Investigating the genetic and genomic basis of osteochondrosis in Thoroughbred horses from Australia and New Zealand

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A thesis submitted to the Faculty of Veterinary Science, The University of Sydney, in fulfilment of the requirements for the Degree of Doctor of Philosophy

December 2012
Declaration

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

(Kao Castle)

17 December 2012
Statement of Contributions

The identities of the studs and horses that participated in the research described in this thesis are protected by confidentiality agreements. On this basis, the names of stud staff and veterinarians who provided data for this research are not listed as authors or named in the acknowledgements, although their input was invaluable.

Chapter 2, Skeletal lesions and injuries in Australasian Thoroughbred weanling and yearling radiographs (draft manuscript). Castle K., Tammen I., Thomson P.C., Jeffcott L.B., Raadsma H.W. and Nicholas F.W.

- Dr Tammen supervised components of this work, contributed to discussions, and provided feedback on the presentation of results.

- Associate Professor Thomson provided advice on statistical techniques to compare results between the weanling and yearling populations, and provided feedback on the presentation of results.

- Professors Jeffcott and Raadsma contributed to planning the data collection strategy, and provided feedback on the presentation of results.

- Emeritus Professor Nicholas supervised components of this work, contributed to discussions and provided extensive feedback on the presentation of the results, and edited the manuscript.
Chapter 3, Osteochondrosis and other common skeletal lesions in Australasian Thoroughbred yearlings: factors affecting prevalence, and genetic/phenotypic parameters (draft manuscript).


- Associate Professor Thomson and Honorary Professor James guided the selection of appropriate statistical techniques in this work. Associate Professor Thomson assisted in developing scripts and running some analyses in the computer programs ASReml 3 and ASReml-R.

- Professor Jeffcott contributed to discussions and planning of this work.

- Professor Raadsma contributed to discussions regarding the planning of this work, and the interpretation and presentation of results.

- Professor Wade supervised components of this work, and contributed to extensive discussions of the statistical techniques in this work, and the interpretation and presentation of results.

- Dr Tammen supervised components of this work.

- Bethany Wilson contributed to extensive discussions of the statistical techniques in this work, and the interpretation and presentation of results.

- Emeritus Professor Nicholas supervised components of this work, and made major contributions to extensive discussions of the statistical techniques in this work and the interpretation and presentation of results, and edited the manuscript.

Chapter 4, Genome-wide association studies of skeletal lesion traits in Australasian Thoroughbred horses (draft manuscript). Castle K., Nicholas F.W., Jeffcott L.B., Raadsma H.W., Tammen I., and Wade C.M.
• Emeritus Professor Nicholas provided useful suggestions regarding the presentation of results, and edited the manuscript.

• Professor Jeffcott, Professor Raadsma, and Dr Tammen contributed to early discussions regarding the usefulness and feasibility of this work.

• Professor Wade supervised this work, providing guidance on quality assurance for samples and for genotyping data, analysis techniques suited to the population, and the use of the PLINK computer software in which the analyses were carried out; and performed editing of the manuscript.


• Emeritus Professor Nicholas and Honorary Professor James provided guidance regarding which coefficients and parameters were appropriate to quantify aspects of this population, and, where relevant which computer programs could be used for their calculation.

• Honorary Professor James provided assistance in modifying the Fortran code of the Pedig computer program.

• Toen Castle discussed the project and provided assistance in using the computer program Mathematica to visualise the pedigree structure over time.

• Associate Professor Thomson, Professor Jeffcott, Dr Tammen, Professor Raadsma, and Professor Wade contributed to discussions of the work in this chapter.

• Emeritus Professor Nicholas supervised this work and edited the manuscript.
Acknowledgements

This thesis and the work it describes relied on the support and input of the participating studs and veterinarians, who are remaining unnamed to protect the identities of the participating studs and horses. Their contribution was extensive, and scores of people were involved. This research and this thesis would not have been possible without their assistance and trust.

Funding for this research and a scholarship was kindly provided by the Rural Industries Research and Development Corporation (RIRDC) of Australia. The RIRDC is the primary source of invaluable funding for research into horse health in this country.

My supervisors (Emeritus Professor Frank Nicholas, Professor Claire Wade, Associate Professor Imke Tammen, Professor Leo Jeffcott and Professor Herman Raadsma) were exceptional. I have nothing but the highest praise for their patience, thoughtfulness and knowledge, shown through their consistent guidance and many instances of vital input throughout the project. Emeritus Professor Frank Nicholas put an enormous amount of work into providing feedback, even after his retirement, and despite the many other projects to which he was committed. Professor Claire Wade arrived part-way through the project, bringing the perfect experience and background to guide the genome-wide association study component of my project, as well as kind words and enthusiasm. Dr Imke Tammen provided useful feedback throughout the project and was primary supervisor for a portion of my project that included a very large amount of paperwork – thank-you for all the
signatures. Professor Leo Jeffcott effectively launched this project by sharing his incredible depth of knowledge regarding equine osteochondrosis and providing a vital clinical perspective to help ensure that any results from this project would make sense to vets in the field. Finally, Professor Herman Raadsma was the project's devil's advocate, always providing the impetus to revisit assumptions and search for efficient ways to carry out this research. Importantly, all of my supervisors were generous enough to work well as a group!

Honorary Professor John James and Associate Professor Peter Thomson provided invaluable guidance on statistical methodology appropriate to this research project. I deeply admire both men for their passion for statistical methods and their generosity with their knowledge.

My colleagues and fellow students in the Faculty of Veterinary Science at the University of Sydney were a pleasure to work with. They were an ongoing source of invaluable advice and good humour. In particular I will cherish memories of time spent with Camilla Whittington, Hannah Siddle, Lee Miles, Karma Nidup, Bethany Wilson, Priscilla Spendlove and Jaclyn Aldenhoven. Colleagues and fellow students elsewhere in Australia and overseas also shared their knowledge and happily participated in energetic discussions about horse research and osteochondrosis: thank-you Else van Grevenhof, Kristin Olstad and Melissa Jackson.

The Hunter Valley Equine Research Centre kindly provided accommodation throughout the data collection phase of this project. Helen Sinclair, the Secretary of the Hunter Valley Equine Foundation, took time to give me the keys at each visit.

Michael Ford and Jacqueline Stewart of the Australian Stud Book generously provided the pedigree file that was the basis for the pedigree analysis of Australian Thoroughbred horses in this thesis.
My friends, neighbours and family have been consistently patient and encouraging. Ryan Curnick, my partner, was my material support and just provided the right amount of humour and patience to get me to the point where this thesis could be submitted. My mother, Riki Davidson, provided encouragement and enormous assistance editing the chapters of this thesis, and my brother, Toen Castle provided sympathy - he is completing his own PhD. My father, Will Castle, was a steady source of gentle good humour and support.

My sincere and heartfelt thanks go to all involved.

Dedication

For my family, near and far.
Abstract

Osteochondrosis (OC) is a skeletal disorder that occurs in young, growing animals. It is defined as a focal disturbance of endochondral ossification, visible as small, discrete areas of abnormal bone and/or cartilage tissue occurring at typical sites on the articular surfaces of joints. In the Australian and New Zealand Thoroughbred horse populations, OC is one of the most common skeletal lesions reported to occur in young horses. The presence of OC lesions can negatively affect a horse's welfare, monetary value, and athletic performance.

Approximately 1,300 diagnostic reports written to assist stud managers in their sales process were used as a data source to determine the prevalence of OC and other skeletal lesions in a population of Australasian Thoroughbred weanlings and yearlings. The prevalence and distribution of skeletal lesions and injuries described in these reports were consistent with those found in studies of related populations. Overall, 20.5% of yearlings were reported as having OC, and the most common sites for OC lesions were the lateral trochlear ridge of the distal femur, the medial femoral condyle, and the sagittal ridge of the third metacarpal bone.

Based on these data, analyses were carried out to determine the extent to which non-genetic factors contribute to the prevalence of OC and other skeletal lesions in Australasian Thoroughbred yearlings; to estimate the heritability of OC and other skeletal lesions overall and at particular anatomical sites; and to estimate phenotypic and genetic correlations between pairs of skeletal lesion traits within and between joints. Correlations between Estimated Breeding Values (EBVs, the estimated sum of additive gene effects for each horse) were used as a proxy for conventional genetic correlation, which can not be determined on the underlying scale with currently available software for binary traits such as those in the current study. Non-genetic factors were found to contribute to the occurrence of some skeletal lesion traits in this population, but the effect of these factors was not consistent between traits. Breeding values were found to contribute significantly to the occurrence of OC, some OC component traits, and bone chip(s) or fragment(s) (FRAG) occurring proximal palmar/plantar to the first phalanx (PPP1) in the hind fetlocks. Heritability estimates for these traits ranged from 0.10 to 0.22. Not all OC traits had positive phenotypic or EBV correlations with one another. However, positive EBV correlations were found within a group of traits including stifle OC lesions and FRAG occurring PPP1 in the hind fetlocks. This group included multiple traits that were among the most prevalent in this population, that are known to have negative impacts on the financial value and/or race performance of affected horses. It appears to be a good potential target for genetic selection.

Single Nucleotide Polymorphism (SNP)-based case-control genome-wide association studies (GWAS) were carried out for 11 OC traits, FRAG occurring PPP1 in the hind fetlocks, and for the chestnut coat colour as a positive control using the Illumina Equine SNP50 beadchip, in a group of 140 horses with skeletal lesion data from the current study. A check for population stratification identified one
large cluster comprising the majority of the population and two small outlier clusters, each comprising the offspring of a single Australian-born Thoroughbred stallion. Despite only a small number of cases being available for these analyses, genome-wide significant quantitative trait loci were found on chromosome 30 for lysis at the sagittal ridge of the third metacarpal bones in the fore fetlocks (within the large cluster only), and on chromosome 3 for the positive control chestnut coat colour (within all three clusters of this population, and within the large cluster only).

A pedigree analysis of the Australian Thoroughbred population was carried out using pedigree data provided by the Australian Stud Book. The impact of past changes in breeding practice, including changes in sire usage and the origins of imported breeding stock, was examined via trends in the rate of inbreeding and loss of genetic variability due to unequal use of founders, population bottlenecks and genetic drift over time. There has been a low rate of loss of genetic variability in the Australian Thoroughbred population since 1973. This rate of loss is now increasing and is likely to increase further in coming decades. The importation of breeding stock from traditional sources (New Zealand, Europe and North America) is no longer increasing genetic variability. The number of sires is decreasing and their co-ancestry is increasing.

Selective breeding could be used to reduce the occurrence of many OC traits and FRAG occurring PPP1 in the hind fetlocks. The group of genetically associated traits that includes stifle OC and FRAG occurring PPP1 in the hind fetlocks appears to be a particularly good target for genetic selection, where minimisation of these lesions would result in financial benefit to breeders as well as improving the welfare of the horses. In recent decades, selective breeding techniques in first world agricultural animal species have generally implemented selection based on EBVs. More recently, techniques have been developed to include SNP data in the calculation of EBVs, resulting in Genomic Estimated Breeding Values (GEBVs) that have the potential to be substantially more accurate than EBVs.

The data required in order to implement genetic selection in this population is easily available, but the creation and maintenance of such a program would require ongoing financial investment. This investment could come from either the industry as a whole, or individual breeders who are open to embracing genetic and/or genomic technologies that are new to the Thoroughbred horse industry. There is also the potential to extend any genetic selection program to include selection for particular athletic traits, or selection against other disorders with a genetic component.
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<td>SRMC3</td>
<td>Sagittal ridges of the third metacarpal bones (in the fore fetlocks)</td>
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<td>SRMT3</td>
<td>Sagittal ridges of the third metatarsal bones (in the hind fetlocks)</td>
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<td>PDP1</td>
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<td>GEBV</td>
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<tr>
<td>GLMM</td>
<td>Generalised linear mixed model</td>
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<td>Ne</td>
<td>Effective population size</td>
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<td>Effective number of founder genomes</td>
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<tr>
<td>SNP</td>
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<td>QTL</td>
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1. Osteochondrosis in Thoroughbred horses

1.1. Context

1.1.1. Introduction

Osteochondrosis (OC) is a skeletal disorder that occurs in young, growing animals. It is characterised by the presence of OC lesions: small, discrete areas of abnormal bone and/or cartilage tissue occurring at specific sites on the articular surfaces of joints. Animals affected by OC may show joint swelling, lameness or joint locking associated with fragments of bone and/or cartilage in the joint space. However, in many cases OC lesions are ‘silent’, with no lameness or other external signs that a lesion may be present. OC is economically important in many animal industries due to the decreased locomotory abilities of some affected individuals, and the subsequent development of osteoarthritis in the affected joints (Kadarmideen and others 2004, Klimiene and Klimas 2006, Persson and others 2007, Stock and Distl 2007, Braun and others 2008).

OC occurs most frequently in horses, dogs, pigs, and cattle, and can also occur in humans. In all of the domesticated animal species that are susceptible to OC, some breeds within each species are affected more frequently than others (Dutra and others 1999, LaFond and others 2002, Kadarmideen and others 2004, Ytrehus and others 2007).

Research into OC in horses has been carried out on a variety of horse breeds. Research that has focussed on Thoroughbred horses has established that OC occurs frequently in that breed, affecting 5-20% of yearlings\(^1\) (Kane and others 2003, Oliver and others 2008, Furniss and others 2011). It is

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\(^1\) Yearlings are young animals (in this case horses) of either sex that are between one and two years old.
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also clear that some types of OC lesions negatively affect Thoroughbred yearling sale prices and subsequent race performance (Jackson and others 2009, Preston and others 2010). Research examining the causes of OC and the assessment of disease susceptibility has been carried out on a range of non-Thoroughbred horse breeds. Some studies have investigated genetic and non-genetic contributions to the occurrence of OC lesions (for example Grondahl and Dolvik, 1993; Wittwer and others 2007a; van Grevenhof and others 2009b). Other work has been carried out to quantify how tissue in OC lesions differs from equivalent healthy tissue on macroscopic, microscopic and molecular levels (for example, Nixon and Pool, 1995; Ytrehus and others 2004b; Mirams and others 2009).

1.1.2. The Thoroughbred horse industries in Australia and New Zealand

Early European settlers brought Thoroughbred horses and horse racing to Australia and New Zealand. The first official Australian race meeting was held in 1810, just 22 years after the arrival of the first penal colony fleet in 1788 (Bailey 1998). Racing is an important part of the Australian and New Zealand national identities, with the most famous horse race in Australia – the Melbourne Cup – known as “the race that stops the nation”.

Each year, between 16,000 and 18,000 Thoroughbred horse foals are registered in Australia, and between 4,000 and 6,000 are registered in New Zealand. These horses form the basis of the Australian and New Zealand Thoroughbred breeding and racing industries. In both countries, horse racing contributes 1-1.5% of GDP each year (Gordon 2001, IER Partnering Solutions 2004).

Horse sales are an integral part of the Thoroughbred breeding and racing industry. These sales most commonly take place as auctions, the largest of which are yearling auctions. Sales of yearlings in
the 2002/2003 Australian season (the most recent season for which collated data are available in 2012) totalled AU$166.5 million, and the average sale price was AU$41,000 (Jackson and others 2009). Yearlings with excellent conformation from families that consistently produce elite performers regularly fetch over AU$1,000,000 at sale.

1.1.3. Osteochondrosis at Thoroughbred yearling sales

Potential purchasers usually engage in rigorous checking of yearlings before purchase. As well as evaluating the general conformation and movement of each yearling of interest, a potential buyer can engage a veterinarian to examine a yearling for a range of problems. This includes examination of a set of radiographs (X-rays) of the horse's limb joints, allowing the diagnosis of bone defects and lesions, including OC.

Yearlings bought at auction in Australia or New Zealand do not have to be replaced or payment refunded by the vendor if a defect is found (Inglis Bloodstock 2009, Magic Millions 2009, New Zealand Bloodstock 2009). Prior to 2003, yearling radiographs were taken on an *ad hoc* basis, both before and after the actual sale. In at least one case, severe skeletal lesions found on radiographs taken after sale led to a purchaser demanding a refund from the vendor, despite the official sale conditions (pers. comm., Anon²). This highlighted the potential for ill-will and litigation between vendors and purchasers. From 2003 onwards, the bloodstock agents involved in the sale of Thoroughbred yearlings in Australia and New Zealand have provided X-ray repositories, where vendors can place radiographs for each yearling prior to sale. This practice assists both buyers and sellers by providing the opportunity for identification of any skeletal defects prior to purchase, thereby facilitating goodwill between all parties.

² A senior staff member at one of the participating studs, whose identity is protected by confidentiality agreements. This event was contentious within the industry and is not described in publicly available written documentation.
As mentioned previously, the presence of skeletal defects or lesions in yearling radiographs can significantly decrease its value. While some defects appear to have little effect on subsequent race performance, others (including OC lesions at some locations) are significant risk factors for poor performance or delayed career starts (Jackson and others 2009). This both delays and decreases the horse's potential to earn prize-money and betting returns.

1.2. Equine osteochondrosis: a review of the literature

This literature review examines the “Who, What, When, Where, and Why” of OC. The questions “who gets OC?”, “what is OC?”, “when does OC occur?”, “where does OC occur?”, and “why does OC occur?” provide a framework in which to identify common threads in the story of OC, and the proposed mechanisms underlying the disorder. Finally, consideration of the question “How can we control the occurrence of osteochondrosis in Thoroughbred horses?” leads to a description of the purpose of this study.

Over the past 20 years, reviews of OC in horses and other animals have tracked the growth of our knowledge regarding the nature and causes of this disorder (Jeffcott 1991, Jeffcott and Henson 1998, van Weeren 2006, Ytrehus and others 2007). There is evidence supporting several different non-genetic factors as possible causes or significant contributors to the occurrence of OC lesions, as well as extensive evidence for genetic contributors to OC.

Although the factors that contribute to OC are diverse, it is thought that there is likely to be a single underlying mechanism leading to the occurrence of OC lesions, that each contributing factor will
support either individually or in combination with others. The extent to which any proposed mechanisms overlap has generally not been addressed.

