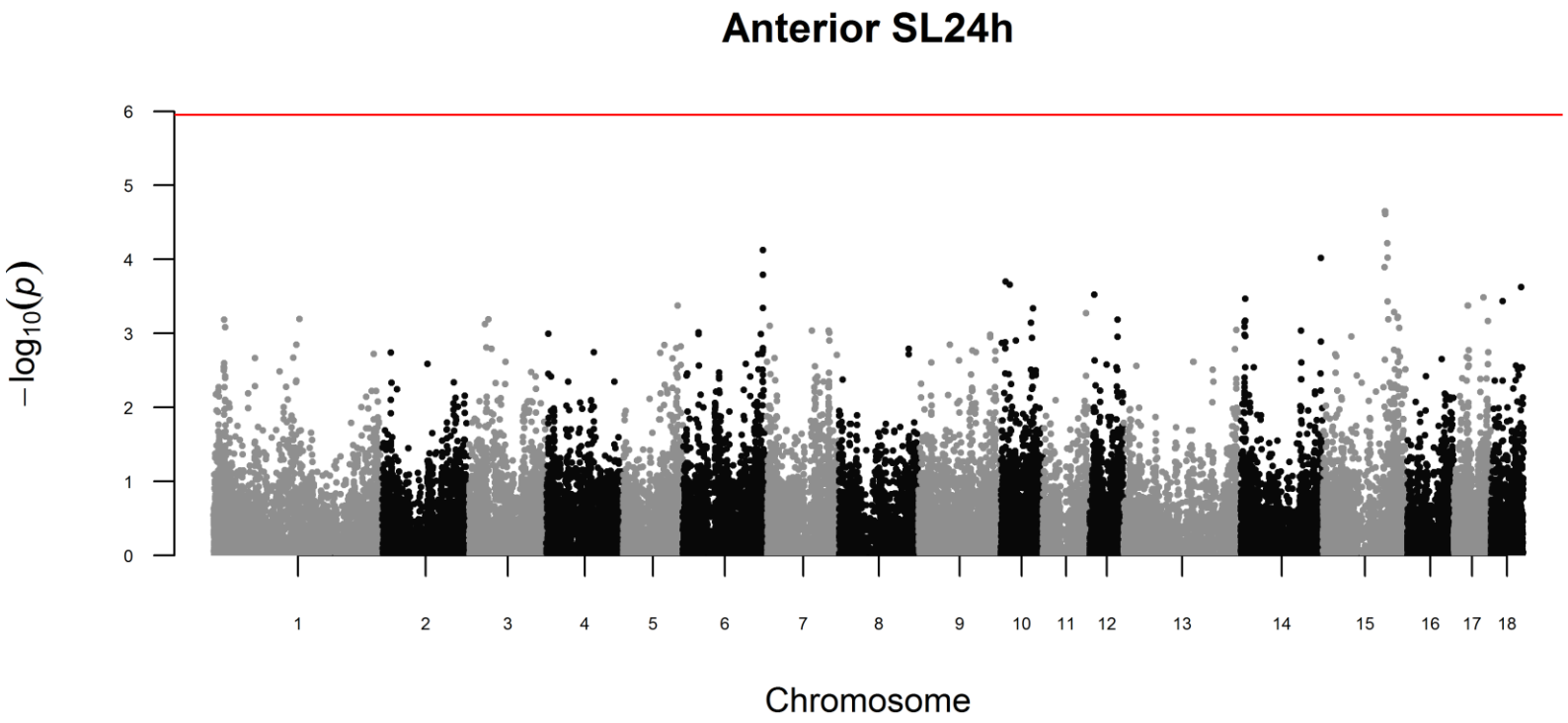


Figure 6.6 Genome wide plot of –log10 P values (y axis) for association of SNPs with anterior skin lesions recorded 24h post-mixing. The horizontal line indicates the threshold for genome wide significance.

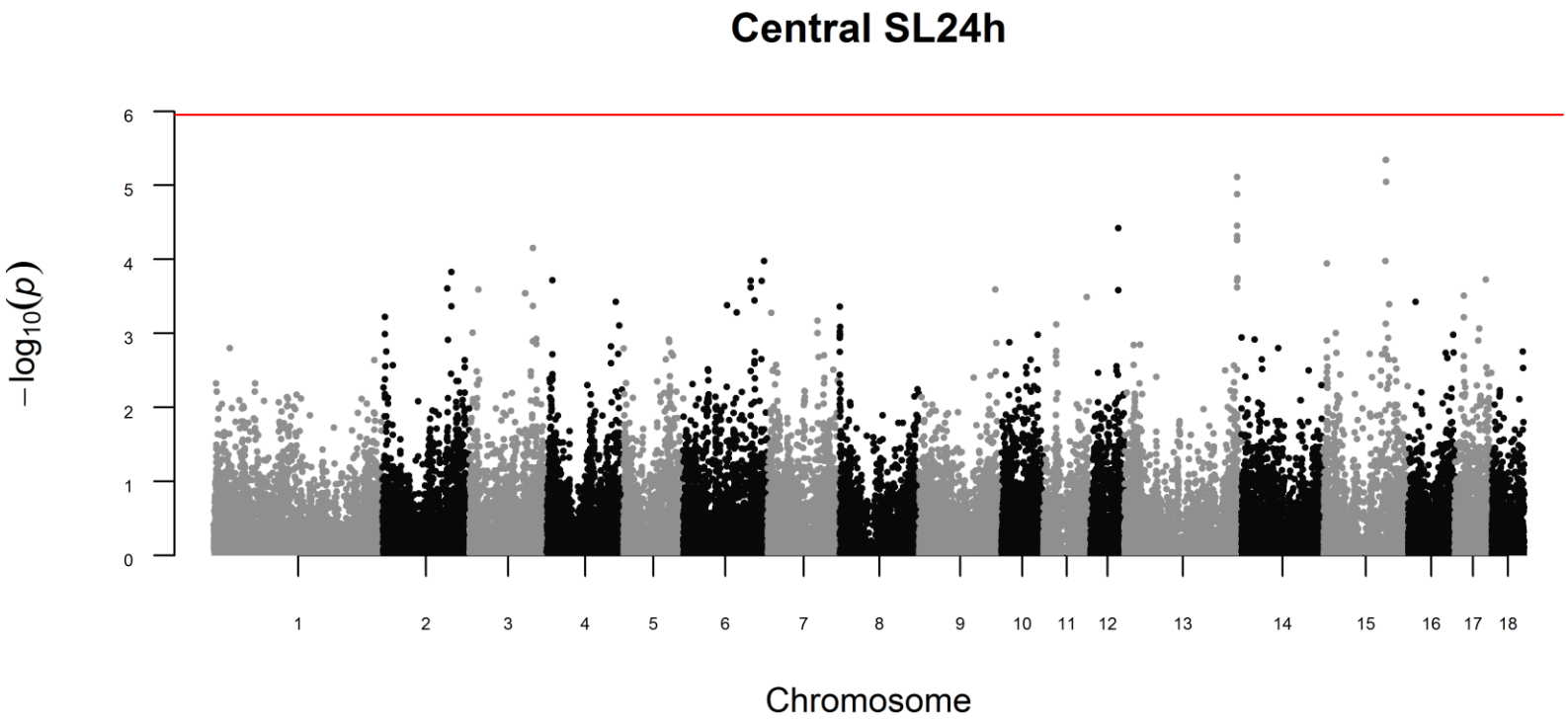


Figure 6.7 Genome wide plot of –log10 P values (y axis) for association of SNPs with central skin lesions recorded 24h post-mixing. The horizontal line indicates the threshold for genome wide significance.

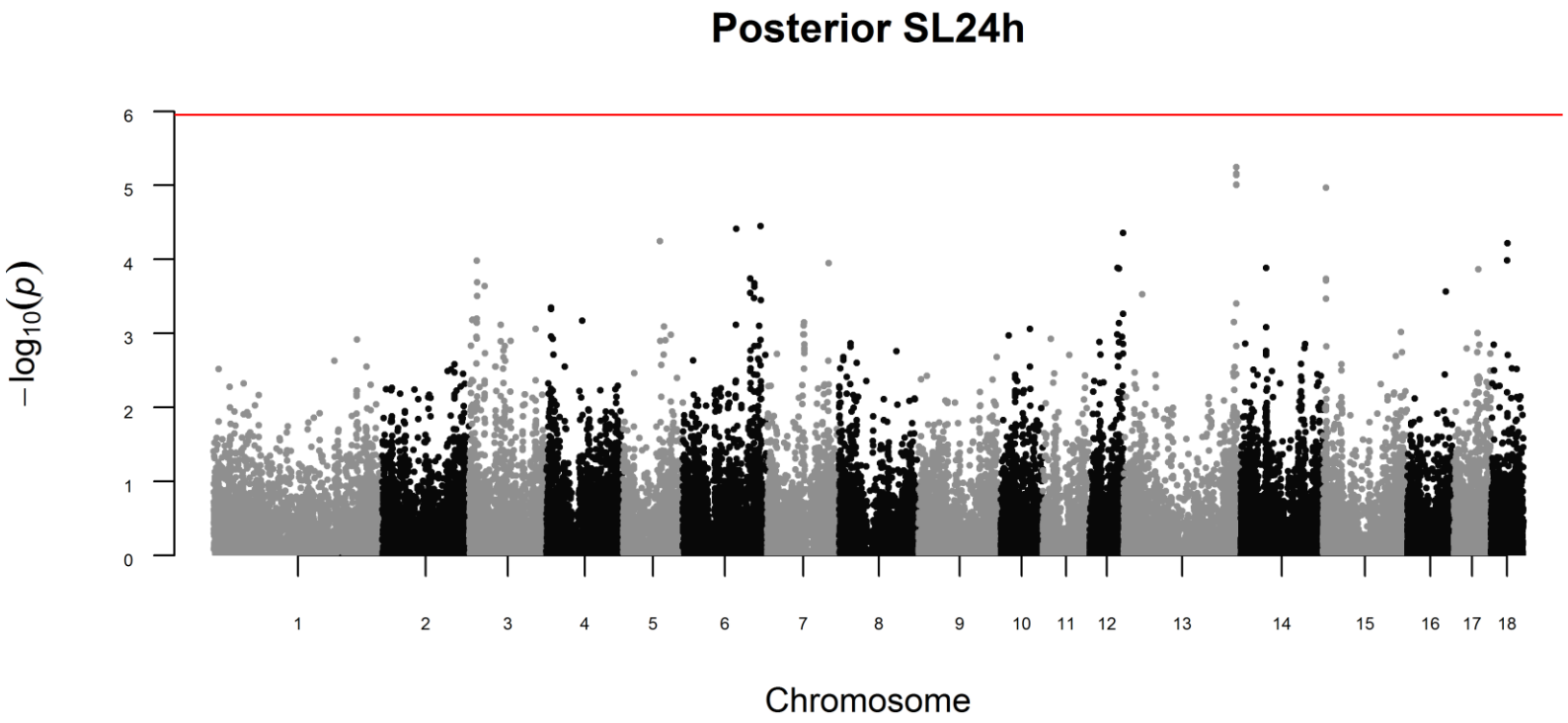


Figure 6.8 Genome wide plot of –log10 P values (y axis) for association of SNPs with posterior skin lesions recorded 24h post-mixing. The horizontal line indicates the threshold for genome wide significance.

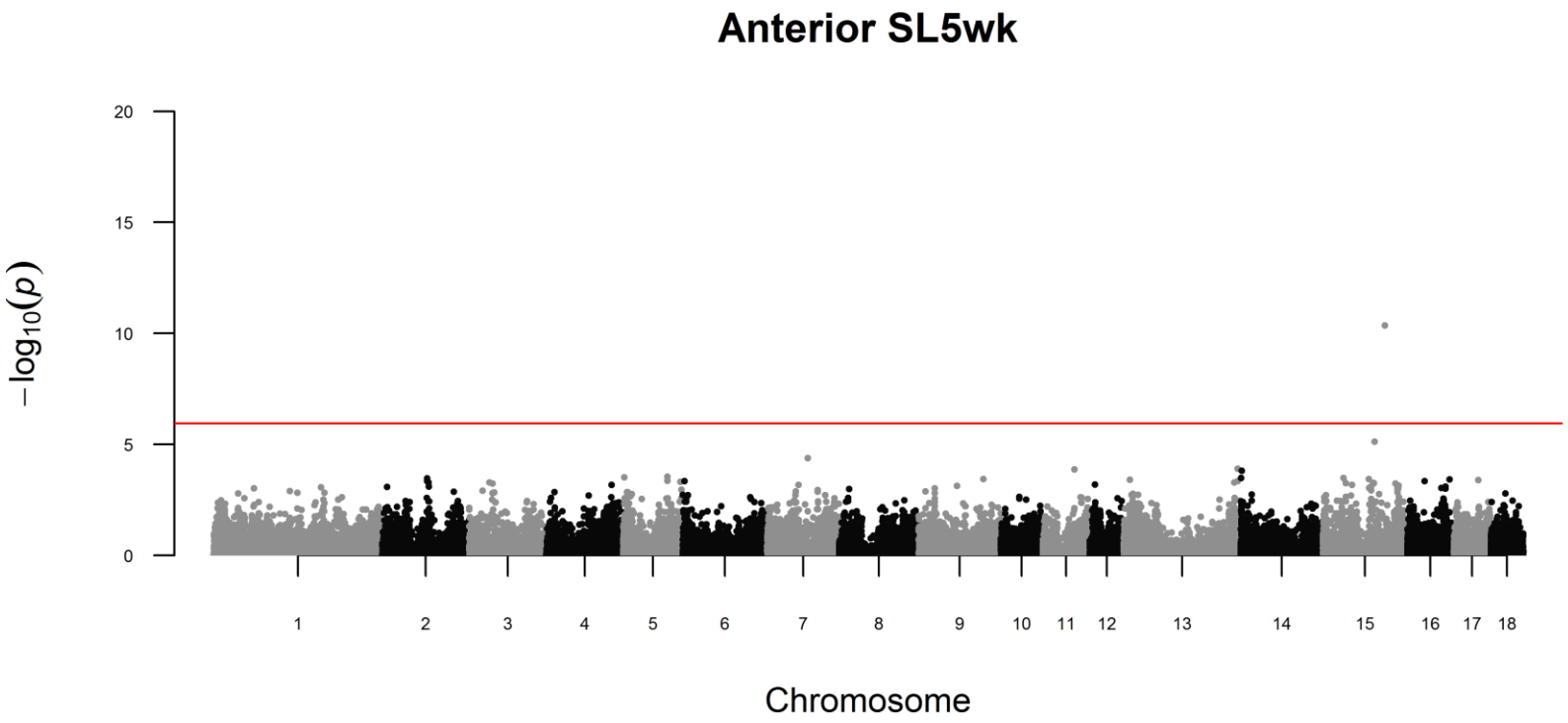


Figure 6.9 Genome wide plot of –log10 P values (y axis) for association of SNPs with anterior skin lesions recorded 5 weeks post-mixing. The horizontal line indicates the threshold for genome wide significance.