1.2.1. Who gets osteochondrosis?

As described earlier, OC can develop in young, growing individuals of many domestic animal species, and humans. A potentially related disease, tibial dyschondroplasia, affects several domesticated bird species (Orth and Cook 1994, Leach and Monsonego-Ornan 2007).

Within each animal species, the breeds that are most affected by OC tend to be large for their species and in many cases they have also been selected for increased muscle mass. Examples are Great Dane dogs (a giant breed), Landrace pigs (a fast-growing commercial breed), Charolais cattle (a fast-growing commercial meat breed) and Warmblood horses (a group of large horse breeds) (Goedegebuure and others 1980, Hoppe 1984, Dutra and others 1999, LaFond and others 2002).

In horses, pony breeds are generally not predisposed to OC, whilst in some larger horse breeds, OC is found in up to 64% of the modern population (Carlsten and others 1993, Dik and others 1999, Pieramati and others 2003, Wittwer and others 2006, Oliver and others 2008, van Grevenhof, Ducro, and others 2009, Preston and others 2010, Furniss and others 2011). However, if ponies do develop OC, it is the same condition as found in larger horse breeds (Voute and others 2011). Most horse breeds are targeted toward athletic pursuits, so the negative impacts of OC (lameness and joint locking) directly impact the animals' ability to fulfil their purpose, as well as creating an animal welfare problem.
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The occurrence of OC in several mammalian species suggests that the biological pathway(s) or physical mechanisms involved in the disorder are not unique to one particular species. If OC lesions develop due to defects in one or more biological pathways, those pathways may be “standard equipment” for mammals, as opposed to having evolved only in dogs (for example), or only in pigs. If tibial dyschondroplasia in birds is indeed directly analogous to OC in mammals, the underlying mechanism may be even older, evolutionarily speaking. The increased occurrence of OC in animal breeds that are large or fast-growing for their species further suggests that the biological pathway(s) associated with OC are likely to be involved with, or interact with, the biological pathways controlling growth.

If a physical mechanism or injury is causing OC (as opposed to a biological defect), this mechanism must also be common between all of the affected species, and affect large or fast-growing breeds more often than their smaller equivalents.

1.2.2. What is osteochondrosis?

OC is defined as a focal disturbance of endochondral ossification in the articular epiphyseal cartilage complex (growth cartilage), i.e. a localised failure in the process of replacing soft, flexible cartilage tissue with hard bone tissue (Olsson 1978, Jeffcott 1991, Douglas 2003). Endochondral ossification is the process by which most bones in mammals grow and lengthen; for a detailed review including relevant molecular mechanisms, see Mackie and others (2008).

The altered tissue that comprises an OC lesion is generally limited to a small area, with the affected area being in the order of millimetres or centimetres in diameter (Hoppe 1984, Nixon and Spencer 1990, Carlsten and others 1993, Braun and others 2008). Initially, OC lesions are restricted to
cartilage tissue, and consist of tissue with an abnormal molecular composition, containing dead or abnormal chondrocytes (cartilage cells) (Laverty and others 2002). These lesions are often associated with necrotic (dead) cartilage canals, and are only millimetres in size, reflecting the diffusion distance through cartilage (Olstad and others 2007, Peansukmanee and others 2009). These early lesions are not visible in a radiograph; cartilage is relatively translucent to X-rays.

Over time, OC lesions extend to include both bone and cartilage. As healthy tissue surrounding the lesion continues growing and ossifying via endochondral ossification, a cartilage core of abnormal tissue begins to extend, relatively speaking, into the bone (Ytrehus and others 2007). Normal chondrocyte metabolism (which is not occurring in the lesion) is required for endochondral ossification to take place (Gerber and others 1999). Inflammation and healing processes become involved, and the OC lesion often becomes much larger than the initial small abnormality (Semevolos and others 2001, Semevolos and others 2005, Mirams and others 2009, Riddick and others 2012). Finally, fissures form in the necrotic tissue, resulting in loose fragments or flaps of bone and/or cartilage. These fragments are typical of chronic or end-stage OC. The term osteochondritis dissecans (OCD) is often used to describe OC lesions with associated fragments (Riley and others 1998, Pieramati and others 2003, Dierks and others 2007, Wittwer, Lőhring, and others 2007). Consequently, the broader term OC includes the common usage of the term OCD. OC lesions that involve bone tissue can be seen by radiograph.

Most literature exploring human OC assumes a different process to that described above, where instead of the lesion starting in the cartilage and growing to include bone, trauma to underlying bone expands to include cartilage tissue (for example Higuera and others 1998; Elias and others 2007). In young human athletes, OC lesions are generally found in the joints under most pressure in
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their sport (e.g. elbow lesions in tennis players). In domestic animals, studies of early lesions support the cartilage-to-bone direction of lesion development, although injury to the bone can also lead to an OC lesion (Ytrehus, Andreas Haga, and others 2004).

In mammalian species where OC is common, it has not been associated with disease or changes in any organs other than the cartilage-bone complex. Importantly, it is not associated with overall alterations in juvenile or mature bone morphology, as are all of the skeletal abnormalities with a documented genetic basis where the genes or pathways affected are those involved in the creation of structural molecules that comprise the cartilage or bone tissue (Superti-Furga and Unger 2007). Therefore it is unlikely (but still possible) that OC lesions could be caused by genetic changes that affect the structure or expression of cartilage or bone components such as collagens.

This exploration of the nature of OC leads to an understanding that the initial causative factors have effects that cover only millimetres, and may involve necrotic or damaged cartilage canals. The process by which an OC lesion expands from a small, cartilage-based abnormality to a large bone-and-cartilage lesion with associated fragments is not well understood, but could include bone breakdown and dissolution processes that are known to be associated with inflammation (Haynes 2004, Chiaradia and others 2012). It has been well established that inflammatory markers are present in the synovial fluid of joints affected by OC in horses, and in their blood (Donabédian and others 2008, de Grauw and others 2011, Chiaradia and others 2012).

1.2.3. When does osteochondrosis occur?

OC lesions in horses have been shown to develop early in life. They have been shown to be common at one month of age in Dutch Warmblood horses, and from zero to seven weeks of age in
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Standardbred foals (Carlsten and others 1993, Carlson and others 1995). The formation of new OC lesions is thought to stop at around 8 months of age (earlier at some anatomical sites) (Dik and others 1999).

The formation of OC lesions has been linked to necrotic cartilage canals; channels that occur within thick cartilage tissue, containing small blood vessels (Carlson and others 1995, Olstad and others 2008, Olstad and others 2011). Cartilage canals are only present in young foals to a few months of age (varying by anatomical site) and regress as the cartilage tissue thins in the normal maturation process. The overlap between the age when OC lesions can be seen, and the age when cartilage canals are present, supports the involvement of cartilage canals or cartilage blood supply in the etiology of OC.

On a historical time scale, OC has arisen as a serious issue in several animal industries relatively recently. OC in pigs, dogs, horses and cattle has been reported since the middle of the 20th century, with reports of the condition in horses first appearing in the 1940s, and dogs in the mid 1970s (Nilsson 1947, Bartels and others 1970). This has coincided with changes in husbandry practices and diet, and continued selection for extreme phenotypic traits such as size, growth rate, musculature and speed in the affected animal species and breeds. OC has quickly become recognised as a significant health problem, particularly in pigs where intensive breeding for meat combined with diets optimised for fast meat production appear to have brought the condition into prominence (Goedegebuure and others 1980).

The development of OC lesions in young foals suggests that the formation of OC lesions is associated with a period of rapid growth. The increase in OC occurrence during a historical period
where fast growth in young domestic animals became more common also points toward growth-associated processes. While there is no evidence that larger individuals are more susceptible to OC (despite larger breeds generally being more susceptible to OC), an association was shown between OC occurrence and growth rate in horses (van Weeren and others 1999). There is strong evidence that the development of OC lesions in young foals is associated with the presence of non-viable cartilage canals during the first few months of a foal's life (Olstad and others 2008). Taken together, these findings suggest that factors contributing to OC may affect the maintenance of cartilage canals during periods of intense growth.

1.2.4. Where does osteochondrosis occur?

OC lesions occur frequently at particular sites on the articulating surfaces of limb joints and between vertebrae that are often described as lesion predilection sites. Fig 1.1 highlights the joints where OC lesions are most frequently reported on the equine skeleton. There are multiple lesion predilection sites in some of these joints.
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Fig 1.1: Common OC sites on the horse skeleton (clockwise from top right) 1. the cervical vertebrae, 2. the shoulders, only one of which can be seen in this illustration, 3. the fore fetlocks, 4. the hind fetlocks, 5. the hocks, and 6. the stifles. (Horse skeleton image obtained from clker.com and used under the terms of the Creative Commons public domain license.)

OC lesions in the cervical vertebrae can occur in any (or multiple) vertebral joints C2-C6, but are less frequent than those found in the other joints indicated in Fig 1.1 (Stewart and others 1991). In the shoulder joint (the scapulo-humeral joint), OC lesions occur on the humerus and in the glenoid cavity (Jenner and others 2008). Lesion predilection sites in the fore and hind fetlocks (the metacarpophalangeal and metatarsophalangeal joints, respectively) are the sagittal ridges of the third metacarpal and metatarsal bones (Wittwer and others 2006, Lykkjen and others 2012).
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In the Thoroughbred horse breed, OC lesions commonly occur in the hock and stifle joints (Oliver and others 2008, Jackson and others 2009, Furniss and others 2011). In the hocks (tarsal joints), OC is commonly reported at the lateral and medial trochlear ridges of the talus, the distal intermediate ridge of the tibia, and the medial malleolus of the tibia. In the stifle (the femorotibial/femoropatellar joint) OC lesions occur primarily on the lateral trochlear ridge of the distal femur, and have also been reported occurring on the medial trochlear ridge of the distal femur, and the patella. Lesions of the medial femoral condyle (also in the stifle) often appear as subchondral cysts, and while they are generally regarded as OC, this may not always be the case (McIlwraith 2010). Radiographic images showing the location and appearance of each individual site can be seen in the freely available downloadable report from Jackson and others (2009).

The distribution and prevalence of OC lesions differs between horse breeds. For example, in Dutch Warmblood horses, OC lesions in the stifle joint were found in the trochlear groove of 21% of young horses (van Grevenhof, Ducro, and others 2009). In contrast, OC lesions in this location have been seen but are extremely rare in Thoroughbred horses (Oliver and others 2008, Jackson and others 2009). Elsewhere in the stifle, the distribution of OC lesions is similar between Dutch Warmblood and Thoroughbred horses. Differences in OC lesion distribution make comparisons between horse breeds somewhat challenging.

There is physical diversity in regards to both form and role amongst the various sites where OC commonly occurs in horses. For example, there is a wide variation in the amount and type of load-bearing that takes place at each site (Firth 2006, Doube and others 2007). During normal limb use, some of the OC predilection sites are subjected to shear force, others to compressive force, while still others have no regular load. The composition of the cartilage varies between sites, because the
cartilage extra-cellular matrix is altered by the compressive and torsional forces to which the chondrocytes in that tissue are exposed (Brama and others 2002).

The curvature or shape of the bone at lesion predilection sites also shows wide variation. OC lesions can be found on both concave and convex lesion predilection sites (Exner and others 1991). Similarly, sites that are typically unaffected by OC also show a range of shapes and curvature.

There is clearly wide variation in the physical environments in which OC lesions form. While it has been suggested that “biomechanical influences are the only way to explain the fact that there are well-defined and very consistent predilection sites in the affected joints” (van Weeren 2006), this is not strictly true. Given that cartilage thickness and composition varies over the articular surface, either mechanical damage or biochemical changes that rendered cartilage microvasculature less viable would result in OC lesions occurring at typical developmental stages, and particular locations.

1.2.5. Why does equine osteochondrosis occur: non-genetic factors

The rate of occurrence of OC lesions in horses can be increased by a range of non-genetic factors. In each case, the young, growing horse must be exposed to the factor for a period of weeks or months in order to develop lesions.

Overfeeding, and insulin resistance occurring subsequent to overfeeding of high-energy feed, are strongly associated with the occurrence of OC in horses. A protocol of overfeeding has been used to induce OC lesions in research horse populations, and an assay targeting insulin resistance is the basis of a patented test for OC in horses (C. J Savage and others 1993, Ralston 1999). While a high
starch and sugar diet has been shown to induce insulin resistance in young Thoroughbred horses, the relationship between diet and insulin resistance is complex, and the biological mechanism(s) by which insulin resistance and/or overfeeding contribute to OC are not known (Treiber and others 2005, Johnson and others 2009). However, it is worth noting that insulin-resistant diabetes in humans is associated with impaired maintenance of microvasculature, that frequently results in damage to hypoxic lens tissue in the eye (diabetic blindness), and severely impaired healing (diabetic ulcers) (Orasanu and Plutzky 2009). If insulin resistance in horses has similar effects on equine microvasculature, the cartilage blood supply could be compromised. Like the lens of the eye, cartilage is a hypoxic tissue.

Severe copper deficiency has been suggested as a causative factor for lesions resembling severe OC in horses (Bridges and others 1984, Bridges and Harris 1988, Hurtig and others 1993). However, copper supplementation does not appear to have a protective effect against OC in Thoroughbred horses that are already receiving some copper in their diet (Gee and others 2005, Gee and others 2007). There are multiple biological mechanisms underpinning copper homeostasis that stabilise the response to copper supplementation when some copper stores already exist (Harvey and McArdle 2008). The use of copper chelation (the artificial induction of a copper deficiency) as a method of reducing the formation of new blood vessels in the treatment of cancer indicates that extreme copper deficiency impairs the biological pathways used in the detection of and response to hypoxia (Lowndes and Harris 2005, Martin and others 2005). The hypoxia response pathways are not tissue-specific, and play an integral part in cartilage maintenance and endochondral ossification, so the impairment of these pathways is likely to result in both the death of cartilage tissue and the prevention of endochondral ossification (Gerber and others 1999, Shum and Nuckolls 2002, Ivkovic and others 2003).
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Diets containing excess phosphorus or excess calcium, and excess dietary energy, have been shown to increase the number and severity of lesions of OC in young mixed-breed horses (C. J. Savage and others 1993). However, a diet containing an excess of calcium, without a concurrent excess of dietary energy, did not have the same result. As discussed above, overfeeding (providing excess dietary energy) can be used to induce lesions of OC, so the diet with excess calcium and dietary energy may be exerting some of its effects via the mechanisms involved in overfeeding. Hyperphosphatemia (the presence of excess phosphorus in the blood) can also result from excess dietary phosphorus, and is associated with changes in bone metabolism and poor vascular health in humans (Demer and Tintut 2010).

Experiments in horses and pigs have also shown that chisel injuries to the bone surface also result in lesions that resemble OC. These physical injuries damage the blood supply to a small region of bone and cartilage. In both animal species, the injury resulted in lesions resembling OC within four weeks of the injury being incurred (Ytrehus, Andreas Haga, and others 2004, Olstad and others 2008). These experiments directly interrupted blood supply to regions of cartilage, suggesting that loss of blood supply to a region of cartilage, perhaps combined with healing responses, results in lesions such as those seen in OC.

Long term exposure (i.e. several months) to glucocorticoid medications, especially dexamethasone, has been shown to result in extensive skeletal lesions in horses that resemble severe extensive OC (Glade and Krook 1982, Glade and others 1983). In common with the other environmental factors that are associated with increased occurrence of OC, dexamethasone profoundly suppresses angiogenesis (Hori and others 1996). Dexamethasone also affects bone metabolism through multiple other mechanisms (Abraham and others 2009, Tóth and others 2010).
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All of the environmental factors associated with increased occurrence of OC are known or are suspected to cause the loss or degradation of cartilage blood supply. There has been a renewal of interest in the involvement of impaired cartilage blood supply in the etiology of OC, backed by strong evidence from micro-computed tomography of early OC lesions in Standardbred foals (Olstad and others 2008).

1.2.6. Why does equine osteochondrosis occur: genetic factors

It has been well established that genetic variation between individual horses contributes to variation in liability to developing OC in some Standardbred and Warmblood horse populations. Numerous studies have examined breed populations in Denmark, Sweden, Norway, the Netherlands, Italy, France and Germany and estimated the heritability\(^3\) (\(h^2\)) of OC, in specific joints or overall, as between 0.00 and 0.52 (Schougaard and others 1990, Grondahl and Dolvik 1993, Philipsson and others 1993, Ricard 2002, Pieramati and others 2003, Schober and others 2003, Stock and others 2005, Jönsson and others 2011). An excellent table summarising these studies and others is provided in van Grevenhof and others 2009b.

Estimates of heritability rely on physical observation of the trait of interest and pedigree data. Horses are large animals and not all their joints can be radiographed easily, so observations are limited to joints that are accessible and known to have a high prevalence of OC lesions in the breed of interest.

In the recent studies of OC in Dutch Warmblood horses the stifles, hocks, fore fetlocks and hind fetlocks were radiographed (van Grevenhof, Ducro, and others 2009, van Grevenhof, Schurink, and others 2009).

\(^3\) In this context heritability is the extent to which a trait is inherited, expressed as the ratio of the additive genetic variance to the variance of the physically observable trait of interest.
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others 2009). Although lesions were recorded at multiple sites in the hocks and the stifles (referred to as the tarsocrural and femoropatellar joints, respectively, in those studies), these lesions were pooled by joint and the results of analyses were presented on a joint-wise basis and overall. The joint-wise and/or overall grouping is also used in other studies: for example the grouping of fore fetlocks, hind fetlocks and hocks in Pieramati and others (2003) and Wittwer and others (2007). These groupings are used to create traits with higher overall prevalence for which more significant results can be obtained.

The studies of OC in Dutch Warmblood horses have started to address the practice of grouping OC lesions in different joints for analysis, by carrying out a cluster analysis to visualise the relationships between the physical manifestations of OC in different joints, and estimating genetic correlations between overall OC status and OC status in each of the stifles, hocks, and fetlocks (i.e. all four fetlocks grouped together) (van Grevenhof, Ducro, and others 2009, van Grevenhof, Schurink, and others 2009). The cluster analysis found strong phenotypic relationships between homologous joints in the left and right limbs, with weaker relationships between fore and hind fetlocks, and between OC lesions with fragments and those without (referred to as FRAG and FLAT, respectively, in those studies).