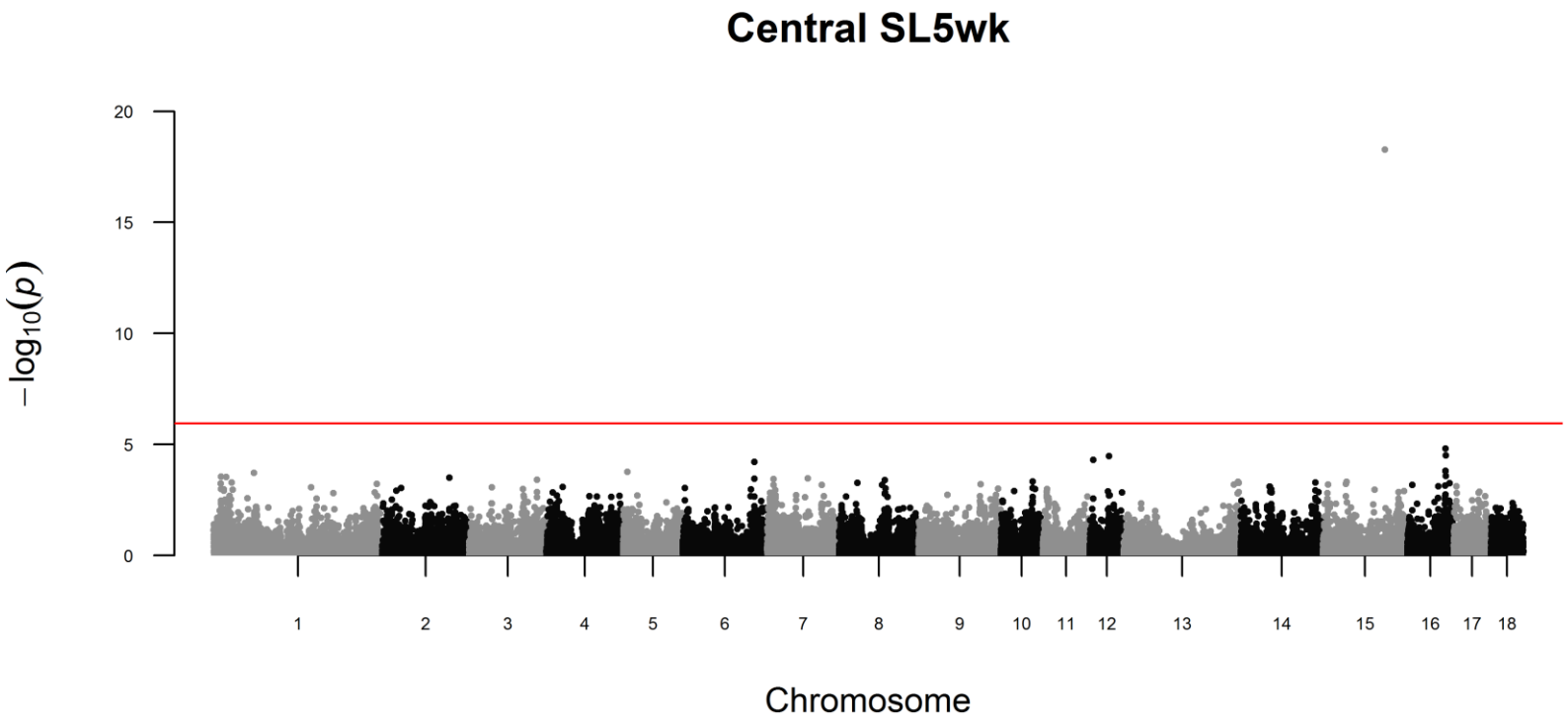


Figure 6.10 Genome wide plot of –log10 P values (y axis) for association of SNPs with central skin lesions recorded 5 weeks post-mixing. The horizontal line indicates the threshold for genome wide significance.

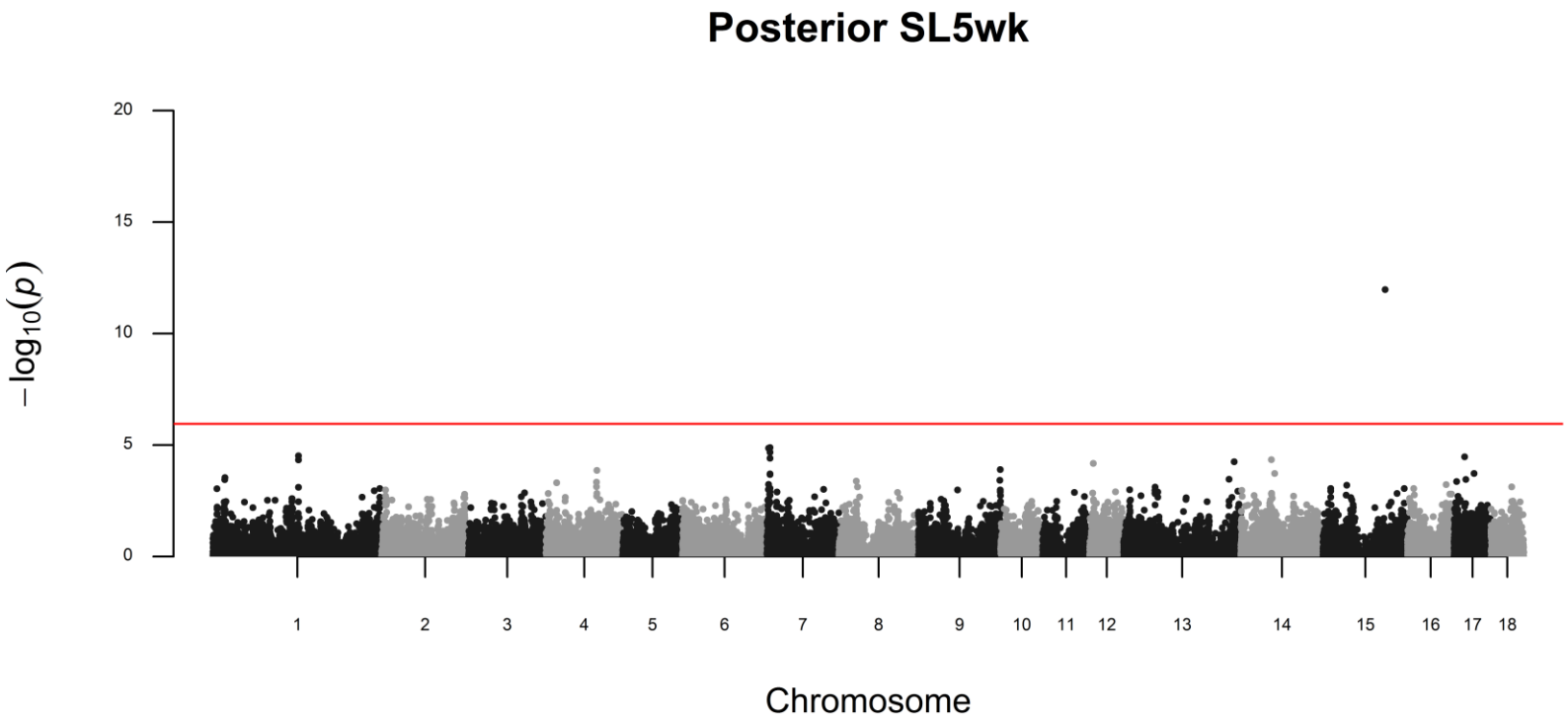


Figure 6.11 Genome wide plot of –log10 P values (y axis) for association of SNPs with posterior skin lesions recorded 5 weeks post-mixing. The horizontal line indicates the threshold for genome wide significance.