High genetic correlations were found between OC lesions with fragments and those without in each joint, and between homologous left and right joints. In contrast, genetic correlations between OC in different joints were moderate to low, with high standard errors (van Grevenhof, Schurink, and others 2009). These results validate grouping homologous left and right joints together, but do not provide any particular support for grouping different joints together for genetic analyses. The
question of whether it is appropriate to pool OC lesions occurring at multiple sites within a single joint also remains unanswered.

Overall, the reported estimates of heritability are sufficiently high to justify searches for DNA sequence variation associated with the disorder. Linkage analyses carried out using microsatellite markers in Hanoverian Warmblood horses identified genome-wide significant quantitative trait loci (QTL) for OC in the fore or hind fetlocks and/or hocks on equine chromosomes (ECA) 2, 4, 5, 16 and 18 (Dierks and others 2007, Lampe and others 2009, Dierks and others 2010). In South German Coldblood horses, genome-wide significant QTL for OC in the fore or hind fetlocks and/or hocks were identified on ECA 2, 18 and 23, with another found on ECA 18 for fetlock OCD (Wittwer, Löhning, and others 2007). The Illumina Equine SNP50 BeadChip was used to carry out genome-wide association studies for OC traits in French Trotters and Norwegian Standardbred trotters, and Thoroughbred horses raised in Kentucky, USA (Lykkjen and others 2010, Corbin and others 2012, Teyssèdre and others 2012). The analysis of Norwegian Standardbred trotters identified QTL on ECA 5, 10, 27 and 28 for hock OC traits, while the study of French Trotters identified QTL on ECA 3 and 14 for hock OC traits, and on ECA 13 and 15 for other OC traits. The study of Thoroughbred horses raised in Kentucky, USA, identified a single genome-wide significant SNP on ECA 3 associated with OCD in one or more of the fore fetlock, hind fetlock, hock, stifle and shoulder joints. Two additional SNP, one on ECA 4 and the other on ECA 18, located within QTL identified within the Hanoverian Warmblood horse population were also found to be significant when each was fitted jointly with the SNP on ECA 3.

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4 Microsatellites are regions of DNA that consist of repeats of the same short sequence (usually just 1-6 base pairs). They are used as markers because the number of repeats in a microsatellite is often highly variable within a population, and the inheritance of regions of DNA from particular ancestors can be inferred from their presence.

5 QTL are regions of chromosomes where polymorphic DNA markers are associated with differences in performance in a trait of interest: in this case, the presence or absence of OC lesions at particular anatomical locations.

6 SNP is a contraction of single nucleotide polymorphism, DNA sequence variation where a single nucleotide differs between individuals in a population, for example the presence of either A or G at a given location in the genome sequence; 50 refers to the approximately 50,000 SNP included on the BeadChip.
Despite the large number of QTL identified in these horse breeds, overlapping QTL are relatively rare. To date, regions identified in more than one horse breed are on ECA 18 at around 36-39 Mb and ECA 4 at around 39Mb in Hanoverian Warmblood horses (for OC occurring at one or more anatomical sites in the fetlocks or hocks) and Thoroughbred horses (for OCD in one or more of a range of limb joints) (Corbin and others 2012). This may reflect discrepancies between the definitions of OC and the anatomical sites examined in the different studies. Alternatively, there may be genuine differences between the genetic factors contributing to OC in different horse breeds.

Many parts of the Thoroughbred genome are under positive selection, reflecting the continual preference for exceptional racing performance in this breed (Gu and others 2009). Among the regions of the genome identified as undergoing positive selection were areas known to be involved with response to hypoxia, and the insulin signalling pathway. The nine most strongly selected regions were on ECA 4, 5, 6, 11, 17 (two regions), 18, 25 and 27. If any of these regions are found to overlap with QTL for OC in Thoroughbred horses, breeders may have to face the proposition that breeding for elite racing performance may have contributed to the occurrence of OC in this breed. However, even if this is the case it would still be possible to carry out genetic selection against OC while continuing to breed for race-winning performance.

A technology called genomic selection has been developed to utilise SNP genotypes, combined with phenotype and relationship data from pedigrees or the SNPs themselves, to produce Genomic Estimated Breeding Values (GEBVs) (Goddard and Hayes 2009). GEBVs could guide both disease prediction in non-breeding horses and genetic selection in the breeding population. The SNP genotypes can account for the vast majority of the additive genetic variance associated with a given
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trait (in this case, OC). In addition, accurate QTL from dense SNP assays such as the Illumina Equine SNP50 BeadChip provide insight into the molecular pathways that play significant roles in disease pathogenesis, potentially providing leads toward treatment options or disease minimisation strategies.

Genetic factors contributing to OC in horses could have their effects through alterations in a range of biological pathways. For example, variation in glucose metabolism could predispose some horses to insulin resistance when they are supplied with excessive dietary energy in the form of carbohydrates. As discussed earlier, this would be expected to have a negative effect on the health and growth of microvasculature in hypoxic tissues such as cartilage. Similarly, changes in the threshold response of hypoxia pathways could also have unforeseen effects on the health and vascularisation of growing cartilage. In addition, QTL affecting the shape or growth pattern of juvenile bones could result in some horses being particularly susceptible to OC at particular anatomical sites: this is particularly likely, given the low to moderate genetic correlations between OC lesions in different joints of Dutch Warmblood horses (van Grevenhof, Schurink, and others 2009). Even QTL affecting temperament could contribute to the occurrence of OC, if biomechanical damage is the main cause of OC. If any of these potential genetic contributors exist, their action would overlap with the known non-genetic contributors to equine OC. As more information becomes available over time regarding the genetic basis of various equine traits, it may be possible to identify genetic correlations between OC and these or other traits.
1.3. How can we control the occurrence of osteochondrosis in Thoroughbred horses?

The Thoroughbred horse breeding industries in Australia and New Zealand are eager to minimise the occurrence of OC in their young horses, in particular those lesions occurring at anatomical sites that have been shown to affect yearling price or racing performance. There is currently a scarcity of published research on factors contributing to OC in Thoroughbred horses, despite the fact that the Thoroughbred horse breed is the most populous horse breed in the world, and has a reasonably high prevalence of OC.

This study sought to contribute to our understanding of OC in Thoroughbred horses with the primary goal of investigating the extent of genetic variation in OC susceptibility in Thoroughbred yearlings in Australia and New Zealand. It also aimed to estimate the effects of various non-genetic factors on the prevalence of OC and some other skeletal lesion traits; to explore the definition of OC particularly with respect to genetic selection against this disorder; and to investigate the association of genomic sequence variation with OC overall and specific OC traits. To achieve these aims, the following investigations were carried out:

a) The prevalence of OC and other skeletal lesions and injuries was determined within sample populations of Australian and New Zealand Thoroughbred weanlings and yearlings (Chapter 2). The data were derived from written diagnostic reports intended to aid stud managers in the sales process, that have not previously been used as a data source in the study of OC or other skeletal lesions. Therefore, prevalence estimates from this study were compared to estimates from surveys of very similar horse populations where radiographs were examined with the primary purpose of gathering data for research purposes.
b) Changes in the radiographic status of horses as they mature from weanlings to yearlings were examined using a sub-population of horses for which diagnostic reports were available for both ages (also in Chapter 2). This allowed conclusions to be drawn regarding the use of data from weanlings as well as yearlings, and comparison with the 8-month-old “age of no return” reported for OC in Dutch Warmblood foals (Dik and others 1999).

c) A logistic generalised linear mixed model was used to estimate the effect of non-genetic factors and heritability of OC and other skeletal lesion traits (Chapter 3). These analyses allowed the identification of traits where prevalence was influenced by factors that could be under the control of a stud manager, such as time of birth (within the foaling season), or where there is sufficient genetic variation to justify selective breeding against OC. Phenotypic and genetic correlations (the latter estimated indirectly) were estimated between joint-wise and overall OC traits, and between component OC traits and other common skeletal lesions (also presented in Chapter 3). The examination of OC component traits provided a foundation for comparing the results of this study with results from analyses of joint-wise OC status published by other authors. It also allowed examination of the definition of OC, with respect to the goal of controlling this disorder: for example, if non-genetic factors contribute to OC lesions occurring at different locations, and if the genetic correlation between the locations is low, it may be appropriate for control strategies to define these as separate traits. Conversely, if there are common non-genetic factors contributing to both OC and other common skeletal lesions, and if the relevant genetic correlations are sufficiently high, a control strategy may extend the definition of OC to include additional skeletal lesion traits.
d) Genome-wide association studies (GWAS) were carried out in a sub-population of horses for which DNA was available (Chapter 4). The high average co-ancestry of the study population (informed by the pedigree analysis described below, Chapter 5) was addressed by matching closely related affected and unaffected horses as a strategy to minimise population stratification. The traits that were examined were OC overall, some joint-wise and component OC traits, and bone fragments occurring proximal palmar/plantar to the first phalanx in the hind fetlocks. The identification of genomic regions associated with OC would be a first step toward future development of genetic testing for OC susceptibility, and may provide insight into some of the biological mechanisms that underlie the formation, development or healing of OC lesions.

e) A pedigree analysis of Australian Thoroughbred horses was carried out to quantify the actual and effective population size, average inbreeding coefficients, generation interval, proportion of horses that go on to reproduce, origins of breeding stock, and trends in sire usage (Chapter 5). Information from these analyses may be useful in the future to inform overall strategies to control OC in Australian and New Zealand Thoroughbred populations.

f) Finally, a general discussion and conclusions are presented in Chapter 6. This chapter addresses the future development of management strategies for OC in Australian and New Zealand Thoroughbred horses, including whether specific OC component traits can or should be targeted, and the use of Estimated Breeding Values or Genomic Estimated Breeding Values (both derived from quantitative genetic analyses) as a tool for selective breeding against OC, genetic testing for OC susceptibility, and the use of non-genetic management strategies either separately or in combination with one another.
1.4. Acknowledgements

Many thanks to the Australian Rural Industries Research and Development Corporation (RIRDC), who kindly provided financial support for this project.

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2. Skeletal lesions and injuries in Australasian Thoroughbred weanling and yearling radiographs

2.1. Summary

*Reasons for performing study:* Diagnostic reports written to assist stud managers in their sales process have not previously been used as a data source for the study of osteochondrosis (OC) or other skeletal lesions. The use of these reports may provide an efficient and cost-effective insight into the prevalence and distribution of skeletal lesions and injuries in Australian and New Zealand Thoroughbred horses.

*Objectives:* To estimate the prevalence and distribution of skeletal lesions and injuries revealed in Thoroughbred weanling and yearling radiographs using written diagnostic reports as a source of data, and to examine the persistence of skeletal lesions and/or injuries as weanlings mature into yearlings.

*Methods:* Written diagnostic reports based on approximately 1,300 sets of weanling and yearling radiographs were collected from Thoroughbred stud managers in Australia and New Zealand. The prevalence and anatomical distribution of skeletal lesions and injuries in weanlings (299 horses) and yearlings (1,004 horses) were determined from these reports. The persistence of skeletal lesions and/or injuries was examined for 84 horses with both weanling and yearling radiographs. Prevalence and distribution estimates from this study were compared to those obtained in similar studies.

*Results:* Overall, there was good agreement between the prevalence and distribution of OC lesions and injuries reported in this study for weanlings and yearlings, and those previously reported for Australian and New Zealand Thoroughbred yearlings. Some categories of skeletal lesions appeared to be systematically under-reported in this study. Overall, the prevalence of OC in yearlings in this study was 20.5%. In this study, the most common sites for OC lesions were the lateral trochlear ridge of the distal femur, the medial femoral condyle, and the sagittal ridge of the third metacarpal bone.

*Potential relevance:* Written diagnostic reports used by stud managers in the sales process appear to be a reasonable source of data regarding OC and bone fragments in the fetlocks. Some skeletal lesions appeared to be under-reported. Findings in weanlings provided a very strong indication of the results in subsequent yearling radiographs.
2.2. Introduction

In Australia, New Zealand, and most other countries with Thoroughbred breeding industries, public bloodstock\textsuperscript{7} auctions are the primary avenue for trade. Globally, the majority of turnover occurs at yearling auctions, except in Japan where weanling auctions are more popular. At auctions in Australia and New Zealand, the most sought-after individual yearlings can achieve prices of more than $2 million (in either AUD or NZD). At some auctions, the median sale price is higher than AUD$100,000 (Inglis Bloodstock 2009a).

With so much money at stake, buyers generally undertake due diligence on their prospective purchases. Stud managers, who commonly act as vendors at yearling auctions, receive written reports from the veterinarian who provided their radiographs, describing any visible skeletal defects, so that they can understand any concerns voiced by potential buyers and better judge the financial value of the horses they are selling. These reports are intended for use by only the stud manager, as an aid prior to and during the sales process, and focus on skeletal defects including osteochondrosis (OC) considered most likely to have a negative impact on financial value.

Studies have previously been carried out to survey the prevalence and distribution of skeletal lesions including OC in Thoroughbred yearlings in Australia (Jackson and others 2009) and New Zealand (Oliver and others 2008), as well as overseas (Cohen and others 2006, Preston and others 2010, Furniss and others 2011). These studies utilised experienced veterinarians to examine sets of Thoroughbred yearling sale radiographs, as opposed to using data from the reports written for stud managers.

\textsuperscript{7} Bloodstock is a term that describes Thoroughbred horses collectively.
In the stifle joints of Australian and New Zealand Thoroughbred yearlings, the two most common findings were forms of OC, namely subchondral cystic lesions (SCL) of the medial femoral condyle (MFC), occurring in 5.6% of 2,401 Australian yearlings and 2% of 1,505 New Zealand yearlings, and OCD of the lateral trochlear ridge of the distal femur (LTRF), occurring in 3.8% (Australia) and 3% (New Zealand) of yearlings. In the hock joints, common findings included osteophytes and enthesiophytes (generally referred to as spurs) occurring in 35% (Australia) and 31% (New Zealand) of yearlings, and OCD at the distal intermediate ridge of the tibia (DIRT) occurring in 3.4% (Australia) and 3% (New Zealand) of yearlings. Common findings in the other joints in the Australian study included bone fragments (FRAG) occurring at the plantar aspect (the back) of the first phalanx in the hind fetlocks, defects of the sagittal ridge of the third metacarpal/metatarsal bones in the fore and hind fetlocks (including OC at this site), and vascular channels or sesamoiditis in the sesamoid bones (also in both the fore and hind fetlocks). Both the Australian and New Zealand studies concluded that the prevalence and distribution of skeletal lesions in their cohorts was in reasonable agreement with reports from overseas Thoroughbred yearling populations, suggesting that these distributions of lesions are typical for Thoroughbred horses of this age, with the stifles more likely to be affected by OC than the hocks.

In the present analysis, the prevalence and distribution of skeletal lesions and injuries as described in written reports provided to stud managers in Australia and New Zealand are compared to the surveys of Australian and New Zealand yearling radiographs summarised above, to identify any systematic differences between the alternative data sources, with a focus on OC. The persistence of skeletal lesions and injuries from weanling to yearling for a subset of horses with reports available for both time points is also examined. In the following chapter, further analyses are carried out to determine the heritability of OC and other common skeletal lesion traits, and the effect of
identifiable non-genetic factors (such as the stud where a horse was raised, the year it was born, the horse's sex) on the same traits.

2.3. Methods

2.3.1. The study population

Seventeen commercial Thoroughbred horse studs located in Australia and New Zealand were recruited as collaborators for this study, based on their presence as vendors at yearling sales in recent years. The study population consisted of 1,004 yearling and 299 weanling male and female Thoroughbred horses raised at the collaborating studs. The horses were born between 2002 and 2007 (inclusive). Ethical approval for the observation of these horses was granted by the University of Sydney Animal Ethics Committee (AEC reference number N00/7-2007/1/4667). The identity of the horses and studs that participated in this research is protected by non-disclosure agreements.

The study population consisted of horses that were radiographed as part of normal operating procedure at the stud at which they were raised. Horses being kept for racing by an owner/breeder (for which radiographs were not requested) and horses of insufficient financial value to justify the cost of radiography were not included. Conclusions drawn from the current study therefore apply to this high-value sub-population, rather than the Australasian Thoroughbred population as a whole.

Approximately 18,000 sales of high-value weanlings and yearlings occurred in Australia and New Zealand during the years in which horses in the study population were eligible for these sales (this number is an estimate of the number of horses sold at sales sessions where the average price was greater than AU or NZ$35,000 (Inglis Bloodstock 2009a, Magic Millions 2010)). In addition, an
unknown number of high-value horses were kept by owners and did not go to sale. The total number of Thoroughbred horses born in Australia and New Zealand in these years was approximately 130,000 (see Chapter 1). The study population therefore comprised a maximum of 7.2% of the high-value sub-population of Australian and New Zealand Thoroughbred horses born in these years, and 1.0% of all Australian and New Zealand Thoroughbred horses born in this period.

2.3.2. The raw data

The collaborating studs provided written diagnostic reports summarising findings from sets of weanling or yearling radiographs. These reports were written to aid stud managers prior to and during the sales process. The majority of studs provided diagnostic reports from two or more veterinarians over the course of the study. Each diagnostic report was authored by one of sixteen experienced equine veterinarians, based on a set of radiographs taken by the veterinarian's own practice. All sets of radiographs were commissioned and paid for by the participating studs.