6.4 Discussion

6.4.1 LD and population stratification

Average LD for adjacent SNPs was very similar to that found in a different population of pigs (r^2 = 0.36; Kapell, 2011). Average LD reduced considerably (r^2 = 0.15) for SNPs within 1Mb windows, compared to those found by Kapell (2011), who calculated an average r^2 of 0.28. Linkage disequilibrium is expected to be lower when several genetic lines under selection for different traits are pooled, and this is probably the reason for the lower LD calculated in the present population. In contrast, genomic data used by Kapell (2011) were pooled from one crossbred line (Yorkshire X Landrace) and its purebred parental line (Yorkshire). Due to time constraints it was not possible to calculate LD across the whole genome for each line individually in this study. Linkage disequilibrium was therefore calculated for line B, and compared to the population as a whole. The results showed that, as expected, LD persisted over longer distances for a single line compared to the whole population.

Population stratification is a known issue in GWAS (Balding, 2006; Segura et al., 2012). In populations where random mating occurs, linkage disequilibrium is less likely to occur between unlinked markers. Where non-random mating occurs (e.g. in farm animals selected for certain traits) we expect variation in allele frequencies between subgroups (e.g. breed type). In the current study, this could result in statistically significant associations between skin lesions and SNPs that are unlinked to a causative loci, simply because that SNP appears with a higher frequency within a given breed (Pritchard and Rosenberg, 1999). As phenotypic records were pooled from animals from 5 distinct genetic lines, some degree of population stratification was expected in this population. A principal components analysis confirmed that there was stratification, which was largely explained by genetic line. Genetic line and the first 5

principal components were included as fixed effects in the model, to ensure that any SNPs found to be significantly associated with skin lesion traits were not an artefact due to stratification. The multi-locus mixed model methodology applied in this study has also been shown to improve the power of GWAS in the presence of population stratification, compared to single locus mixed models (Segura et al., 2012). Quantile-quantile plots are often used in GWAS in order to assess the deviation of the values of the observed test statistic (in this instance –log10 *P* value) from the expected *P* value. If population structure was a cause for concern it would be anticipated that the observed values would deviate from the expected values, which was not the case for any of the skin lesion traits analysed in this study.

6.4.2 GWAS

The aim of the present study was to identify SNPs significantly associated with skin lesion traits recorded 24 hour and 5 weeks post mixing, in a commercial herd of pigs. Sixteen SNPs were significantly associated with skin lesion traits on a chromosome-wide level, while a single SNP was found to be significantly associated with anterior, central, and posterior SL5wk on the genome-wide level. Six of the SNPs found to be significant on a chromosome wide level were located within introns of genes with a known function. Four of the SNPs were located within holocarboxylase synthetase (HLCS), which is an essential part of multi-protein complexes in chromatin, that has been implicated in shortened life span and low stress resistance in Drosophila (Xia et al.,2013), and gluconeogenesis, fatty acid synthesis in humans (Yang et al., 2001). A further SNP associated with central SL24h was found within caspase 10 (CASP10), which is involved in apoptosis and has been associated with cancer susceptibility in humans (Yan et al., 2012). Finally, a SNP associated with posterior SL24h was located within an intron of the *Drosophila* homologue, Single-Minded Family BHLH

Transcription Factor 2 (SIM2), which is involved in the regulation of fly neurogenesis, and implicated in Down's syndrome in humans (Chrast et al., 1997). A further 4 SNPs were located within genes for uncharacterised proteins. A SNP associated with central SL24h, was located on the uncharacterised gene LOC100624904, which is predicted to be a caspase 8 like protein, which has been shown to be involved in initiating apoptosis (Kruidering and Evan, 2000). Two SNPs associated with posterior SL24h were located within LOC100516477. One of these SNPs was located within an exon, and is a missense variant meaning, in this instance, that an allele change from A to G results in a change in amino acid sequence from asparagine to serine. This gene is predicted to be analogous to ankyrin repeat, SAM and basic leucine zipper domain-containing protein 1-like (ASZ1), which has been shown to enhance the expression of genes involved in germ cell development (Wang et al., 2013). Finally the single genome-wide significant SNP detected in the present study, as well as one SNP associated with central SL24h on a chromosome wide level was located within an intron of a gene of uncharacterised protein, predicted to belong to the aldehyde oxidase family of genes, which are involved in catalysing the oxidation of aldehyde and are involved in drug metabolism (Garattini and Terao, 2012). All other SNPs significantly associated with skin lesion traits were located outside of known gene regions. None of the genes mentioned have been previously implicated in pig behaviour.

There have been 4 main studies exploring the genomic basis of social aggression in pigs. The most similar to the present study was conducted by Pong-Wong et al (2010), who performed GWAS on a population of 552 animals phenotyped for aggression 24 hours post-mixing, and 3 weeks post-mixing, as well as for three behavioural aggressive traits: receipt of non-reciprocal (one-sided) aggression, delivery of reciprocal (two-sided) aggression, and involvement in reciprocal aggression. Skin

lesions were recorded using an identical recording protocol used in this study. That study found no genome or chromosome wide significant SNPs associated with any skin lesion or aggressive behavioural traits. The authors hypothesise that this may have been due to insufficient statistical power, given the low sample size. Alternatively, they suggest that the trait may be truly polygenic, and it may be that no single gene contributes to a sufficient amount of variation to enable detection.

The remaining studies have focused on genes involved in the hypothalamic-pituitary-adrenocortical (HPA) axis and the serotonergic system, both of which have been previously implicated in the regulation of aggressive behaviour (Fernandez et al., 1994; Alekseyenko & Kravitz, 2014). Terenina et al. (2012), investigated the relationship between 17 candidate genes known to regulate the serotonergic system and aggressive behaviour, measured via skin lesions as per the present study. The study found one SNP located within the gene for the dopamine receptor DRD2 (chr 9) to be associated with posterior skin lesions recorded 24 hours post-mixing. A further 8 polymorphisms within genes for the dopamine receptor DRD2, serotonin transporter SLC6A4 (chr 12), serotonin transporter HTR2C (X chr), and vasopressin receptor VPR1A (chr 5) were found to be significantly ($P < 0.05$) associated with aggressive behavioural traits. Similarly, Muráni et al. (2010) used a candidate gene approach to look for associations between SNPs located in 10 genes related to the HPA axis with physiological measures of stress (cortisol, creatine kinase, glucose, and lactate), adrenal weight, and aggressive behaviour, as measured by skin lesions. They found an association between the glucocorticoid receptor NR3C1 (chr 2) and lesions to the anterior region of the body 24 hours post-mixing, and between the arginine vasopressin receptor AVPR1B (chr 9) and central lesions recorded 24 hours post-mixing. None of the SNPs found to be significantly associated with aggression in the present study shared a chromosome

with genes associated with aggression as found by Terenina et al. (2012) and Muráni et al. (2010).