The radiographic projections on which each written report was based were a standard set, suitable for submission to yearling sale X-ray repositories in Australia or New Zealand (Magic Millions 2009, Inglis Bloodstock 2009b). This standard set contains a minimum of 34 projections, and is intended to allow veterinarians to detect the majority of skeletal lesions and injuries in the limb joints (interpretation of these projections is discussed in detail in Jackson and others (2009)). Each projection provides an image from a particular angle when the joint is in a particular position, presenting a profile of parts of the joint surface with sufficient contrast to allow visualisation of any abnormalities, allowing the diagnosis of skeletal lesions. Many of these skeletal lesions can be seen in only one projection.
The written report provided for each yearling was based on radiographs submitted with the horse when it was presented for sale at a yearling auction. Where more than one set of yearling pre-sale radiographs was available for any horse, only data from the latest set of radiographs were used. The written reports for weanlings were based on radiographs submitted with each horse when it was presented for sale as a weanling, or on weanling 'survey' radiographs that were taken to identify horses requiring corrective surgery prior to sale. Multiple sets of sale radiographs were available if horses were sent to multiple sales (i.e. multiple weanling and/or yearling sales). This could occur if the horse was passed in at auction at their first sale or if they were purchased with the intention of resale at a profit at a subsequent sale. All sale radiographs were taken in the 42 days (6 weeks) prior to sale commencement. In cases where a yearling had undergone corrective surgery for a skeletal lesion, their written report included this fact and they were recorded as being 'affected' for the lesion that had been surgically treated, to reflect the fact that a lesion requiring surgical intervention had occurred. The data collected in this study were considered to indicate prevalence (total cases occurring in a particular area at a particular point in time) as opposed to incidence (new cases reported in a particular area over a specific time period).

No quality control was undertaken on the radiographs or the diagnostic reports, so errors resulting from poor-quality radiographs and missed or incorrect diagnoses were propagated to the standardised data set (see following section). Based on the findings of Jackson and others (2009), it is expected that such errors would be more prevalent for subtle skeletal lesions, but rare for larger or more severe skeletal lesions.
2.3.3. **Standardising the data**

For the purposes of this analysis, findings from each written diagnostic report were entered into a MySQL database. Each lesion was recorded separately as occurring at a particular lesion site, and having a particular lesion phenotype (referred to here as the lesion type). The original text of the veterinary report was also recorded for quality control purposes. The reports did not consistently state lesion size or depth, and therefore size was not recorded in the standardised data set. From this database, the binary status of each horse (0 = unaffected, 1 = affected) was determined, with respect to any combination of lesion type and lesion site. The absence of consistent data regarding lesion size or depth precluded the use of ordinal or other scales that could indicate lesion severity.

Fig 2.1 illustrates the location of the five pairs of joints described in each written diagnostic report, on a diagram of the equine skeleton. Table 2.1 lists these joints and the anatomical sites within each joint that were used to categorise lesion location, and introduces common terms or acronyms where relevant for these joints and sites. Where possible, site acronyms are the same or similar to those used elsewhere (for example Pieramati and others 2003; Wittwer and others 2006; Oliver and others 2008; van Grevenhof and others, 2009; Jackson and others, 2009). In all, lesions were categorised into eighteen distinct anatomical sites within five joints on either side of the body (left and right), totalling 36 site categories.
Fig 2.1: The five joints examined in a standard set of radiographs were (clockwise from top right) 1. the front knees, 2. the fore fetlocks, 3. the hind fetlocks, 4. the hocks, and 5. the stifles. The formal anatomical name of each joint is given in Table 2.1. (Horse skeleton image obtained from clker.com and used under the terms of the Creative Commons public domain license.)
Table 2.1: Joint names, the sites nested within each joint that are used to categorise lesions, and acronyms for specific sites frequently discussed in the text.

<table>
<thead>
<tr>
<th>Joint</th>
<th>Common name</th>
<th>Site</th>
<th>Site acronym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carpal joint</td>
<td>Front knee</td>
<td>Any location within the front knee</td>
<td></td>
</tr>
<tr>
<td>Metacarpophalangeal joint</td>
<td>Fore fetlock</td>
<td>Sagittal ridge of the third metacarpal bone</td>
<td>SRMC3</td>
</tr>
<tr>
<td>Metatarsophalangeal joint</td>
<td>Hind fetlock</td>
<td>Sagittal ridge of the third metatarsal bone</td>
<td>SRMT3</td>
</tr>
<tr>
<td>Tibiotarsal joint</td>
<td>Hock</td>
<td>Lateral trochlear ridge of the talus</td>
<td>LTRT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medial trochlear ridge of the talus</td>
<td>MTRT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distal intermediate ridge of the tibia</td>
<td>DIRT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medial malleolus of the tibia</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any other location in the hock</td>
<td></td>
</tr>
<tr>
<td>Femorotibial and femoropatellar joint</td>
<td>Stifle (hind knee)</td>
<td>Lateral trochlear ridge of the distal femur</td>
<td>LTRF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medial trochlear ridge of the distal femur</td>
<td>MTRF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medial femoral condyle</td>
<td>MFC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any other location in the stifle</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2 describes the lesion types, comprising skeletal disease and injuries, and introduces acronyms or common names for lesion types that are frequently discussed in the text. Where these common names or acronyms are used in other publications with a different definition, the effect of these differences is discussed in the text. The term 'lesion type' is used here in a different manner to the term 'lesion'. A lesion is any localised area of abnormal tissue, including osseous fragments in the joint space. A lesion type refers to a particular lesion phenotype, such as subchondral cystic lesions.
Skeletal lesions and injuries in Australasian Thoroughbred weanling and yearling radiographs

Table 2.2: The twelve lesion types into which skeletal lesions or injuries were categorised. Acronyms are provided for lesion types frequently discussed in the text.

<table>
<thead>
<tr>
<th>Lesion type reported as:</th>
<th>Phenotype/comments</th>
<th>Lesion type acronym / common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteochondritis dissecans</td>
<td>Isolated or attached radiodense fragments overlying irregular radiolucency at the bone margin</td>
<td>OCD</td>
</tr>
<tr>
<td>Marginal bone lysis</td>
<td>Irregular radiolucency at the bone margin, occurring most frequently on a bony ridge that is part of an articulating joint surface</td>
<td>LYS</td>
</tr>
<tr>
<td>Bone chip(s) or fragment(s)</td>
<td>Small isolated or attached radiodense fragments indicating calcified tissue that has separated from the bone</td>
<td>FRAG</td>
</tr>
<tr>
<td>Subchondral cystic lesion</td>
<td>Radiolucency contained beneath the bone surface, often approximately spherical in shape, sometime with a visible cloaca joining the lucency to the joint surface</td>
<td>SCL</td>
</tr>
<tr>
<td>Flattened ridge</td>
<td>Radiolucency showing smooth flattening of a bony ridge that is part of an articulating joint surface</td>
<td>FLAT</td>
</tr>
<tr>
<td>Bone modelling</td>
<td>The formation of new bone tissue, generally reported as occurring on an articular surface, indicated radiographically as surface irregularity, localised radiodensity or tide marks</td>
<td>MOD</td>
</tr>
<tr>
<td>Osteophytes or enthesiophytes</td>
<td>Spur-like bony growths that occur due to joint inflammation or subchondral bone damage; osteophytes can occur on any bone surface while enthesiophytes occur at sites of insertion of soft tissue structures including muscles and ligaments</td>
<td>Spurs</td>
</tr>
<tr>
<td>Sesamoiditis</td>
<td>Inflammation of the sesamoid bones, indicated radiographically by lucency or osteophytes; visible vascular channels (both regular and irregular) were also included in this category</td>
<td></td>
</tr>
<tr>
<td>Bone collapse or wedging</td>
<td>Specific to the hock (TJ), the collapse or partial collapse of the third and/or central tarsal bones</td>
<td></td>
</tr>
<tr>
<td>Fracture</td>
<td>Appearing as a radiolucent crack in the bone, fractures occur as the direct result of trauma</td>
<td></td>
</tr>
<tr>
<td>Exostosis</td>
<td>Lump or dome-shaped bone growth that generally occur as a result of trauma to the bone</td>
<td></td>
</tr>
<tr>
<td>Sclerosis and periostitis</td>
<td>Thickening or hardening of tissue; sclerosis is a more general term, while periostitis refers specifically to the periosteum, the membrane surrounding the bone</td>
<td></td>
</tr>
</tbody>
</table>
2.3.4. Defining osteochondrosis

The definition of OC used in the current study was determined through consultation with an equine veterinarian experienced in diagnosing OC (L.B. Jeffcott), informed by (McIlwraith 1987, Jeffcott 1991, Jeffcott and others 1993).

The following anatomical sites were defined as OC predilection sites: in the fore and the hind fetlocks, the sagittal ridges of the third metacarpal bones (SRMC3) and sagittal ridges of the third metatarsal bones (SRMT3), respectively; in the hocks, the lateral and medial trochlear ridges of the talus, the distal intermediate ridge and medial malleolus of the tibia (LTRT, MTRT, DIRT and MM, respectively), the lateral malleolus of the tibia and the base of the talus; and in the stifles, the lateral and medial trochlear ridges of the distal femur, and the medial femoral condyle (LTRF, MTRF and MFC, respectively), the trochlear groove, and articular surface of the patella. The definition of OC sites can differ between studies, depending on breed-based differences in the distribution of lesions, and the radiographic projections used to obtain diagnoses (for example Pieramati and others 2003; Wittwer and others 2006; van Grevenhof and others 2009).

At each of the above OC sites, only lesion types reported as osteochondritis dissecans, marginal bone lysis, bone chip(s) or fragment(s), or subchondral cystic lesions (OCD, LYS, FRAG or SCL respectively) were defined as OC lesion types. Reports of bone modelling or flattened bone contours (MOD and FLAT, respectively) at these sites were not defined as OC, although analogous phenotypes are sometimes treated as OC in other studies (Wittwer and others 2006, van Grevenhof and others 2009). Where there are differences between the definition of OC used in this study and the definitions used elsewhere, they are noted in the relevant part of the discussion.
The four large results tables provided in the appendices of the current study include a visual guide to the definition of OC used in the current study (i.e. cells referring to OC traits at single anatomical sites have grey backgrounds; those referring to OC traits grouping multiple anatomical sites have heavy borders). Any of these tables could be used as a reference while interpreting the results; for example supplementary Table S2.5 on page 61.

2.3.5. Comparing diagnoses from written reports to those from direct examination of radiographs

Diagnostic reports intended to aid stud managers prior to and during the sales process have not previously been used as a data source to determine the prevalence of OC or other skeletal lesions or injuries in a horse population. Direct comparison between the written reports and the radiographs on which they were based were beyond the scope of this project. It was, however, possible to compare the prevalence and distribution of skeletal lesions and injuries reported in this study to surveys on very similar horse populations where radiographs were examined with the primary purpose of gathering data for academic evaluation, to identify any systematic differences between the results obtained from using both methods.

Two recent studies surveyed the prevalence of skeletal lesions and injuries in Australian and New Zealand Thoroughbred yearling radiographs, based on direct assessment of yearling sale radiographs (Oliver and others 2008, New Zealand; Jackson and others 2009, Australia). It is possible that there is some overlap between these two study populations (1,505 yearlings sold at the 2003 to 2006 New Zealand national sales; and 2,401 yearlings sold at the 2003 Australian Magic Millions sales, respectively) and the population in this study.
2.3.6. Changes between weanling and yearling radiographs

Written reports describing findings in both weanling and yearling radiographs were available for 84 horses (i.e. two reports based on sets of radiographs taken at different times). These reports were used to determine if there were changes in diagnosis as the horses matured from weanlings to yearlings.

Measures of association and tests of independence for weanling versus yearling were carried out on paired count data for particular lesion types occurring at particular anatomical sites, hereafter referred to as traits. Gwet's AC1 statistic was chosen to quantify association because it provides a chance-corrected estimate (that is, it accounts for the likelihood of chance agreement in weanling and yearling radiographs given the prevalences of a given trait in weanlings and yearlings), can be used on categorical data, and has less bias than the commonly-used kappa or pi statistics where the prevalence of a trait is particularly high or low, as is the case for the majority of these analyses (Gwet 2008). Fisher's exact test for count data was used to test for independence between weanling and yearling diagnoses (Fisher 1922). These statistics were determined for 22 traits where more than 10 of the 84 horses were affected as weanlings, and more than 10 of the 84 were affected as yearlings.

2.4. Results

2.4.1. The prevalence and distribution of skeletal lesions and injuries

The average age and standard deviation of the yearling research cohort was 473 ± 52 days (15.5 ± 1.7 months), and 538 horses (54%) were from New Zealand. The average age and standard
deviation of the weanling research cohort was 314 ± 57 days (10.3 ± 1.9 months), and 67 horses (22%) were from New Zealand.

Supplementary Table S2.4 summarises the number of affected yearlings for each lesion type at each site (with left and right sites listed separately), while supplementary Table S2.5 provides the same data with equivalent sites in the left and right limbs pooled. Supplementary Table S2.6 and Supplementary Table S2.7 provide the same summaries for weanlings. The Supplementary Material is located immediately after the References section on page 59. As stated previously, each table includes a visual guide to which lesions are defined as OC in the current study (i.e. cells referring to OC traits at single anatomical sites have grey backgrounds; those referring to OC traits grouping multiple anatomical sites have heavy borders).

The prevalence and distribution of skeletal lesions and injuries were very similar between the yearling and weanling populations in this survey. Overall, 69.9% of weanlings and 64.5% of yearlings were reported as having one or more skeletal lesions or injuries. OC occurred in 29.4% of weanlings and 20.5% of yearlings. Radiographic diagnoses indicating injury (fracture, exostoses and sclerosis or periostitis) were rare compared to skeletal lesions.

Common findings in the front knee joints of yearlings and weanlings were MOD (reported for 5.4% of yearlings and 2.0% of weanlings) and spurs (reported for 5.7% of yearlings and 2.0% of weanlings). LYS was common in weanlings (4.0%) but not yearlings (1.4%). Overall, 14.9% of yearlings and 10.4% of weanlings were reported as having skeletal lesions or injuries of the front knee.
In the fore fetlock joints of yearlings and weanlings, common findings were OC at the SRMC3 (reported for 6.0% of yearlings and 8.0% of weanlings), FLAT of the SRMC3 (reported for 2.6% of yearlings and 6.0% of weanlings) and sesamoiditis (reported for 14.0% of yearlings and 13.7% of weanlings).

In the hind fetlocks, common findings in both yearlings and weanlings were FRAG at the front of the fetlock (PDP1) (reported for 2.6% of yearlings and 2.7% of weanlings), FRAG at the back of the fetlock (PPP1) (reported for 6.5% of yearlings and 6.7% of weanlings), sesamoiditis (reported for 7.9% of yearlings and 8.0% of weanlings). OC of the SRMT3 was reported for 2.3% of weanlings, but only 1.3% of yearlings.

In the hock joints of yearlings, common findings included OC of the DIRT and MTRT, with 2.0% and 3.1% affected respectively. In weanlings, OC was common at the DIRT (4.3% affected) but not the MTRT (0.7% affected). Overall, 7.2% of yearlings and 9.0% of weanlings were diagnosed with OC at a site in the hock. No OC lesion types were reported at the lateral malleolus of the tibia or the base of the talus. MOD and spurs were also common in the hocks: MOD was reported in the hocks of 3.9% of yearlings and 3.0% of weanlings, while spurs were reported in 13.5% of yearlings and 9.0% of weanlings. Exostoses in the hock were reported in 2.0% of weanlings but only 0.6% of yearlings.

Common findings in the stifle joints of yearlings and weanlings included OC of the LTRF (reported in 3.7% of yearlings and 5.7% of weanlings), and OC of the MFC (reported in 4.3% of yearlings and 8.7% of weanlings). In total, 8.7% of yearlings and 14.4% of weanlings were diagnosed with OC at a site in the stifle joint. No OC lesion types were reported for the trochlear groove or articular
surface of the patella. FLAT of the MFC was common in both yearlings (3.5% affected) and weanlings (5.7% affected). Some horses diagnosis with FLAT at one MFC were also diagnosed with OC at the other MFC (see Table 2.3).

2.4.2. Comparing diagnoses from written reports to those from direct examination of radiographs

Table 2.3 shows major findings from the New Zealand and Australian surveys of yearling sale radiographs, and this study, where approximately half of the population of yearlings was from each country (Oliver and others 2008, Jackson and others 2009). Both the (Oliver and others 2008) and (Jackson and others 2009) reports used the term 'OCD' in the manner in which 'OC' is used in this study. Equivalent anatomical site / lesion-type combinations are shown in the table rows with the terminology used in the original publication, excepting lesions occurring at the MFC in the stifle where direct comparison was not possible.
Table 2.3: A comparison of prevalence estimates reported in Jackson and others (2009), Oliver and others (2008) and the present study. NA: results not available. Cells referring to OC traits at single anatomical sites have grey backgrounds; those referring to OC traits grouping multiple anatomical sites have heavy borders. Bracketed terms are the terms used in the study under which they are listed.