Several studies have led to the discovery of QTL associated with other aspects of pig behaviour, such as feed intake (Houston et al., 2005); exploration during a stressful isolation test (Désautés et al., 2002); behaviour before and after infection with a parasite known to affect behaviour (Reiner et al., 2009). Désautés et al. (2002) measured locomotion, vocalization, defecation rate, and exploration behaviour of pigs for 10 minutes after they were placed in a novel environment. In addition, blood taken before and after isolation was measured for adrenocorticotropic hormone (ACTH), cortisol and glucose. Of these traits, locomotion, exploratory behaviour, ACTH taken after the test, and cortisol measured before and after the test; were found to be significantly ($P < 0.05$) associated with genetic markers, although markers associated with behaviour each accounted for less than 9% of the variation in the trait (compared to almost 21% of the variance in cortisol change). Cortisol levels before and after the stress test were associated with markers located on chromosomes 7 and 18; however SNPs located on chromosomes 7 and 18 in the present study were between 26 and 101 million base pairs from the nearest cortisol-associated QTL. The same study also found a QTL associated with ACTH, an important part of the HPA system, located on chromosome 17, however the SNP detected on chromosome 17 in this study was located at least 23 million base pairs from this QTL. Reiner et al. (2009) identified QTL significantly related to the amount of activity whilst lying down, time spent drinking, and time spent walking on a genome wide level, and additional QTL significant on a chromosome wide level for rooting and social behaviour. One SNP found to be significantly associated with posterior SL5wk in the present study was found within

the region of the QTL associated with time spent walking, which accounted for 12.3% of the phenotypic variance in that trait.

Three main studies have identified QTL associated with aggression in Drosophila and in mice. Brodkin et al. (2002) identified 2 QTL that affect intermale aggression. They proposed five candidate genes for these QTL, including diglycerol kinase a subunit (dgka), kinesin family member 5A (Kif5A), olfactory receptor 9 (Olfr9), Glutamate subunit AMPA3 (Gria3), hypoxanthine guanine phosphoribosyl transferase (Hprt). Orthologues of dgka, Kif5A, Gria3, and Hprt have all been identified in pigs, located on the X chromosome and chromosome 5. A similar sequence to Olfr9 also exists in pigs, however the function of this gene has not yet been characterised. Dow et al. (2012) identified 3 loci that affected the expression of intermale aggression in mice. Three candidate genes were proposed: protein tyrosine phosphatase, receptor type, F polypeptide (Ppfia2), which is orthologous to an uncharacterised protein in the pig genome (LOC102164936), and citrase synthase (Cs) and Erb-B2 Receptor Tyrosine Kinase 3 (Erbb3), both of which are highly similar to known proteins in pigs, both located on chromosome 5. In Drosophila, Edwards et al., (2006) identified four candidate genes for QTL found to affect variation in aggressive behaviour, Sin3A-associated protein 130 (Sap130), CG10754, mutagen-sentitive 312 (mus312) , and Ral guanine nucleotide exchange factor 2 (Rgl). A BLAST search of each of these protein sequences returned orthologues with a maximum of 37% similarity in pigs.

In this study, the maximum combined effect of SNPs associated with any skin lesion trait was 6%. These results suggest that skin lesion traits are not under the control of a single gene, or a few genes, that exert a large effect. This, together with the wide range of biological pathways implicated in aggression as discussed above, suggest that

aggressive behaviour is likely to be controlled by many genes, each with a small effect. If this is the case, genomic selection, which utilises dense markers across the entire genome to calculate genomic breeding values, is likely to be of use more value with regards to making genetic progress in this trait. Kapell et al., (2011) assessed the potential for genomic selection for this trait in pigs, using SNP genotypes from 552 pigs phenotyped for aggression using both behaviour and skin lesions. Genomic information was found to increase the accuracy of prediction (correlation between observed phenotype and predicted phenotypic based on the model) to a greater degree for skin lesion traits compared to directly measured aggressive behavioural traits. It was concluded that genomic selection is likely to be a useful tool when selecting based on skin lesions, and this may be of interest in future work. As work is currently on going to improve the accuracy of genomic selection between breed types, it was not possible to explore this further in the current population.

6.4.3 Conclusion

Although several SNPs were found to be significantly associated with skin lesion traits in this population, the overall variance explained was low. It is likely that aggressive behaviour is controlled by many genes with small effect. Genomic information may aid selection against this trait in the future, however this is more likely to be in the form of genomic selection.

Chapter 7 - General discussion and conclusion

7.1 Introduction

Skin lesions, which are inflicted during the course of aggressive interactions between pigs, provide a rapid method of identifying individuals that have been involved in physically aggressive interactions. Previous analyses suggest that skin lesions are heritable, and are genetically associated with aggressive behaviour. The evidence suggest that skin lesions may provide a useful trait for the purposes of selecting against social aggression in pigs (Turner et al., 2008; Turner et al., 2009). Gaps in the current knowledge pose a barrier to implementing these traits into practical breeding programmes. This thesis aimed to further understand the genetic basis of skin lesion traits, and how these traits correlate with other production and behavioural traits, including aggressive behaviour. The chapters in this thesis containing data analyses can be grouped under 3 broad topics. In Chapters 2 and 3 the relationships between aggressive behaviour at mixing, and skin lesions under newly mixed and stable social conditions were explored. Chapter 2 focused on these relationships on a phenotypic level, while Chapter 3 focused on the expected behavioural response, using skin lesions as selection traits. The main findings of these studies will be briefly outlined in section 7.2. Chapters 4 and 5 focused on the genetic relationships between short and long term lesions, and traits of commercial interest, as well as other behavioural traits. Chapter 4 used information on growth, feed intake and carcass data for this purpose, while Chapter 5 used data from behavioural experiment, designed to measure fearful behavioural responses in pigs. The main findings of Chapter 5 will be briefly discussed in section 7.3. Chapter 6 focused on the genomic basis of skin lesion traits, specifically exploring statistical associations between single nucleotide polymorphisms (SNPs) with short and long term lesions. The GWAS results presented in Chapter 6 will be discussed separately in section 7.4. Finally, section 7.5 will discuss the overall implications of the results, and provide suggestions for further study.

7.2 Exploring the relationship between skin lesions and aggressive behaviour at mixing

The population of pigs described in Chapters 2 and 3 had previously been described in Turner et al. (2009) where genetic correlations were found between skin lesions and certain aggressive behavioural traits (namely, the time spent: involved in reciprocal aggression, delivering non-reciprocal aggression, and receiving non-reciprocal aggression). Negative genetic correlations between aggressive behaviour at mixing identified by SL24h and skin lesions 3 weeks (SL3wk) post mixing prompted the question of whether increased aggression at mixing might lead to increased social stability in the long term. There was very little information in the existing literature regarding long-term social stability in pigs, and how this related to skin lesions in particular. The strength and direction of genetic correlations between skin lesions and reciprocal aggressive behaviour (r_G = 0.67), combined with the heritabilities (h^2 = 0.19 – 0.48) estimated for skin lesion traits, suggests that it should be possible to reduce aggressive behaviour at mixing via selection against skin lesions. However no study has previously attempted to quantify the potential genetic change. Quantifying an accurate expected response to section using genetic correlations is difficult due to the high degree of sampling error involved in the estimation of those correlations. Furthermore, the correlation among skin lesion traits and behavioural traits are very complex and therefore an estimation of the selection response given the data was preferred. The size of the dataset was also not large enough to fit a full model including a direct and social environmental effect so that expected selection response based on genetic correlation may not reflect the true relationship between traits. Although negative correlations between aggression at mixing and skin lesions under socially stable groups have previously been published (Turner et al., 2009), no published literature has discussed

the possibility of using long-term skin lesions as a method of selecting against aggression.

Chapter 2 of this thesis investigated the relationship between aggression at mixing and skin lesions in more detail. The aim was to establish whether increased aggression at mixing resulted in more stable hierarchies in the long term, as determined by the number of skin lesions recorded 3 weeks post mixing. The results showed that, on both a group and individual animal level, increased involvement in reciprocal aggression at mixing results in fewer skin lesions 3 weeks post mixing. If increased aggression results in increased group stability, it would seem that involvement in reciprocal contests is important for low long-term aggression. If aggression serves to establish dominance relationships, then we may expect that short, decisive fights that have a clear winner and are not repeated would be more likely to lead to an unambiguous dominance status. With this in mind it was expected that the 'quality' of the fights - for example proportion of repeated fights fight intensity, or ambiguous outcomes - would also correlate with skin lesions at three weeks, however the results indicated no significant association. It may be that active involvement in reciprocal aggression aids long-term recognition of dominance relationships, and the quality of the aggression is less important. Alternatively the behavioural traits defined here simply may not accurately describe behaviours that facilitate stable dominance hierarchies. On an individual animal level, residual correlations and the results of stepwise regression analyses combined suggested that animals that are involved in aggression receive the most skin lesions to all body regions, however within the aggressive cohort animals that win a high proportion of fights receive fewer lesions at mixing. Animals that avoid aggression altogether receive the fewest lesions at mixing, but receive the most lesions at three

weeks. This suggests that avoiding aggression altogether at mixing is the most detrimental strategy in the long term based on the results on phenotypic level.

The aim of Chapter 3 was to quantify the expected response to selection, and identify the skin lesion trait that would result in the biggest overall reduction in aggression on a phenotypic level. To achieve this, a comparison of EBVs for all aggressive behavioural traits with EBVs in the lowest 10% for skin lesions at mixing, and the highest 10% for skin lesions 3 weeks post mixing were carried out. At first, the genetic correlations were estimated to examine the consistency of the expected response given the data and genetic correlations. Genetic correlations between anterior SL24h and aggressive behavioural traits were of a moderate to high magnitude and were found to unambiguously predict aggressive behaviour. Receipt of non-reciprocal aggression was not significantly correlated with anterior SL24h. This is to be expected, as non-reciprocal aggression is directed towards the rear of the body. However, this result is in contrast to the phenotypic correlations presented in Chapter 2, which suggested that animals that received anterior lesions were also likely to have been the recipients of non-reciprocal aggression. The genetic correlations described in Chapter 3 were more closely aligned with the phenotypic results of Turner et al., 2006, where they investigated the proportion of skin lesions inflicted on each body region, and found that anterior SL24h were indicative of reciprocal aggression, while posterior SL24h were indicative of receipt of non-reciprocal aggression.

Chapter 3 provided further insight into how central and posterior lesions are related to aggressive behaviour at mixing. The results suggested that central lesions are an ambiguous measure of aggression in individual animals, as they appear to measure active involvement in aggression as well as the receipt of one-sided aggression, perhaps

due to the low fight success rate of animals with many lesions to this body region. If posterior lesions are an unambiguous measure of aggression received, and anterior lesions are an unambiguous measure of involvement in reciprocal aggression, it is likely that central lesions result from a mixture of these behaviours. Animals that receive many lesions to the central region of the body appear to be those animals that are actively involved in aggression, but are unsuccessful. In contrast, selection for low posterior SL24h was predicted to result in selection for animals that are genetically predisposed to be involved in much aggression, and win a large number of fights.

With regard to lesions received under stable social conditions, the results suggested that selection for increased skin lesion numbers would reduce aggressive behaviour at mixing. The magnitude of the predicted response was lower than that predicted for SL24h. Selection for anterior and central SL3wk were predicted to have a similar response in terms of the magnitude of the response, while posterior SL3wk were found to be a poor predictor of aggressive behaviour performed at mixing. The implications of this will be discussed further later in the discussion. Overall, the results suggested the selection based on low EBVs for anterior SL24h has the potential to substantially reduce aggressive behaviour. For example, on a phenotypic level, the number of attacks received by pen mates for individuals with low anterior SL24h EBVs were -0.74 standard deviations below the population mean. On a genetic level, the duration of time spent receiving reciprocal attacks was -1.17 standard deviations below the population mean.

7.3 Correlations between skin lesions and traits of commercial interest

There was some evidence from the literature that social aggression between pigs may be associated with other traits of commercial interest, in particular growth and other

behavioural responses (Tan et al., 1991; Hessing et al., 1994; Wellock et al., 2003; D'Eath et al., 2009), however few studies have looked at these relationships on a genetic level. The existing literature is often contradictory on these relationships, and the aims of Chapter 4 and 5 were to determine whether phenotypic or genetic associations exist between skin lesion traits and several production and behavioural traits of interest using a different population than those in Chapter 2 and 3.

The correlations presented in Chapter 4 showed very little evidence of a phenotypic association between SL24h, SL5wk and production traits. If aggression *per se* affected growth we would expect to see differences in growth between animals that engaged in a lot of aggression at mixing and animals that fought very little, however this was not evident in this population. As daily growth is averaged over the entire growing period and skin lesions are a measure of the environment over 24 hours, it is possible that aggression temporarily effects growth, however this cannot be detected over the entire growing period. Alternatively, previously found effects of aggression on growth may be due to the effects of mixing, and not aggression *per se* (Stookey and Gonyou, 1994). For example, in addition to comparing growth between mixed and unmixed groups, Stookey and Goyou (1994) looked at growth in groups of pigs that were mixed for a single day, and then returned to their original groups. Despite aggression levels rapidly falling to those observed prior to mixing, growth in temporarily mixed groups was reduced to a similar extent as permanently mixed groups. As data from an unmixed control were not available in order to compare growth with it was impossible to analyse whether mixing affected growth on a phenotypic level in this population. No behavioural measures of fearfulness were found to correlate with any skin lesion traits on a phenotypic level.