<table>
<thead>
<tr>
<th>Joint</th>
<th>Oliver and others (2008)</th>
<th>Jackson and others (2009)</th>
<th>Yearlings, the present study</th>
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<tr>
<td>Front knees</td>
<td>NA</td>
<td>Fragment: 0.7%</td>
<td>FRAG: 0.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osteo-/enthesiophyte: 5.9%</td>
<td>Spurs: 5.7%</td>
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<tr>
<td></td>
<td></td>
<td>OCLL: 22.2%</td>
<td>SCL: 1.0%</td>
</tr>
<tr>
<td>Fore fetlocks</td>
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<td>Sagittal ridge MCIII (Defect): 37.1%</td>
<td>SRMC3 (All): 8.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proximal dorsal P1 (Fragment): 0.7%</td>
<td>PDP1 (FRAG): 1.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palmar P1 (Fragment): 0.1%</td>
<td>PPP1 (FRAG): 0.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proximal sesamoid bones (Vascular channels): 97%</td>
<td>Sesamoiditis: 14.0%</td>
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<td>Hind fetlocks</td>
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<td>Sagittal ridge MTIII (Defect): 7.5%</td>
<td>SRMT3 (All): 1.5%</td>
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<td></td>
<td>Proximal dorsal P1 (Fragment): 2.2%</td>
<td>PDP1 (FRAG): 2.6%</td>
</tr>
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<td></td>
<td>Palmar P1 (Fragment): 6.1%</td>
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<td></td>
<td>Proximal sesamoid bones (Vascular channels): 88%</td>
<td>Sesamoiditis: 7.9%</td>
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<tr>
<td>Hocks</td>
<td>Spurs: 31%</td>
<td>Spurs: 35%</td>
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<td>OCD total: 4.4%</td>
<td>OCD total: 8.5%</td>
<td>OC total: 7.2%</td>
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<td>LTR (OCD): 0.9%</td>
<td>Lateral trochlear ridge talus (OCD): 1.6%</td>
<td>LTRT (OC): 1.6%</td>
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<td>MTR (OCD): 0.1%</td>
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<td>Distal intermediate ridge tibia (OCD): 3.4%</td>
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<td>Stifles</td>
<td>MFC (Lucency): 10.7%</td>
<td>Medial condyle femur (OCLL): 5.6%</td>
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<td>MFC (Flattening): 52%</td>
<td></td>
<td>MFC (FLAT): 4.6%</td>
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<td>OCD total (min. to max. possible): 10.7% to 13.9%</td>
<td>OCD total (min. to max. possible): 5.6% to 10.1%</td>
<td>OC total: 8.7%</td>
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<td>LTR (OCD): 2.5%</td>
<td>Lateral trochlear ridge femur (OCD): 3.8%</td>
<td>LTRF (OC): 3.7%</td>
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<tr>
<td></td>
<td>MTR (OCD): 0.7%</td>
<td>Medial trochlear ridge femur (OCD): 0.7%</td>
<td>MTRF (OC): 0.9%</td>
</tr>
</tbody>
</table>
2.4.3. *Changes between weanling and yearling radiographs*

Supplementary Table S2.8 shows contingency tables, AC1 and P values obtained using Fisher's exact test for change in diagnosis between weanling and yearling radiographs for the 22 traits. The independence of a horse's status as a weanling and a yearling was strongly rejected (P < 10\(^{-6}\) for all 22 traits). There were moderate to very strong associations (0.644 to 0.967) between a horse's status as a weanling and a yearling for all traits. For the four OC traits that were examined, association between weanling and yearling status was strong, ranging from 0.820 to 0.931.

For each of the 22 common traits (including four OC traits), no horses that were unaffected as weanlings were affected as yearlings, and some of the horses that were affected as weanlings were unaffected as yearlings. That is, no horses developed new lesions or injuries as yearlings when they were found to be unaffected as weanlings, and some lesions and injuries present in weanlings had healed by the time the horse was radiographed as a yearling.

2.5. *Discussion*

2.5.1. *The prevalence and distribution of skeletal lesions and injuries*

Many of the skeletal lesion and injury traits commonly seen in this population were OC traits. Overall, 20.5% of yearlings and 29.4% of weanlings were diagnosed with OC lesions occurring at one or more anatomical sites. In comparison, a recent study of Dutch Warmblood horses with an average age between that of the weanlings and yearlings in this study, a definition of OC equivalent to that used here with the addition of the FLAT lesion type, and a similar distribution of OC lesion sites, reported an overall prevalence of OC of 70% (van Grevenhof and others 2009). Excluding the
FLAT lesion type (that is, the 'B' lesion grading in that study), the prevalence of OC in that Dutch Warmblood population was 54%. Interestingly, that Dutch Warmblood population included horses with up to 58% Thoroughbred ancestry, but the prevalence of OC was still approximately double that seen in this analysis. These two populations (i.e. Australasian Thoroughbred and Dutch Warmblood horses) may be raised in very different environments.

The most common anatomical site for OC lesions in the aforementioned population of Dutch Warmblood foals was the trochlear groove (TG). OC or other lesion types were not reported as occurring at this anatomical site in the present study, but have been noted to occur rarely in other surveys of Australian, New Zealand and USA Thoroughbred yearling radiographs (Oliver and others 2008, Jackson and others 2009, Preston and others 2010). Another example of breed-specific difference in OC lesion distribution is a reversal of the relative prevalence of OC in the fore and hind fetlocks between the Thoroughbred population in the current study and Dutch Warmblood horses (at SRMC3 and SRMT3, referred to as MCP-PSR and MTP-PSR, respectively, in van Grevenhof and others (2009)).

Despite some differences between disparate horse breed populations, such as those described above, the majority of studies of OC show strong commonalities in the anatomical sites where OC lesions are reported (Pieramati and others 2003, Wittwer and others 2006, van Grevenhof and others 2009, Lykkjen and others 2012). In the hock, most OC lesions occur at the LTRT or DIRT. In the stifle, OC lesions with bone fragmentation occur primarily at the LTRF, while lesions characterised by radiolucency (for example SCL) occur at the MFC. In the fore and hind fetlocks, OC lesions occur at the sagittal ridge of the third metacarpal and metatarsal bones, respectively.
The existence of some breed-specific differences in both the distribution and prevalence of OC lesions suggests that genetic factors contributing to OC in different breeds may include or be influenced by those governing the skeletal conformation of the breed. Non-genetic (i.e. husbandry and environmental) factors may also be affecting OC prevalence.

Aside from OC traits, other traits that occurred frequently in this population were bone fragments occurring in both PPP1 and PDP1 in the hind fetlocks, sesamoiditis in both the fore and hind fetlocks (sesamoiditis was very broadly defined as changes or vascular channels in the sesamoid bones in the current study), and spurs and modelling in the hocks. Each of these traits was common enough that their prevention may warrant further research. Phenotypic traits equivalent to bone fragments occurring PDP1 in the fetlocks have also been reported in several other horse breeds (Philipsson and others 1993, Wittwer and others 2007, Lykkjen and others 2012).

Some of the skeletal lesions identified as occurring frequently in this study are associated with lower sale price or decreased race performance. Stifle OCD (at any anatomical site in the stifle), and bone fragments either PPP1 or PDP1 in both the fore and hind fetlocks have been associated with lower sales price in the USA (Preston and others 2010). In Australia, OCD and SCL in the stifles, OC at SRMT3 in the hind fetlocks, and some lesion types in the sesamoid bones have been associated with decreased race performance at 2 and 3 years of age, but their effect on price is unknown (Jackson and others 2009). Lower sales prices have a direct effect on a stud's profitability, and poorer race performance can have the same effect indirectly, by harming a stud's reputation. These particular skeletal lesions may therefore be of particular interest to Thoroughbred stud managers in Australia and New Zealand.
2.5.2. Comparing diagnoses from written reports to those from direct examination of radiographs

There were some systematic differences between this study and those reporting lesions and injuries from direct examination of yearling radiographs. In particular, the current study found a lower reported prevalence of: SCL in the front knees, sesamoiditis (defined broadly as changes to the sesamoid bones in the fore or hind fetlocks), lesions of the SRMC3 and SRMT3, spurs in the hocks, and FLAT of the MFC (in the stifles).

However, the reported prevalence and distribution of FRAG in the fore and hind fetlocks was almost identical, as was the prevalence and distribution of OC lesions and SCL in the hocks and stifles. These differences support the suggestion by Jackson and others (2009), that missed or incorrect diagnoses were more prevalent for subtle lesions, but rare for larger or more severe lesions. Also in support of this suggestion, one veterinarian associated with the current study commented that they did not report minor changes to SRMC3 or SRMT3 because in their view these were 'normal abnormalities'. Findings known to affect yearling sale price, such as bone fragments in the fetlocks, were not systematically under-reported in the current study (Preston and others 2010).

The similarities between the findings regarding the prevalence and distribution of lesions in this study and the two aforementioned studies support the use of written reports targeted at stud managers as an efficient and cost-effective data source for the study of OC, particularly for lesions in the hock and stifle. They are also likely to be a good source of data on bone fragments occurring in the fore and hind fetlocks. However, OC occurring at the SRMC3 and SRMT3 is likely to be under-reported, and the interpretation of any further analyses of these data should take this into account.
2.5.3. Changes between weanling and yearling radiographs

Written reports based on weanling radiographs provide a strong indication of the results of future reports based on radiographs taken when the horse is a yearling, for OC and other skeletal lesion and injury traits. For the 22 traits examined in this study, none of the 84 horses was found to have new lesions as a yearling, and in some instances lesions had resolved.

A study of Dutch Warmblood foals by Dik and others (1999) found that after age of 8 months, OC lesions were permanent and did not spontaneously resolve. This does not hold true in the Thoroughbred population of the current study. Some OC lesions reported in weanlings (average age 10.3 months) were not subsequently seen in the same horses as yearlings (average age 15.5 months), suggesting that these defects had healed in the interim. While surgery may have assisted the healing process for some of these horses, sites showing signs of surgery in the current study were recorded as indicating OC, and not all OC lesion sites are amenable to surgery that will leave the horse suitable for sale as a yearling (Oliver and others 2008). It should be emphasised, however, that no new OC lesions appeared during that same time period.

Few of the common traits were more prevalent in the yearling cohort than in the weanling cohort: exceptions were SCL and flattening of the MFC, and skeletal lesions or injuries in the front knee. However, these increases in prevalence were not seen in the cohort of horses with both weanling and yearling radiographs, and they may in fact reflect differences in the makeup of the entire weanling and yearling cohorts, that contained different proportions of horses from Australian and New Zealand.
The apparent resolution of some OC lesions between the weanling and yearling radiographs should certainly be investigated as it may assist in better understanding of why horses develop OC.

### 2.6. Conclusions

Overall, 64.5% of yearlings and 69.9% of weanlings in the current study were reported as having one or more skeletal lesions or injuries. The prevalence of OC overall amongst yearlings was 20.5%, and amongst weanlings was 29.4%. OC lesions occurred most frequently at the SRMC3 in the fore fetlocks (reported for 6.0% of yearlings and 8.0% of weanlings), the LTRF in the stifles (reported in 3.7% of yearlings and 5.7% of weanlings), and the MFC in the stifles (reported in 4.3% of yearlings and 8.7% of weanlings). OC lesions in the hocks and hind fetlocks were less common (e.g. OC at the DIRT in the hocks was reported in 2.0% of yearlings and 4.3% of weanlings, OC at the SRMT3 in the hind fetlocks was reported in 1.3% of yearlings and 2.3% of weanlings).

Other skeletal lesions that were reported frequently in the current study included MOD and spurs at sites in the hocks and in the front knees; FRAG occurring PDP1 and PPP1 in the hind fetlocks; and sesamoiditis (indicated radiographically by lucency or osteophytes or visible vascular channels (both regular and irregular)) occurring in either the fore or hind fetlocks.

The similarities between the findings for prevalence and distribution of lesions in this study and in other surveys of radiographic abnormalities in Thoroughbred horses support the use of written reports targeted at stud managers as an efficient and cost-effective data source for the study of OC, particularly for lesions in the hock and stifle. These reports are also likely to be a good source of data on bone fragments occurring in the fore and hind fetlocks. However, OC occurring at SRMC3
and SRMT3 are likely to be under-reported, and the interpretation of any further analyses based on data from these written records should take this into account.

Findings in weanling radiographs were found to provide a very strong indication of findings in yearling radiographs. Some lesions healed in the period between weanling and yearling radiographs, but the majority of lesions remained.

2.7. Acknowledgements

Sincere thanks are extended to the Australian Rural Industries Research and Development Corporation (RIRDC) for their financial support of the project, and to the participating studs and veterinarians for providing their data, and for their commitment, advice and questions.

Thanks also go to Professor Claire Wade and Honorary Professor John James for their feedback on this chapter, especially the regarding presentation of the large tables.

2.8. References


Furniss, C., Carstens, A., Berg, S.S. van den (2011) Radiographic changes in Thoroughbred
Skeletal lesions and injuries in Australasian Thoroughbred weanling and yearling radiographs


2.9. Supplementary Material

The following common keys apply from Table S2.4 to Table S2.7. 'Trait' refers to any combination of one or more lesion types occurring at one or more anatomical sites. Cells referring to OC traits at single anatomical sites have grey backgrounds; those referring to OC traits grouping multiple anatomical sites have heavy borders.

Columns: OCD – osteochondritis dissecans; LYS – marginal bone lysis; FRAG – bone chip(s) or fragment(s); SCL – subchondral cystic lesions; FLAT – flattened ridge; MOD – bone modelling; SES – sesamoiditis; Spurs – osteophytes or enthesiophytes; COLL – bone collapse or wedging; FRAC – fracture; EXS – exostosis; SC/PE – sclerosis or periostitis; OC – any of OCD, LYS, FRAG, or SCL; All – any lesion type.


Rows (anatomical sites) nested within CJ: ANY – any location within the carpal joints.

Nested within MCPJ: SRMC3 – sagittal ridge of the third metacarpal bone, PPP1 – proximal palmar/plantar to the first phalanx in the MCPJ, PDP1 – proximal dorsal to the first phalanx in the MCPJ, ANO – any location within the MCPJ not otherwise named.

Nested within MTPJ: SRMT3 – sagittal ridge of the third metatarsal bone, PPP1 – proximal palmar/plantar to the first phalanx in the MTPJ, PDP1 – proximal dorsal to the first phalanx in the MTPJ, ANO – any location within the MTPJ not otherwise named;

Nested within TJ: LTRT – lateral trochlear ridge of the talus, MTRT – medial trochlear ridge of the talus, DIRT – distal intermediate ridge of the tibia, MM – medial malleolus of the tibia, ANO – any location within the TJ not otherwise named, OC – any of LTRT, MTRT, DIRT or MM.

Nested within FTPJ: LTRF – lateral trochlear ridge of the femur, MTRF – medial trochlear ridge of the femur, MFC – medial femoral condyle, ANO – any location within the FTPJ not otherwise named, OC – any of LTRF, MTRF, or MFC.

Nested within All: OC – any of SMRC3, SRMT3, LTRT, MTRT, DIRT, MM, LTRF, MTRF, or MFC, All – any anatomical site.
Table S2.4: Affected yearlings (out of a total 1,004) for each trait, shown as “left limb :: right limb”. NA - Not Applicable (lesion type only occurs at specific anatomical sites).

<table>
<thead>
<tr>
<th>Yearling</th>
<th>Lesion types</th>
<th>OCD</th>
<th>LYS</th>
<th>FRAG</th>
<th>SCL</th>
<th>FLAT</th>
<th>MOD</th>
<th>Spurs</th>
<th>SES</th>
<th>COLl</th>
<th>FRAC</th>
<th>EXS</th>
<th>SC/PE</th>
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Table S2.5: Affected yearlings (out of a total 1,004) for each trait with left and right sites pooled, shown as “number affected (% prevalence)”. Individual traits with a prevalence of 2% or more are in bold text. Traits where zero (0) horses were affected are left blank.

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<th>MOD</th>
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Table S2.6: Affected weanlings (out of a total 299) for each trait, shown as “left limb :: right limb”. NA - Not Applicable (lesion type only occurs at specific anatomical sites).

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<th>MOD</th>
<th>Spurs</th>
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<th>COLL</th>
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<td>0 : 0</td>
<td>3 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>5 : 6</td>
<td>19 : 17</td>
<td>NA</td>
<td>2 : 1</td>
<td>1 : 3</td>
<td>2 : 6</td>
<td>0 : 1</td>
<td>3 : 1</td>
<td>31 : 32</td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>13 : 7</td>
<td>1 : 6</td>
<td>6 : 0</td>
<td>1 : 0</td>
<td>0 : 0</td>
<td>3 : 3</td>
<td>0 : 0</td>
<td>NA</td>
<td>NA</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>21 : 13</td>
<td>24 : 16</td>
<td></td>
</tr>
<tr>
<td>FTPJ</td>
<td>LTRF</td>
<td>4 : 7</td>
<td>3 : 4</td>
<td>1 : 0</td>
<td>0 : 0</td>
<td>2 : 3</td>
<td>3 : 1</td>
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<td>NA</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>8 : 11</td>
<td>13 : 16</td>
<td></td>
</tr>
<tr>
<td>MTRF</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>1 : 0</td>
<td>0 : 0</td>
<td>0 : 3</td>
<td>0 : 0</td>
<td>NA</td>
<td>NA</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>1 : 0</td>
<td>1 : 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFC</td>
<td>0 : 1</td>
<td>2 : 12</td>
<td>0 : 0</td>
<td>8 : 8</td>
<td>9 : 10</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>NA</td>
<td>NA</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>10 : 21</td>
<td>13 : 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANO</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 1</td>
<td>0 : 0</td>
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<td>NA</td>
<td>0 : 0</td>
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<td>0 : 0</td>
<td>0 : 1</td>
<td>0 : 0</td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>4 : 8</td>
<td>5 : 16</td>
<td>1 : 0</td>
<td>9 : 8</td>
<td>11 : 13</td>
<td>3 : 4</td>
<td>0 : 0</td>
<td>NA</td>
<td>NA</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 1</td>
<td>19 : 31</td>
<td>27 : 45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>OC</td>
<td>17 : 15</td>
<td>26 : 39</td>
<td>9 : 4</td>
<td>10 : 8</td>
<td>25 : 22</td>
<td>8 : 10</td>
<td>0 : 0</td>
<td>NA</td>
<td>NA</td>
<td>0 : 0</td>
<td>0 : 0</td>
<td>0 : 2</td>
<td>58 : 62</td>
<td>78 : 87</td>
<td></td>
</tr>
</tbody>
</table>
Table S2.7: Affected weanlings (out of a total 299) for each trait with left and right sites pooled, shown as “number affected (% prevalence)”. Individual traits with a prevalence of 2% or more are in bold text. Traits where zero (0) horses were affected are left blank.