Significant negative genetic associations were found between central and posterior SL24h, or skin lesions on all body regions 3 weeks post mixing with hot carcass weight and lifetime daily gain. No significant genetic associations were estimated between skin lesion traits recorded at either time with loin depth or back fat. Genetic correlations (r_g between 0.31 and 0.37) have previously been found between growth and carcass weight with loin depth and back fat in commercial crossbred pigs (Miar et al., 2014) so that based on the association of hot carcass weight and skin lesion it could be derived that body composition traits may be also genetically correlated to lesion traits. However, the results presented in this thesis suggest that loin depth and backfat are not under the same genetic control as skin lesion traits. Phenotypic and genetic correlations as presented in Chapters 2 and 3, suggest that animals with many lesions under stable social conditions are those that had a low proportion of fight success, were involved in little reciprocal aggression and received substantial one-sided aggression at mixing, suggesting that these animals are subordinate and unaggressive. It may be the case that unaggressive animals have more resources left for growth (Rauw et al., 1998). Negative residual correlations between growth and skin lesions suggest that environmental factors work in opposition to genetic correlations, resulting in little to no phenotypic correlation between these traits. Perhaps receiving attacks is physically demanding and reduces the rate growth of individuals that receive much aggression in the long term. If this is the case, then reducing the amount of aggression experienced by these individuals may have a favourable result on growth. In contrast, animals that receive many lesions to the anterior region of the body are likely to be the most aggressive individuals at mixing. Lesions to the anterior region of the body were not found to significantly correlate with any production trait. Likewise, similar results obtained in Chapter 5 did not produce any evidence that selection for anterior SL24h is associated with or measures of behaviour associated with fearfulness.

There was some evidence in Chapter 5 that there are genetic correlations between the receipt of many lesions under socially stable conditions and low reactivity in challenging situations: being isolated from pen mates and subjected to a human approach, or restrained, isolated, and handled in a weighing crate. Once again, these relationships were not observed on a phenotypic level. Pen and residual correlations were positive, although they did not significantly differ from zero, therefore it would appear that environmental factors work in opposition to genetic factors, and explain why these relationships were not observed on a phenotypic level.

7.4 Genome wide association studies (GWAS) and genomic selection

Several SNPs were associated with skin lesions recorded 24 hours and 5 weeks post mixing, however each of these SNPs only accounted for between 1 and 4% of the phenotypic variation observed when considered singly, and up to 6% when all SNPs were considered for each trait. The wide range of biological systems shown to be implicated in the regulation of aggressive behaviour (Anholt and Mackay, 2012), combined with the results of Chapter 6 and previous work (Pong-Wong et al., 2010) suggest that social aggression is likely to be under the control of many genes with small effects. With this in mind, genome wide studies attempting to identify few genes with large effect may be of limited use when studying this, or any, complex behaviour, and genomic selection may be of more value with regards to making genetic progress. In contrast to genome wide association studies (GWAS), which identifies SNPs associated with specific QTLs or genes, genomic selection aims to use dense markers across the whole genome to estimate genomic breeding values. Traditional methods of estimating breeding values rely on a combination of pedigree information and performance

records of relatives over generations, with selection of animals occurring after performance records are available. When referring to mass phenotypic selection, rate of genetic gain per annum is affected by the heritability of the trait, the selection intensity, phenotypic variation, and the generation interval. In many cases, for example dairy cattle, the generation interval is the limiting factor, as it can take up for 7 years to obtain a highly accurate EBV for a sire (Schaeffer, 2006). In contrast, genomic selection makes use of genomic (SNP genotypes) and phenotypic information to estimate breeding values, allowing for accurate prediction of an animal's performance at birth. This eliminates the need to wait for phenotypic data to be recorded (Schaeffer, 2006), thereby decreasing the generation interval and increasing the rate of genetic gain per annum. The technique has had the most success when applied to dairy cattle populations and is extensively used in breeds such as the Holstein (Hayes et al., 2015), but has more recently been adopted by laying hen and pig breeding companies. In pigs initial work has been carried out looking at the viability of using genomic selection to select against aggression (Kapell et al. 2011). In that study 552 pigs were genotyped using the PorcineSNP60 panel and phenotyped for aggression using both behaviour and skin lesions. Genomic information was found to increase the accuracy of prediction (correlation between observed phenotype and predicted phenotypic based on the model) to a greater degree for skin lesion traits compared to directly measured aggressive behavioural traits. It was concluded that genomic selection is likely to be a useful tool when selecting based on skin lesions. The success of genomic selection so far has mainly been in purebred populations, and work is still on-going to find ways of computing accurate predictions across lines and breeds. Very recently, Hidalgo et al. (2015) obtained accuracies of between 0.11 and 0.31 for litter birth weight, total number of piglets born, and litter size variation when calculating genomic breeding values for crossbred animals from their purebred parental lines. The genotyped

animals used in this thesis came from 5 genetically distinct crossbred lines under selection for different traits, and so the estimation of genomic breeding values across lines may not reflect the true genomic breeding values within line. In addition, the number of animals in this experimental data is not large enough to predict accurate genomic breeding values. Across population genomic selection is currently of great interest in the animal breeding community, therefore a consistent and accurate method of prediction, which is suitable for use across lines will hopefully soon be developed (Jonas and de Koning, 2015).

7.5 Conclusion

7.5.1 Development of the most appropriate selection criteria for reducing aggression of pigs

In this section, the most appropriate selection criteria for reducing aggression of pigs based on the results of this thesis is concluded. In contrast to directly measuring behavioural traits, which are difficult and costly to record, skin lesions provide a relatively rapid and simple method of phenotyping involvement in aggressive behaviour. Skin lesions measured 24 hours post mixing, can be used as an indication of the level of aggression an animal was involved in the 24 hours post mixing, when dominance relationships are formed. In contrast, skin lesions measured several weeks after mixing reflect the amount of aggression an animal has received, and lesions measured on a group level may provide an indication of the stability of the social relationships within the group (Table 2.6). When recording traits for selection purposes, it is of benefit to measure the trait as early as possible, so that breeding values can be rapidly incorporated into breeding indexes, and earlier decisions on breeding stock made. For this reason, recording skin lesions a mixing has an advantage over skin lesions recorded later in the growth period. Skin lesions measured several

weeks post mixing have the disadvantage of being collected later in the production cycle, however lesions are easier to record at this point, as there are fewer lesions to count, and older animals tend to be easier to handle. In addition, as animals can spend several months in the same social groups, chronic aggression is likely to be more important from a welfare perspective than aggression at mixing.

Heritabilities of lesion numbers at mixing were of low to moderate magnitude in the two analysed populations (Table 3.2 and Table 4.2, respectively), whereas heritabilities of lesions scores in the stable groups were of moderate to high magnitude (Table 3.2 and Table 4.2, respectively). Heritability of anterior SL24h has been estimated to be between 0.08 and 0.26 from growing pigs housed under different management systems. The lowest heritability was estimated for the second population used in the present thesis (Chapters 4 to 6; Table 4.2). This may be due variation between breed type, which was included in the model as a fixed effect, and may have accounted for some of the genetic variation. Heritabilities estimated for anterior SL24h are within a range to obtain sufficient response to selection within breeding programmes.