<table>
<thead>
<tr>
<th>Weanling</th>
<th>Lesion types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joint</td>
<td></td>
</tr>
<tr>
<td>CJ</td>
<td>ANY</td>
</tr>
<tr>
<td>MCPJ</td>
<td>SRMC3</td>
</tr>
<tr>
<td></td>
<td>PPP1</td>
</tr>
<tr>
<td></td>
<td>PDPI</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
</tr>
<tr>
<td>MTPJ</td>
<td>SRMT3</td>
</tr>
<tr>
<td></td>
<td>PPP1</td>
</tr>
<tr>
<td></td>
<td>PDPI</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
</tr>
<tr>
<td>TJ</td>
<td>LTRT</td>
</tr>
<tr>
<td></td>
<td>MTRT</td>
</tr>
<tr>
<td></td>
<td>DIRT</td>
</tr>
<tr>
<td></td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
</tr>
<tr>
<td>OC</td>
<td>16 (5.4)</td>
</tr>
<tr>
<td>FTPJ</td>
<td>LTRF</td>
</tr>
<tr>
<td></td>
<td>MTRF</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
</tr>
<tr>
<td>OC</td>
<td>11 (3.7)</td>
</tr>
<tr>
<td>All</td>
<td>OC</td>
</tr>
<tr>
<td>All</td>
<td>28 (9.4)</td>
</tr>
</tbody>
</table>
Table S2.8: Contingency tables comparing weanlings and yearlings for 22 traits, with AC1 and P values for Fisher exact tests for each trait. The traits are listed in the same order as they are presented in previous tables; by lesion type and then by anatomical site. This table continues over two pages.

<table>
<thead>
<tr>
<th>LYS at OC sites in all joints</th>
<th>LYS at all sites in all joints</th>
<th>FRAG at all sites in all joints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
</tr>
<tr>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
</tr>
<tr>
<td>Affected</td>
<td><strong>Affected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>59</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td><strong>AC1 = 0.830</strong></td>
<td><strong>P = 3.20 x 10^{-11}</strong></td>
<td><strong>AC1 = 0.810</strong></td>
</tr>
<tr>
<td><strong>MOD at all sites in all joints</strong></td>
<td><strong>Spurs at all sites in all joints</strong></td>
<td><strong>Sesamoiditis in the fore fetlocks</strong></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
</tr>
<tr>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
</tr>
<tr>
<td>Affected</td>
<td><strong>Affected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td><strong>AC1 = 0.817</strong></td>
<td><strong>P = 6.18 x 10^{-10}</strong></td>
<td><strong>AC1 = 0.967</strong></td>
</tr>
<tr>
<td><strong>Sesamoiditis in the fore or hind fetlocks</strong></td>
<td><strong>OC at OC sites in the hock joints</strong></td>
<td><strong>OC at OC sites in the stifle joints</strong></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
</tr>
<tr>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
</tr>
<tr>
<td>Affected</td>
<td><strong>Affected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>56</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>11</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td><strong>AC1 = 0.785</strong></td>
<td><strong>P = 8.41 x 10^{-11}</strong></td>
<td><strong>AC1 = 0.878</strong></td>
</tr>
<tr>
<td><strong>OC at OC sites in all joints</strong></td>
<td><strong>OC lesion types at all sites in all joints</strong></td>
<td><strong>All lesion types in the front knees</strong></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
</tr>
<tr>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
</tr>
<tr>
<td>Affected</td>
<td><strong>Affected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td><strong>AC1 = 0.820</strong></td>
<td><strong>P = 1.98 x 10^{-15}</strong></td>
<td><strong>AC1 = 0.746</strong></td>
</tr>
<tr>
<td><strong>Spurs at all sites in all joints</strong></td>
<td><strong>Sesamoiditis in the fore fetlocks</strong></td>
<td><strong>OC lesion types at all sites in all joints</strong></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
</tr>
<tr>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
</tr>
<tr>
<td>Affected</td>
<td><strong>Affected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>16</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td><strong>AC1 = 0.644</strong></td>
<td><strong>P = 1.49 x 10^{-10}</strong></td>
<td><strong>AC1 = 0.878</strong></td>
</tr>
<tr>
<td><strong>FRAG at all sites in all joints</strong></td>
<td><strong>All lesion types in the front knees</strong></td>
<td><strong>OC lesion types at all sites in all joints</strong></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Weanling/Yearling</strong></td>
</tr>
<tr>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Unaffected</strong></td>
</tr>
<tr>
<td>Affected</td>
<td><strong>Affected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>16</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td><strong>AC1 = 0.644</strong></td>
<td><strong>P = 1.49 x 10^{-10}</strong></td>
<td><strong>AC1 = 0.878</strong></td>
</tr>
</tbody>
</table>

**AC1** is the continuity-corrected value, and **P** values are for Fisher exact tests.
<table>
<thead>
<tr>
<th>All lesion types at SRMC3</th>
<th>All lesion types at ANO (fore fetlocks)</th>
<th>All lesion types at PPP1 (hind fetlocks)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>AC1 = 0.928</td>
<td>P = 7.59 x 10^-14</td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>59</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>AC1: 0.830</td>
<td>P = 3.20 x 10^-11</td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>AC1 = 0.901</td>
<td>P = 6.66 x 10^-10</td>
<td></td>
</tr>
<tr>
<td><strong>All lesion types at ANO (hind fetlocks)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>AC1 = 0.805</td>
<td>P = 1.20 x 10^-8</td>
<td></td>
</tr>
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<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>AC1 = 0.933</td>
<td>P = 3.80 x 10^-12</td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>AC1 = 0.792</td>
<td>P = 5.22 x 10^-10</td>
<td></td>
</tr>
<tr>
<td><strong>All lesion types at the MFC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>AC1 = 0.984</td>
<td>P = 6.46 x 10^-13</td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>AC1 = 0.963</td>
<td>P &lt; 2.2 x 10^-16</td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td>AC1 = 0.763</td>
<td>P = 8.96 x 10^-15</td>
<td></td>
</tr>
<tr>
<td><strong>All lesion type at any OC site (all joints)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weanling/Yearling</strong></td>
<td><strong>Unaffected</strong></td>
<td><strong>Affected</strong></td>
</tr>
<tr>
<td>Unaffected</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Affected</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>AC1 = 0.798</td>
<td>P = 7.98 x 10^-7</td>
<td></td>
</tr>
</tbody>
</table>

AC1: Area Under the Curve; P: Probability
3. Osteochondrosis and other common skeletal lesions in Australasian Thoroughbred yearlings: factors affecting prevalence, and genetic/phenotypic parameters

3.1. Summary

Reasons for performing study: There are no published studies that address additive genetic variation and non-genetic factors contributing to the diagnosis of osteochondrosis (OC) and other skeletal lesions in Thoroughbred horses, or the phenotypic and genetic relationships between skeletal lesions occurring at different anatomical sites.

Objectives: To estimate the extent to which non-genetic factors contribute to the prevalence of OC and other skeletal lesions in Australian and New Zealand Thoroughbred yearlings; to estimate the heritability of OC and other skeletal lesions overall and at particular anatomical sites; and to estimate phenotypic and genetic correlations between pairs of skeletal lesion traits within and between different joints.

Methods: The status of 1,004 Australian and New Zealand Thoroughbred yearlings was obtained with respect to skeletal lesions in the carpal, metacarpophalangeal, metatarsophalangeal, tarsal and femorotibial joints (i.e. the front knees, fore and hind fetlocks, hocks and stifles). A logistic generalised linear mixed model was used to estimate the effect of non-genetic factors and the heritability of each trait. Phenotypic correlations were estimated between pairs of skeletal lesion traits both within and between joints. Correlations between Estimated Breeding Values (EBVs, the estimated sum of additive gene effects for each horse) were obtained for the same pairs of traits as a proxy for conventional genetic correlation, which cannot be determined on the underlying scale for binary traits.

Results: Non-genetic factors and breeding values were found to contribute to the occurrence of many skeletal lesion traits in this population. Heritability estimates were generally higher for OC than non-OC skeletal lesion traits, and for specific traits than for broader traits (e.g. osteochondritis dissecans (OCD) at the lateral trochlear ridge of the distal femur (LTRF), 0.22 vs. OC at any OC predilection site in the stifle, 0.10). A group of genetically associated traits including OCD at the LTRF, subchondral cystic lesions of the medial femoral condyle, lysis of the sagittal ridge of the third metacarpal bone in the fore fetlocks and bone chip(s) or fragment(s) occurring proximal palmar/plantar to the first phalanx in the hind fetlocks was found. The three OC traits in this cluster
Osteochondrosis and other common skeletal lesions in Australasian Thoroughbred yearlings: factors affecting prevalence, and genetic/phenotypic parameters

were the most prevalent OC traits in this population. Not all OC traits had positive phenotypic and EBV correlations with one another. Some non-OC traits had positive phenotypic and/or EBV correlations with some OC traits.

Potential relevance: Non-genetic factors affect the prevalence of different skeletal lesions in different ways. Several OC traits may be amenable to genetic selection. Positive genetic associations were found for a group of traits including multiple OC and non-OC traits known to negatively affect race performance and/or the financial value of affected horses, that are also among the most common skeletal lesions found in this population. This group of traits may be of particular interest as a target for genetic selection to minimise OC.
3.2. Introduction

In Australia and New Zealand, Thoroughbred foals are born in a foaling season that extends from approximately late July to early December. These foals are the result of matings occurring on or after September 1 the previous year (New Zealand Racing 2009, Australian Stud Book 2010). Yearling sales take place between January and June two calendar years after the foals are born, so that, for example, horses born July – December 2005 were sold January – June 2007.

A range of skeletal lesions are commonly reported in Thoroughbred yearlings at sale in Australia, New Zealand and the USA (Oliver and others 2008; Jackson and others 2009; Preston and others 2010, and Chapter 2). Some of these lesions have a negative impact on sale price and/or race performance. Many Thoroughbred stud managers are eager to find strategies to reduce the prevalence of these lesions in their yearlings.

Osteochondrosis (OC) is one of the most common and important skeletal lesions reported in young horses (Jeffcott 1991). OC lesions occur at specific sites on the articular surfaces of joints, where they develop in the articular cartilage of the epiphysis, and over time grow to include both bone and cartilage. When fissures form in the OC lesion, loose fragments or flaps of bone and/or cartilage can result. These fragments are typical of end stage OC, i.e. osteochondritis dissecans (OCD). Stifle OCD is one category of skeletal lesions that has been associated with both lower sale price and poorer race performance in Thoroughbred horses (Jackson and others 2009, Preston and others 2010).
Osteochondrosis and other common skeletal lesions in Australasian Thoroughbred yearlings: factors affecting prevalence, and genetic/phenotypic parameters

Both non-genetic factors and breeding values have been shown to contribute to liability to develop OC in non-Thoroughbred horse breeds. However, at the time of writing, analyses presenting evidence for non-genetic factors affecting the prevalence of OC (or other skeletal lesions or injuries) in Thoroughbred yearlings were limited to the examination of diet and dietary supplementation, and sex (for example Savage and others 1993a, 1993b; Gee and others 2007; Oliver and others 2008).

Several non-genetic factors that can contribute to increased prevalence of OC were described in Chapter 1. In addition to these specific dietary and other triggers, a study of South German Coldblood horses identified variation in the prevalence of OC associated with age at diagnosis, month of birth, and sex, as well as interactions between month of birth and sex, and age and sex (Wittwer and others 2007). This study also examined the effect of the stud where a horse was raised, but concluded that this was not significant. A study of Dutch Warmblood horses identified sex, the equine veterinary practice responsible for taking radiographs, age, and year of diagnosis as being significantly associated with the prevalence of OC (albeit with a higher than normal threshold, namely $P \leq 0.10$), while the month of diagnosis was not associated (van Grevenhof, Schurink, and others 2009). Other studies of OC in non-Thoroughbred horse breeds have also examined the effects of these factors in various combinations, with varied conclusions (for example Hoppe, 1984; Philipsson and others 1993; van Weeren and others 1999).

The presence of additive genetic variation contributing to variation in the occurrence of OC lesions in specific joints has been demonstrated for several Warmblood and Standardbred horse populations, as well as South German Coldblood horses, with heritability estimates ranging from 0.0 to 0.52 depending on both the population and the joint (a comprehensive summary table is
Osteochondrosis and other common skeletal lesions in Australasian Thoroughbred yearlings: factors affecting prevalence, and genetic/phenotypic parameters

provided in van Grevenhof and others (2009b). Many of these studies also provided estimates of heritability for OC overall, although the definition of 'overall' differed according to which joints were examined in each study. All currently published estimates of heritability do not address OC lesions in the shoulder or neck joints, and other joints are also excluded in some studies, so estimates of the heritability of OC overall may be affected by incomplete diagnosis of the disorder.

Examples of overall and joint specific estimates of the heritability of OC include those from the recent studies of Dutch Warmblood horses and South German Coldblood horses. The heritability of OC overall (the stifles, hocks, fore fetlocks or hind fetlocks) in Dutch Warmblood horses was 0.23, and 0.10 for South German Coldblood horses (for OC in the hocks, fore fetlocks or hind fetlocks) (Wittwer and others 2007, van Grevenhof, Schurink, and others 2009).

The current study examined the effect of sex, region where raised, stud where raised, year of birth, diagnosing veterinarian, age at diagnosis, date of birth (day of year), and breeding values on the prevalence of OC and other skeletal lesions and injuries in a population of Australian and New Zealand Thoroughbred yearlings. These analyses were included to allow the identification of traits where the prevalence was influenced by factors that could be under the control of the stud manager (e.g. time of birth within the foaling season), and traits where the effect of genetic variation is sufficient to indicate that selective breeding could be used as a management tool.

The current study also estimated correlations between OC lesions in homologous left and right joints, and between OC lesions and other skeletal lesions both within and between joints. This information was intended to inform the definition of OC in Australasian Thoroughbred horses, with respect to the goal of controlling or minimising this disorder.
3.3. Materials and methods

3.3.1. The study population

The study population consisted of 1,004 Australian and New Zealand Thoroughbred yearlings, as described in Chapter 2. The prevalence and distribution of a variety of skeletal lesions and injuries for the study population were described in Chapter 2. Definitions of anatomical sites used to categorise lesion locations are provided in Table 2.1 on page 41, and definitions of each lesion type are provided in Table 2.2 on page 42. The phrase 'lesion type' is used to refer to both skeletal lesions and injuries.

For each horse, the following data were recorded: sex, geographic region where raised (the Australian states NSW and QLD, and New Zealand), stud where raised (within the three regions), date of birth (year, day of year), age in days at diagnosis, diagnosing veterinarian, and pedigree to five generations (comprising a total of 5,584 horses).

Ethical approval for the collection of these data was granted by the University of Sydney Animal Ethics Committee (AEC reference number N00/7-2007/1/4667). The identity of the horses and studs that participated in this research is protected by non-disclosure agreements.

3.3.2. Factors affecting the prevalence of skeletal lesions in yearlings

The effect of several non-genetic factors and breeding values on the prevalence of skeletal lesion and injury traits in yearlings was examined using a logistic generalised linear mixed model (GLMM). The term 'trait' is used henceforward to describe any single lesion type or pooled group of
lesion types occurring at any particular anatomical site or pooled group of anatomical sites, with a prevalence ≥ 2% in the study population. In total 64 traits were examined (Table S3.8 summarises these traits by joint). No injuries were examined as individual traits (none were prevalent enough to reach the analysis threshold) but injuries are included in traits where all lesion types were pooled together.

OC overall, OC in the fore fetlocks, OC in the hocks and OC in the stifles were included in the 64 traits identified in the current study. A further 16 OC component traits pertaining to specific anatomical sites and/or lesion types (e.g. OCD at the lateral trochlear ridge of the distal femur (LTRF)) were also examined, for a total of 20 OC traits (within the 64 traits examined). Fourteen traits were super-sets of OC, that is they included both OC and non-OC traits, for example a pooled category of all lesion types at an OC predilection site such as the LTRF in the stifles. The remaining 30 traits were non-OC traits.

For each trait, each yearling was scored as affected (1) or unaffected (0). While scoring on an ordinal scale would have allowed more powerful analyses (e.g. Dik and others 1999; van Grevenhof and others 2009a), the collection of data on lesion severity (such as size or depth) that would have facilitated the use of ordinal scoring was not within the scope of this project. Each trait was assumed to be a threshold trait, where horses with lesions reaching a certain threshold of severity were scored as affected.

The effects of sex, region, stud, diagnosing veterinarian, age at diagnosis, date of birth (i.e. day of year), year of birth and pedigree were estimated for each trait using an animal model in the form of a logistic GLMM. Analysis was carried out using ASReml 3 and ASReml-R software (Butler and
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others 2007, Gilmour and others 2009). The logistic GLMM used a logit link function to transform binary scores onto an underlying continuous scale. The following model was used in all cases. Model terms and the logit distribution are defined in Table 3.1.

\[
\ln \left( \frac{\mu}{1-\mu} \right) = \eta + Year + Sex + Region + \sum_{\text{Overall}} \beta_i \cdot Age + \sum_{\text{Region}} \beta_i \cdot Age + \sum_{\text{Overall}} \beta_i \cdot DOB + \sum_{\text{Region}} \beta_i \cdot DOB + \text{Vet} + \text{Stud Region} + \sum_{\text{Region}} \text{spl}(Age) + \sum_{\text{Region}} \text{spl}(DOB) + \text{Horse}
\]
Table 3.1: Terms in the model used to examine the effect of additive genetic variation and non-genetic factors on the prevalence of skeletal lesion and injury traits in Thoroughbred yearlings.

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>The probability of a horse being scored as affected, where ( \mu/(1-\mu) ) is the corresponding odds. The logit of the probability is the log of the odds, i.e. ( \text{logit}(\mu) = \ln\left(\frac{\mu}{1-\mu}\right) )</td>
<td>-</td>
</tr>
<tr>
<td>( \eta )</td>
<td>The population mean logit for the trait under analysis</td>
<td>-</td>
</tr>
<tr>
<td>Year</td>
<td>The horse's year of birth</td>
<td>Categorical; fixed</td>
</tr>
<tr>
<td>Sex</td>
<td>The horse's sex</td>
<td>Categorical; fixed</td>
</tr>
<tr>
<td>Region</td>
<td>The region where the horse was raised</td>
<td>Categorical; fixed</td>
</tr>
<tr>
<td>overall ( \beta_1 \cdot \text{Age} )</td>
<td>Regression on age in days at diagnosis</td>
<td>Covariate; fixed</td>
</tr>
<tr>
<td>region ( \beta_2 \cdot \text{Age} )</td>
<td>Regression on age in days at diagnosis ( \times ) Region interaction</td>
<td>Covariate; fixed</td>
</tr>
<tr>
<td>overall ( \beta_3 \cdot \text{DOB} )</td>
<td>Regression on date of birth (day of year)</td>
<td>Covariate; fixed</td>
</tr>
<tr>
<td>region ( \beta_4 \cdot \text{DOB} )</td>
<td>Regression on date of birth (day of year) ( \times ) Region interaction</td>
<td>Covariate; fixed</td>
</tr>
<tr>
<td>Vet</td>
<td>The veterinarian who authored the diagnostic report</td>
<td>Categorical; random</td>
</tr>
<tr>
<td>Stud</td>
<td>The stud where the horse was raised (nested within Region)</td>
<td>Categorical; random</td>
</tr>
<tr>
<td>region ( \text{spl}(\text{Age}) )</td>
<td>Smoothing spline for age at diagnosis ( \times ) Region interaction</td>
<td>Non-linear spline; random</td>
</tr>
<tr>
<td>region ( \text{spl}(\text{DOB}) )</td>
<td>Smoothing spline for date of birth (day of year) ( \times ) Region interaction</td>
<td>Non-linear spline; random</td>
</tr>
<tr>
<td>Horse</td>
<td>The additive breeding value of the horse</td>
<td>Additive genetic; random</td>
</tr>
</tbody>
</table>

This model allows for different effects of age at diagnosis and date of birth for each region, by including Region \( \times \) Age and Region \( \times \) DOB interactions. These interactions allow differences in climate, foaling distribution over time, and local sales times between regions to be taken into account. The Stud factor is nested within Region; both Stud and Region are included because the Region term is necessary for both interaction terms. The Vet factor is not nested within Region as
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some veterinarians travelled between regions in their provision of radiographic and diagnostic services.

Heritability ($h^2$) on the underlying continuous scale was estimated for each trait as:

\[
 h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_V^2 + \sigma_S^2 + \left(\frac{\pi^2}{3}\right)}
\]

with $\sigma_A^2$, $\sigma_V^2$ and $\sigma_S^2$ being the variance components for Horse, Vet and Stud respectively, while $\pi^2/3$ corresponds to the ‘residual variance’ for an underlying logistic distribution. In this instance it is possible for the Vet and Stud variances to considerably increase the total phenotypic variance on the underlying scale (i.e. the denominator in the equation above), so the significance of the Horse factor was used as an indicator of the presence of significant additive genetic variation, rather than using the standard error of heritability.

In the interpretation of results from analyses using this model, fixed effects with $P \leq 0.05$, and random effects with a Variance Component:Standard Error ratio ($z$-ratio) $\geq 1.64$, equivalent to $P \leq 0.05$ for a one-sided test, were considered significant. The significance of each fixed effect was determined using the Wald test, or using chi-squared when the Wald test failed (the denominator degrees of freedom could not be determined in all cases). A $P$ value based on a composite of the covariate and random splines was calculated for $Age \times Region$ and $DOB \times Region$, weighting the covariate and spline components equally.
Due to the large number of traits under consideration and the number factors for each trait (i.e. multiple testing), some factors will have been falsely estimated to be significant. To identify where these errors were most likely to have occurred, the distribution of P values for each factor was examined. Where necessary z-ratios produced by the initial ASReml-R analyses were converted to P values.

3.3.3. Correlation between EBVs as an indication of genetic correlation between skeletal lesion traits

It is not currently possible to obtain estimates of conventional genetic correlations on the underlying scale for binary traits (software able to estimate such correlations may become available in the future). As a proxy for genetic correlation, correlation between Estimated Breeding Values (EBVs) was estimated, where the EBV is the estimated sum of additive gene effects for each horse. In the present analysis the EBVs were logit values (calculated by ASReml 3 software (Gilmour and others 2009)). EBV correlations were estimated for pairs of traits with non-zero additive genetic variation (the Horse factor) was calculated to illustrate relationships between OC in homologous left and right joints; OC overall and OC in specific joints; between individual lesion types occurring at one or more OC lesion sites; between OC lesion types (pooled together) at OC lesion sites and OC lesion types at other anatomical sites; and between single lesion types occurring at single lesion sites. In order to examine EBV correlations between skeletal lesions occurring in homologous left and right pairs of joints, the significance of the Horse factor and EBVs for OC in either the left joint(s) or right joint(s) were estimated using the same GLMM methodology described above in the section 'Factors affecting the prevalence of skeletal lesions in yearlings'.
Correlation between EBVs was estimated using Pearson's correlation coefficient excluding outliers falling more than 3.0 standard deviations away from the mean for each trait. While correlation between EBVs may not accurately quantify the true genetic correlation between two traits, it provides an indication of the nature of any genetic relationship between them. The analyses were limited to the yearling population in this study and excluded ancestors.

EBVs are based on phenotypic data from the individual and its relatives, with closer relatives being weighted more heavily than distant relatives, and the individual's own phenotype being given the most weight. Since these analyses were limited to horses with known phenotypes (i.e. the yearlings in the study population), we expect to see similar results from the estimates of EBV correlation (described above) and phenotypic correlation (described below). Since these analyses are carried out on traits where additive genetic variation was found to have a significant effect on phenotype, it would be reasonable to find similar genetic and phenotypic correlation between traits even without the use of EBVs. In this study, similarity between estimates of genetic and EBV correlations may be caused by either or both of these factors.

3.3.4. Phenotypic correlation between skeletal lesion traits

Many of the yearlings in the research cohort were diagnosed with more than one skeletal lesion or injury, including instances where the same lesion type was diagnosed at two or more different lesion sites. Tetrachoric correlation (which assumes that the two binary variables for which a correlation is sought represent underlying threshold traits with a normal bivariate distribution) was used to quantify the phenotypic correlation between the same pair-wise combination of traits described above in the section 'Correlation between EBVs as an indication of genetic correlation between
skeletal lesion traits'. Tetrachoric correlation values range between -1 and 1. Analyses were carried out using the polycor library in the statistical software package R (Fox 2010).

### 3.4. Results

As described previously, the 64 skeletal lesion traits examined include OC overall (pooling OC lesion types at all OC lesion predilection sites); OC in the fore fetlocks, OC in the hocks, and OC in the stifles (pooling OC lesion predilection sites within joints); 16 OC component traits (e.g. OCD at the LTRF) for a total of 20 OC traits, as well as 30 non-OC traits, and 14 OC super-set traits (where both OC lesions and non-OC lesions are encompassed by the trait definition). OC in the hind fetlocks was included in analyses of OC overall, but did not occur frequently enough to be examined independently.

The first results presented below are the effects of non-genetic factors on OC traits, followed by the effects of some non-genetic factors on non-OC traits. The high number of traits examined in this study prevents the detailed examination of all factors affecting each non-OC trait, so the results below focus on factors affecting both OC and non-OC/OC super-set traits where that factor may be under the control of stud managers. Results pertaining to the issue of multiple testing are then shown. Table S3.8 in the Supplementary Material for this chapter tabulates the estimates of significance for all factors and heritability estimates for each of the 64 skeletal lesion traits examined in the current study.

Results pertaining to homology between left and right joints, including heritability estimates, as well as the phenotypic and EBV correlations between left and right joint(s) are provided after the
results obtained from the GLMM analyses. Finally, heritability estimates and phenotypic and EBV correlations between pairs of traits are presented for OC overall and OC in specific joints; for individual lesion types occurring at one or more OC lesion sites; for OC lesion types (pooled together) at OC lesion sites and other anatomical sites; and between single lesion types occurring at single lesion site categories.

These analyses were valuable when considering strategies to control or reduce the prevalence of OC in Thoroughbred horses. For example, if non-genetic factors contribute to OC lesions occurring at different locations, and if the genetic association between the locations is low, it may be appropriate for control strategies to define these as separate traits. Conversely, if there are common non-genetic factors contributing to both OC and other common skeletal lesions, and if the relevant genetic associations are sufficiently high, a control strategy may extend the definition of OC to include additional skeletal lesion traits.

3.4.1. Non-genetic factors affecting the prevalence of OC and its component traits

No non-genetic factors were found to significantly affect lesion prevalence for OC overall. *Sex, Age × Region* and *Stud* significantly affected the prevalence of OC in the fore fetlocks; no factors were found to significantly affect the prevalence of OC in the hocks; and *Sex* significantly affected the prevalence of OC in the stifles.

Four OC component traits comprised specific OC lesion types occurring at one or more OC predilection sites in any joint. For the OCD lesion type, the *DOB × Region* interaction was significant, while for the marginal bone lysis (LYS) lesion type, the *Year, Sex, Age × Region*, and
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Stud factors/interactions were all significant. No non-genetic factors were found to significantly affect the prevalence of bone chip(s) or fragment(s) (FRAG) or subchondral cystic lesions (SCL) occurring at one or more OC predilection sites in any joint.

Four OC component traits occurred in the hocks. These were OC at the medial trochlear ridge of the talus (MTRT), OC at the distal intermediate ridge of the talus (DIRT), OCD at any OC predilection site in the hock (i.e. one or more of the MTRT, DIRT, lateral trochlear ridge of the talus (LTRT), or medial malleolus (MM)), and FRAG at any OC predilection site in the hock. Of these four traits, only the prevalence of OCD at any OC predilection site in the hock was found to be significantly affected by any non-genetic factors, that being the interaction $DOB \times Region$.

The seven remaining OC component traits occurred in the stifles. Three of these traits were one specific lesion type (OCD, LYS or SCL) at one or more OC predilection site in the stifles: that is, the lateral or medial trochlear ridge of the distal femur (MTRF), and the medial femoral condyle (MFC). $DOB \times Region$ was found to significantly affect the prevalence of both OCD and LYS at any OC predilection site in the stifles, and $Age \times Region$ also significantly affected LYS at any OC predilection site in the stifles. None of the factors examined in this analysis were found to significantly affect the prevalence of SCL at any predilection site in the stifles. The remaining four stifle OC component traits were location-specific: OC at the LTRF; OCD at the LTRF; OC at the MFC; and SCL at the MFC. $Age \times Region$ and Stud were found to be significant for the prevalence of OC at the LTRF, but not for OCD at the same site, where none of the examined factors were found to be significant. The prevalence of SCL at the MFC differed significantly by Region, but when the lesion type definition was broadened to OC, none of the examined factors were found to
be significant. Sex, which was found to be significant for OC in the stifles overall, was not significant for any individual component trait.

Some studies of equine OC include phenotypes equivalent to flattened ridges (FLAT) in their definition of OC lesions. In this population, FLAT was reported frequently at the sagittal ridge of the third metacarpal bone (SRMC3) in the fore fetlocks, and at the MFC in the stifles. When all lesions types occurring at the SRMC3 (where LYS and FLAT were the most common lesion types) were considered, the non-genetic factors/interactions that were significant for OC in the fore fetlocks (that is, Sex, Age × Region and Stud) were found to be even more significant. When a pooled category of all lesion types at the MFC was considered (where OC lesion types and the FLAT lesion type dominated), the Stud factor was identified as significantly affecting prevalence.

Overall, non-genetic factors were found to affect the prevalence of some OC component traits. Any changes to husbandry methods undertaken with the aim of minimising OC are likely to alter the prevalence of some, but not all, OC component traits.

3.4.2. Non-genetic factors affecting the prevalence of skeletal lesions

Three non-genetic factors affecting skeletal lesion traits in these analyses (Age, DOB and Region) were examined individually and in an interaction term (Age × Region and DOB × Region). Where both the interaction term and one or more component factors were found to be significant, the effect of the interaction factor is discussed while the component factors are not. In addition, the Stud factor was nested within the Region factor. Where both the Stud and Region factors were found to be significant, the effect of Stud is discussed while Region is not. In each case, the overall effect is
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the sum of the main factors and the interaction or nested factor. There were no instances of both the interaction or nested factor and the main factor being significant amongst the OC traits discussed above.

Fig 3.1 illustrates the proportion of OC traits, non-OC traits, and OC super-set traits for which each factor or interaction was found to be significant. It would be useful for stud managers to know whether the factors that affect the prevalence of OC and OC component traits also contribute to other skeletal lesions and injuries. Age was only significant for some non-OC traits, while DOB (day of year) was significant for some non-OC traits and some OC super-set traits. Vet (the diagnosing veterinarian) was not found to be significant for any trait. In this figure, Age and DOB are counted independent of whether an interaction term including this factor was also found to be significant for that trait.

Fig 3.1: The proportion of OC traits, non-OC traits and OC super-set traits for which nine non-genetic factors/interactions were found to be significant.
Given that stud managers often do not choose which horses come into their care, only some of the non-genetic factors examined here can be influenced by changes in husbandry practices. Excluding Age and DOB, which were not found to significantly affect the prevalence of OC traits, these factors/interactions are: Age × Region (the age at which a yearling is sold, by region), DOB × Region (the time at which a foal is born if the mare is not already pregnant, by region), and aspects of Stud such as diet. These three factors are examined in more detail below.

Age × Region significantly influenced the prevalence of four OC traits, three non-OC traits and three OC super-set traits in the fore fetlocks, front knees, stifles and overall. The OC traits were: LYS occurring at one or more OC predilection site in any joint; LYS at any OC predilection site in the stifles; and OC/LYS at the SRMC3 in the fore fetlocks (counted here as two traits, although every horse affected by OC at this site was diagnosed with (at least) the LYS lesion type, rendering the two traits identical for analysis purposes). The non-OC traits significantly influenced by Age × Region were OC and ALL lesion types occurring anywhere in the front knees, and OC lesion types occurring in the fore fetlocks at sites other than the SRMC3, proximal dorsal to the first phalanx (PDP1) or proximal palmar/plantar to the first phalanx (PPP1). The OC super-set traits affected by Age × Region were ALL lesion types occurring at the SRMC3 in the fore fetlocks; OC lesion types occurring at any site in one or more joints; and the FRAG lesion type occurring at any site in one or more joints.

Fig 3.2 illustrates the prevalence of the above OC traits by age in each region, and all lesion types in the front knees as an example non-OC trait. Two of the OC traits are subsets of a third, that is, LYS at OC stifle sites and OC/LYS at the SRMC3 are subsets of LYS at all OC sites in any joint. The effect of the Age × Region interaction (the age of yearlings in each region when sold) differed for
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each of these traits. For example, for stud managers in Region 2 the prevalence of LYS at stifle OC sites increases with yearling age while the prevalence of OC/LYS in the fore fetlocks decreases. Altering yearling age at sale time by targeting earlier or later sales may therefore decrease the prevalence of one trait at the expense of increasing the prevalence of the other.

The $DOB \times Region$ interaction had a significant effect on five OC traits, seven non-OC traits, and two OC super-set traits. The OC traits influenced by the $DOB \times Region$ interaction were: OCD at any OC site in the hocks; OC at the LTRF in the stifles; OCD and LYS occurring at any OC site in the stifles; and OCD at any OC site in any joint. The non-OC traits where the $DOB \times Region$ interaction had a significant effect on prevalence were: OC lesion types occurring in the front knees; FRAG, OC and ALL lesion types occurring PPP1 in the hind fetlocks; OC lesion types

Fig 3.2: The effect of age in each region for OC traits where Age x Region was significant, and for all lesion types (ALL) in the front knees.
occurring elsewhere in the hind fetlocks; FLAT at the MFC in the stifles; and the FLAT lesion type occurring at any site in one or more joints. The OC super-set traits where the \( \text{DOB} \times \text{Region} \) interaction was significant were the OCD and FRAG lesion types occurring at any site in one or more joints.

Fig 3.3 shows the effect of the \( \text{DOB} \times \text{Region} \) interaction for the five OC traits where this factor was significant, and for FRAG occurring PPP1 in the hind fetlocks. The effect of the \( \text{DOB} \times \text{Region} \) interaction (the day of the year when a foal was born within each region) differed for each trait. For example, for stud managers in Region 1, the prevalence OCD at hock OC sites increased the later in the season a foal was born, while the prevalence of OCD at stifle OC sites decreased. Meanwhile in Region 2, the prevalence of OCD at hock OC sites remained relatively constant for foals born at any point in the foaling season while the prevalence of OCD at stifle OC sites fell sharply the later in the season that a foal was born. Altering foaling times by changing mating schedules (where possible) would therefore allow stud managers in Region 2 to minimise both traits, but would require a trade-off between traits for stud managers in Region 1.
The *Stud* factor significantly affected the prevalence of four OC traits, one non-OC trait and three OC super-set traits. Fig 3.4 illustrates variation in the prevalence of OC traits between studs. The OC traits were OC/LYS at the SRMC3 in the fore fetlocks (counted here as two traits), OC at the LTRF in the stifles, and LYS at one or more OC sites in any joint. The non-OC traits where prevalence varied significantly by *Stud* were all lesion types occurring at the SRMC3 in the fore fetlocks, all lesion types occurring at the MFC in the stifles, and the OCD lesion type occurring at one or more sites in any joint. The OC super-set trait significantly affected by *Stud* was the FLAT lesion type occurring at one or more sites in any joint.

*Fig 3.3*: The effect of DOB in each region for OC traits where the DOB x Region was significant, and for FRAG occurring PPP1 in the hind fetlocks. DOB is shown as day of year.
Sex and Year were significant factors for some OC and other traits. Examination of the raw data for these traits indicated that for each trait where Sex was significant, colts (males) were more likely to be affected than fillies (females). Significant variation between years is likely to reflect the effect of weather on diet and biomechanical factors (e.g. less grass and harder ground in drought years).

Overall, three non-genetic factors/interactions that may be under the control of stud managers (Stud, Age × Region, DOB × Region) affected the prevalence of some OC and non-OC traits, but the effect of these factors differed between traits. Any changes to husbandry methods undertaken with the aim of minimising OC are likely to alter the prevalence of some, but not all, OC component traits.