Genetic correlations between skin lesions at mixing, and corresponding lesions 5 weeks later were of large magnitude for anterior and central lesions, and of moderate magnitude for posterior lesions (Table 4.4). Therefore, selection for reduced anterior and central lesions at mixing are expected to also reduce long-term aggression as predicted by lesions in the stable group. The large genetic correlations calculated between lesions recorded at mixing and 3 weeks post-mixing indicate that the short period of recording lesions (a single day at each time point) is large enough to be highly informative of aggressive behaviour at mixing. Higher genetic correlations were found between anterior SL24h and aggressive behavioural traits, compared to central or

posterior SL24h, particularly with regards to severe, reciprocal aggression (for example the number and duration of reciprocal attacks). Anterior SL24h was not significantly correlated with the proportion of fights won at mixing, therefore selection against this trait is not expected to simply select for meek or subordinate individuals (Table 3.3). Consequently, it is expected that a range of dominance ranks would be maintained following selection, however it is hoped that selection will lead to social relationships being formed in a less aggressive manner. Selecting for reduced anterior SL24h was predicted to have the largest response on aggressive behaviour at mixing, compared to selection for any other lesion trait, either at mixing or 3 weeks post mixing (Figure 3.3 [predicted genetic response] and Figure 3.4 [predicted phenotypic response]).

As anterior SL24h showed lower heritability than central SL24h (Table 3.2), the differences in predicted response on aggressive behaviour must be due to the differences in genetic correlations with aggressive behavioural traits (Table 3.4), and the differences in variation of these traits. However, following transformation, the variation in anterior lesions at mixing was slightly lower than central lesions (Table 3.1). In contrast, the phenotypic variance of anterior SL24h was substantially higher than for central lesions. Although the phenotypic variation was highly skewed, this may result in clearer identification of the most aggressive animals, and result in the highest phenotypic reduction in aggressive behaviour (Table 3.5). Anterior SL24h showed no genetic correlations with performance traits, such as average daily gain, and body composition (Table 4.5 and Table 4.6), therefore selection for this trait is not expected to compromise production traits. The genome wide association study did not reveal any QTL to be significantly associated with anterior SL24h (Figure 6.6). It is likely that this trait is under the regulation of many genes, each with a small effect, as per the

infinitesimal model. This means that marker assisted selection cannot be used for this trait; however traditional pedigree selection or genomic selection should be successful (Kapell, 2011).

As discussed in Chapter 3, social effects of aggression are likely to be of importance, however it has been shown that an accurate estimation of the genetic correlation between direct and social effects is challenging (Canario et al. 2012). Due to the large number of groups composed of few families required to estimate social effects (Bijma et al., 2007) it was not possible to estimate social breeding values in either population represented in this thesis. However, the heritabilities of the skin lesion and behavioural traits were substantially higher than the proportion of the phenotypic variance attributed to common environmental (pen) effects (Table 3.2 vs. Table 2.6). Additionally, genetic correlations between lesion traits and aggressive behaviour were substantially higher than corresponding correlations related to the pen environment (Table 3.2 vs. Table 2.6). These results indicate that, relative to the genetic effects, the pen environment has less of an influence on skin lesion numbers. This means that the use of a direct-social effects model, which would theoretically be the best model to use, may not be necessary. In summary, based on the results of analyses performed throughout this thesis, anterior SL24h is the best skin lesion trait for selection purposes.

7.5.2 Selection for increased SL3/5wk

The results presented in this thesis suggest that selection for increased lesions under stable social conditions may also result in reduced aggression at mixing. Although the impacts of long term aggression are yet to be quantified, it is likely that chronic

aggression is of a higher welfare concern than aggression at mixing. From a practical perspective, there are several advantages to recording skin lesions several weeks post-mixing. Skin lesions recorded at this time point have been found to have higher heritabilities than those recorded at mixing, and lesions are easier to record in socially stable groups of older animals, as there are fewer lesions to count, and older animals are easier to handle. With respect to improved performance, selection for increased lesions under stable social conditions would be more efficient, in particular if selection was based on posterior lesions at this time. However, skin lesions within stable groups have only been studied in relation to aggressive behaviour at mixing. More research is therefore needed to determine whether these lesions are informative for long-term reduction in aggression. There are several key questions raised by these analyses that would be of interest in future studies. If mixing and long term aggression are problematic, then animals of particular interest in future behavioural research are those that receive few lesions both at mixing and under stable social conditions. Whether they are dominant or subordinate, presumably these animals are able to convey their social status via other means, such as behavioural (Camerlink et al., 2014) or olfactory (McGlone, 1985) cues.

7.5.3 Suggestions for further work

As discussed above in section 7.5.2, based on the analyses performed throughout this thesis, anterior lesions would be the recommended trait for selection purposes. However, in reality more work is needed before skin lesions can be incorporated into breeding programmes. In Chapter 2 an expected response to selection based on selection for low anterior SL24h was estimated, based on individuals with anterior SL24h EBVs in the bottom 10% of the population. In practice, anterior skin lesions would need to be included into a breeding index, which includes all traits under

selection. Further work is required to estimate genetic correlations with current index traits, including reproductive traits. This information could then be used to a) estimate the actual expected reduction in aggression, once this trait is appropriately weighted and incorporated into a selection index, b) derive the expected economic cost/benefit of selecting against skin lesions, depending on how this trait impacts current section traits.

Although genetic correlations between skin lesions and certain traits were explored in this thesis, this work does not begin to explore what is likely to be the highly complex relationship between the genes underlying aggressive behaviour, and any number of biological systems. Given that aggression has been implicated in a wide range of neuroendocrine pathways (see section 1.7 of the introduction for more detail), it is entirely possible that selection aimed at modifying this behaviour could have effects on other processes, possibly to the detriment of the animals and producers. Work is on-going to disentangle these relationships, particularly in Drosophila (Anholt and Mackay, 2015) however the complexity of these relationships means that it is likely to be some time before these processes are fully understood in higher mammals, including pigs.

Detailed behavioural interactions such as those recorded at mixing and described in this thesis have not yet been recorded for aggression within stable groups. It is therefore not certain which individuals initiate aggression and inflict skin lesions within social stable groups, and why chronic aggression persists. Genetic correlations between aggression at mixing and skin lesions in stable groups offer some evidence that subordinate animals receive lesions from dominant individuals, however the correlations are often of a low magnitude and do not fully explain the skin lesions within socially stable groups. Positive low to moderate genetic correlations have been found between skin lesions recorded at mixing and in stable groups, suggesting that

animals that receive many lesions at mixing go on to receive many lesions in stable groups, which is clearly in conflict with behavioural correlations discussed in this thesis. If lesions are inflicted by dominant pigs to subordinate group members, the motivation behind these attacks are unknown. For example, it is possible that subordinate pigs provoke aggression through direct challenges to the social hierarchy (for example by trying to displace dominant pigs at feeding), or perhaps these pigs are socially incompetent and are unable to convey their subordination, or have not learned to accept the social order. Alternatively these individuals may be the victims of unprovoked bullying behaviour from chronically aggressive individuals. It is also possible that skin lesions are the result of fighting between subordinate individuals that are attempting to increase their social standing in the group. It is unknown at this time whether these individuals experience reduced welfare as a result of the chronic aggression they receive. Behavioural information from stable groups is vital if we are to further dissect the relationships between skin lesions and behaviour under newly mixed, and stable social conditions. Information on social networks and the implications of positive behaviours are also of interest, as it is likely that positive behaviours also affect social relationships in the long term. Finally, it is of importance to determine the extent to which short and long term aggression affects the wellbeing and productivity of pigs.

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