3.4.3. Multiple testing

Table 3.2 shows the percentage of P values ≤ 0.01, 0.05, 0.10 and 0.50 amongst the 64 examined traits, for each of the 10 factors in the GLMM model. Z-ratios were converted to P values where necessary. For Age × Region and DOB × Region, P values based on a composite of the covariate and
random splines, weighting each equally. When the percentage of low P values is considerably higher than the threshold itself, this indicates that at least some of the significant effects of this factor are real.

Amongst the ten terms in the model, only the \textit{Age} and \textit{Vet} factors did not show a disproportionate percentage of low P values. Neither of these factors was significant for any OC traits. Results for the \textit{Vet} factor were presented above in the section 'Non-genetic factors affecting the prevalence of skeletal lesions' with reference to this lack of significance. For the other model terms, at least some of the results presented above pertaining to OC traits, and pertaining to factors affecting both OC and non-OC traits, are likely to be truly significant.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Factor & \% \leq 0.01 & \% \leq 0.05 & \% \leq 0.10 & \% \leq 0.50 \\
\hline
Year & 6.25 & 10.94 & 15.63 & 53.13 \\
Sex & 3.13 & 9.38 & 20.31 & 70.31 \\
Region & 4.69 & 9.38 & 18.75 & 71.88 \\
Age & 0.00 & 6.25 & 10.94 & 56.25 \\
DOB & 0.00 & 12.50 & 21.88 & 59.34 \\
Age x Region & 14.06 & 14.06 & 17.19 & 39.06 \\
DOB x Region & 21.88 & 21.88 & 21.88 & 34.34 \\
Vet & 0.00 & 0.00 & 6.25 & 39.06 \\
Stud & 4.69 & 14.06 & 14.06 & 46.88 \\
Horse & 12.50 & 12.50 & 17.19 & 73.44 \\
\hline
\end{tabular}
\caption{The percentage of P values falling at or below 0.01, 0.05, 0.10, and 0.50 for 64 skeletal lesion traits.}
\end{table}
3.4.4. Heritability, and phenotypic and EBV correlations between OC and other skeletal lesion traits

In the current study, phenotypic and EBV correlation between pairs of traits was estimated with the aim of gaining insight into appropriate ways to group traits for management via genetic selection and/or changes in husbandry strategies. For example, if there were negative phenotypic and EBV correlations between OC lesions occurring at different locations, it would be appropriate for control strategies to define these as separate traits. Conversely, if there were positive phenotypic and EBV correlations between OC traits and other common skeletal lesions, a control strategy may extend the definition of OC to include additional skeletal lesion traits. EBV correlations were estimated between pairs of traits where the heritability of each trait was not zero, but did not require that both heritability estimates be significant.

Table 3.3 shows phenotypic correlations (lower triangle) and EBV correlations (upper triangle) for OC overall, OC in the fore fetlocks, OC in the hocks, and OC in the stifles, and the heritability of each trait on the diagonal. The heritability of OC overall in Australasian Thoroughbred horses was 0.11, and the Horse factor was significant (Horse factor variance was 0.43 ± 0.26). The heritability of OC in the fore fetlocks was 0.15, and was also significant (Horse factor variance was 0.64 ± 0.26). In both the hocks and stifles, the heritability estimate was 0.10, but the Horse factor did not reach the significance threshold (Horse factor variances were 0.32 ± 0.47 and 0.36 ± 0.41 for OC in the hocks and stifles, respectively). There were both positive and negative phenotypic and EBV correlations between OC occurring in different pairs of joints.
Table 3.3: EBV correlations (upper triangle) and phenotypic correlations (lower triangle), between OC overall, OC in the fore fetlocks, OC in the hocks, and OC in the stifles. Heritability is shown on the diagonal.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Fore fetlocks</th>
<th>Hocks</th>
<th>Stifles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.11</td>
<td>0.60</td>
<td>0.48</td>
<td>0.64</td>
</tr>
<tr>
<td>Fore fetlocks</td>
<td>1.00</td>
<td>0.15</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>Hocks</td>
<td>1.00</td>
<td>0.14</td>
<td>0.10</td>
<td>-0.19</td>
</tr>
<tr>
<td>Stifles</td>
<td>1.00</td>
<td>0.12</td>
<td>-0.06</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Phenotypic correlations between OC overall and OC in each joint were part-whole correlations and were therefore estimated at the positive extreme. Phenotypic correlations between OC in the fore fetlocks and OC in the hocks and stifles were both slightly positive, but there was a slightly negative correlation between OC in the hocks and OC in the stifles. EBV correlations showed a similar pattern, with moderate to highly positive part-whole correlations between OC overall and OC in each joint, slight or low positive correlations between OC in the fore fetlocks and OC in the hocks and stifles, and a slight negative correlation between OC in the hocks and OC in the stifles. These results indicate that hock OC and stifle OC should not be grouped together for the purposes of minimising OC.

Table 3.4 shows phenotypic correlations (lower triangle) and EBV correlations (upper triangle) between individual OC lesion types (OCD, FRAG, LYS and SCL) and also the FLAT lesion type pooled over OC lesion sites in any joint, and the heritability of each trait on the diagonal. EBV correlations are shown only for traits where additive genetic variation was identified. Amongst these traits, the Horse factor was significant for only the LYS lesion type (Horse factor variance was 0.38 ± 0.14).
Table 3.4: Correlation between EBVs (upper triangle), phenotypic correlation (lower triangle), and heritability (diagonal) for particular lesion types occurring at OC sites in any joint. NA: no genetic variation was found for at least one of the compared traits. *No horses were affected by both traits. Grey: an OC component trait.

<table>
<thead>
<tr>
<th>Lesion type</th>
<th>OCD</th>
<th>FRAG</th>
<th>LYS</th>
<th>SCL</th>
<th>FLAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCD</td>
<td>0.07</td>
<td>NA</td>
<td>0.17</td>
<td>-0.13</td>
<td>NA</td>
</tr>
<tr>
<td>FRAG</td>
<td>-0.04</td>
<td>0.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LYS</td>
<td>-0.09</td>
<td>0.29</td>
<td>0.09</td>
<td>0.42</td>
<td>NA</td>
</tr>
<tr>
<td>SCL</td>
<td>0.11</td>
<td>-0.94*</td>
<td>0.12</td>
<td>0.14</td>
<td>NA</td>
</tr>
<tr>
<td>FLAT</td>
<td>0.05</td>
<td>0.07</td>
<td>0.24</td>
<td>0.20</td>
<td>0.0</td>
</tr>
</tbody>
</table>

There were both positive and negative estimates of phenotypic correlation between the different lesion types. There were low positive phenotypic correlations between the LYS lesion type and the FRAG and FLAT lesion types, and between the SCL and FLAT lesion types. There was a very high negative correlation between FRAG and SCL, where no horses were affected by both traits, but this negative correlation is not significant in a population this size with these low trait incidences. Other pairwise combinations of lesion types at OC lesion sites in any joint showed slight or negligible phenotypic correlation between traits. There was a low positive phenotypic correlation (0.20) between the FLAT lesion type and the pooled OC lesion types over OC lesion sites in any joint (not shown in table).

EBV correlations between traits, where available, did not demonstrate quite the same patterns as phenotypic correlations for the same pairs of traits. There was a moderate positive EBV correlation between LYS and SCL, a low positive correlation between OCD and LYS, and a slightly negative correlation between OCD and SCL.
Overall, these results indicated that it is not appropriate to group all OC lesion types together for the purposes of managing OC. The negative EBV correlation between OCD and SCL indicates that selection against one trait may have the opposite effect on the other (the EBV correlation between FRAG and SCL could not be calculated). To the extent that phenotypic correlations are typically similar to genetic correlations, the low positive phenotypic correlations between the FLAT lesion type and OC, LYS and SCL support the inclusion of this phenotype as a form of OC (albeit one with minimal clinical consequences). Unfortunately, the relevant EBV correlations also could not be calculated from the present data set.

Table 3.5 shows phenotypic correlations (lower triangle) and EBV correlations (upper triangle) between OC lesion types (a pooled category of OCD, FRAG, LYS and SCL) at OC lesion sites and non-OC anatomical sites, and the heritability of each trait on the diagonal. Correlations between OC overall and non-OC traits are provided in the final column and row. EBV correlations are shown only for traits where additive genetic variation was identified in both traits. Instances where no horses were affected by both traits are indicated.
Table 3.5: EBV correlation (upper triangle), phenotypic correlation (lower triangle), and heritability (diagonal), for the pooled OC lesion types (OCD, FRAG, LYS and SCL) occurring at particular anatomical sites. NA: no genetic variation was found for at least one of the compared traits. *No horses were affected by both traits. Grey: an OC component trait. Anatomical location key provided below.

<table>
<thead>
<tr>
<th>Joint</th>
<th>Site</th>
<th>CJ</th>
<th>MCPJ</th>
<th>MTPJ</th>
<th>TJ</th>
<th>FPTJ</th>
<th>ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ANY</td>
<td>SRMC3</td>
<td>ANO</td>
<td>PPP1</td>
<td>PDP1</td>
<td>ANO</td>
</tr>
<tr>
<td>CJ</td>
<td>ANY</td>
<td>0.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>MCPJ</td>
<td>SRMC3</td>
<td>0.38</td>
<td>0.15</td>
<td>0.03</td>
<td>0.43</td>
<td>-0.07</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
<td>0.24</td>
<td>0.36</td>
<td>0.03</td>
<td>-0.11</td>
<td>0.22</td>
<td>0.06</td>
</tr>
<tr>
<td>MTPJ</td>
<td>PPP1</td>
<td>-0.15</td>
<td>0.16</td>
<td>0.17</td>
<td>0.16</td>
<td>-0.25</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
<td>0.01</td>
<td>0.20</td>
<td>0.50</td>
<td>0.09</td>
<td>0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>TJ</td>
<td>MTRT</td>
<td>0.09</td>
<td>-0.04</td>
<td>0.07</td>
<td>-0.90*</td>
<td>-0.90*</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>DIRT</td>
<td>0.09</td>
<td>-0.03</td>
<td>0.16</td>
<td>0.23</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>FTPJ</td>
<td>LTRF</td>
<td>-0.90*</td>
<td>-0.03</td>
<td>0.04</td>
<td>0.18</td>
<td>0.00</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>-0.90*</td>
<td>0.23</td>
<td>0.28</td>
<td>0.15</td>
<td>-0.93*</td>
<td>-0.06</td>
</tr>
<tr>
<td>ALL</td>
<td>OC</td>
<td>0.16</td>
<td>0.25</td>
<td>0.17</td>
<td>-0.02</td>
<td>0.12</td>
<td>NaN</td>
</tr>
</tbody>
</table>


Amongst these traits, the Horse factor was significant only for OC (i.e. OC/LYS) at the SRMC3 in the fore fetlocks (heritability estimated at 0.15, Horse factor variance was 0.64 ± 0.26), and the pooled OC lesion types occurring PPP1 in the hind fetlocks (heritability estimated at 0.16, Horse factor variance 0.63 ± 0.24). In the hocks, the highest heritability estimate was 0.17 for OC at the MTRT (Horse factor variance 0.71 ± 0.40). Heritability was estimated to be 0.00 for OC at the DIRT (zero variance was assigned to the Horse factor). The heritability of OC at the LTRF in the stifles was estimated at 0.07 (Horse factor variance 0.37 ± 0.63), while the heritability of OC at the
MFC the heritability was 0.17 (Horse factor variance $0.70 \pm 0.70$). The highest heritability estimate for the pooled OC lesion types at a non-OC site was PDP1 in the hind fetlocks, at 0.18 (Horse factor variance $0.72 \pm 1.04$).

There were no clear clusters of positively or negatively correlated traits. There were low positive phenotypic correlations between OC lesion types at any site in the front knees and two site categories in the fore fetlocks (SRMC3 and ANO, a category for lesions occurring at sites other than SRMC3, PDP1 or PPP1); between OC lesion types at any site in the front knees and PDP1 in the hind fetlocks; between each pairwise combination of SRMC3 in the fore fetlocks, ANO in the fore fetlocks, and ANO in the hind fetlocks (a category for lesions occurring at sites other than SRMT3, PDP1 or PPP1), with the correlation between ANO in the fore and hind fetlocks being strongest; between ANO in the fore fetlocks and the MFC in the stifles; between PPP1 in the hind fetlocks and the DIRT in the hocks; and between ANO in the hind fetlocks and MTRT in the hocks. There were very highly negative correlations between pairs of traits where no horses were affected by both traits, but these were not significant in this population size. Phenotypic correlations between other pairwise combinations of OC lesion types at different sites were slight or negligible. Phenotypic correlations between OC overall and individual anatomical sites showed extreme positive correlation for each part-whole pair of traits, low to moderate positive correlation with OC at ANO in the fore fetlocks and at PPP1 in the hind fetlocks, and negligible correlation with OC occurring PDP1 in the hind fetlocks. Examination of the results of the GLMM analyses generally did not show common factors acting on a pair of phenotypically correlated traits in the same manner.
EBV correlations between the pooled OC lesion types at various anatomical sites generally did not follow the same pattern as the phenotypic correlations for the same traits, and two groups of positively correlated traits were apparent. The first group consisted of the SRMC3 in the fore fetlocks, PPP1 in the hind fetlocks, and the LTRF and SCL in the stifles. Three of the four sites in this cluster are OC predilection sites, with lesions occurring PPP1 in the hind fetlocks being the exception. The second group consisted of ANO in the fore fetlocks, and ANO and PDP1 in the hind fetlocks, none of which are regarded as OC predilection sites. OC at the MTRT did not fall clearly into either group. There were low to moderately positive EBV correlations between OC overall and sites in the first group, slight positive EBV correlations between OC overall and ANO in the fore and hind fetlocks, and a low negative EBV correlation between OC overall and PDP1 in the hind fetlocks. The pooled OC lesion types occurring PDP1 in the hind fetlocks showed negative correlations with the same lesion types at each individual OC predilection site and with OC overall.

Overall, the results of the phenotypic correlation analyses did not indicate any clear groups of traits. Two useful groupings were obtained based on positive EBV correlations within each group. It is apparent that the pooled OC lesion types occurring PDP1 in the hind fetlocks are unrelated to OC overall or OC at any anatomical site. The exclusion of other non-OC anatomical site categories (e.g. PPP1 in the hind fetlocks) is less clear-cut, with both positive and negative phenotypic and EBV correlations between these sites and known OC sites.

Table 3.6 shows phenotypic correlations (lower triangle) and EBV correlations (upper triangle) between thirteen traits where a single lesion type occurred at a single anatomical site category. Correlations between OC overall and non-OC traits are provided in the final column and row. EBV correlations are not available for traits where no genetic variation was identified (i.e. sesamoiditis.
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(SES) in the fore fetlocks and FLAT at the MFC in the stifles). Instances where no horses were affected by both traits are indicated. The Horse factor reached the threshold for significance for LYS (i.e. OC/LYS) at SRMC3 in the fore fetlocks, discussed earlier, and for the FRAG lesion type occurring PPP1 in the hind fetlocks (heritability was estimated at 0.18, Horse factor variance \(0.73 \pm 0.26\)). The highest estimates of heritability for OC traits were OCD at the LTRF (0.22, Horse factor variance 1.08 \(\pm 1.11\)), and SCL at the MFC (0.20, Horse factor variance 0.84 \(\pm 1.13\)), both in the stifles, where the Horse factor did not reach the significance threshold.

There were no clear clusters of positive or negative phenotypic correlation between traits. There were low positive phenotypic correlations between a large number of these traits: bone modelling (MOD) in the front knees and FLAT at SRMC3 in the fore fetlocks; MOD at ANO in the hocks (a lesion site category for sites other than the DIRT, MTRT, LTRT and MM) and SCL at the MFC in the stifles; osteophytes or enthesiophytes (spurs) in the front knees and LYS at SRMC3 in the fore fetlocks; OC/LYS at SRMC3 in the fore fetlocks and FLAT in the same location and at the MFC in the hind fetlocks; and both LYS and FLAT at the SRMC3 in the fore fetlocks and SCL at the MFC in the stifles. SES in the fore fetlocks had a low positive correlation with SES in the hind fetlocks, FRAG occurring PDP1 in the hind fetlocks, spurs at ANO in the hocks, and FLAT at the MFC. FRAG occurring PPP1 in the hind fetlocks was associated with SCL at the MFC in the stifles, and both FRAG occurring PDP1 in the hind fetlocks and FLAT at the MFC in the stifles were associated with MOD at ANO in the hocks. Phenotypic correlations between the remaining pairs of traits were low or negligible, and were both positive and negative. Examination of the results of the GLMM analyses generally did not show common factors acting on a pair of phenotypically correlated traits in the same manner. Phenotypic correlations between non-OC traits and OC overall were generally

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around zero, with the exception of FRAG occurring PPPI in the hind fetlocks, where a positive correlation was seen.
Table 3.6: Correlation between EBVs (upper triangle), phenotypic correlation (lower triangle), and heritability (diagonal) where a single lesion type occurred at a single anatomical site in 2% or more of the population. NA: no genetic variation was found for at least one of the compared traits. *No horses were affected by both traits. Grey: an OC component trait. Anatomical location key provided below.

<table>
<thead>
<tr>
<th>Joint</th>
<th>Site</th>
<th>CJ</th>
<th>MCPJ</th>
<th>MTPJ</th>
<th>TJ</th>
<th>FTPJ</th>
<th>ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ANY</td>
<td>SRMC3</td>
<td>ANO</td>
<td>PPP1</td>
<td>PDP1</td>
<td>ANO</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LYS</td>
<td>FLAT</td>
<td>SES</td>
<td>FRAG</td>
<td>FRAG</td>
</tr>
<tr>
<td>CJ</td>
<td>ANY</td>
<td>MOD</td>
<td>0.12</td>
<td>0.27</td>
<td>0.31</td>
<td>0.30</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>SPUR</td>
<td>0.06</td>
<td>0.09</td>
<td>0.23</td>
<td>-0.08</td>
<td>NA</td>
<td>0.43</td>
</tr>
<tr>
<td>MCPJ</td>
<td>SRMC3</td>
<td>LYS</td>
<td>0.12</td>
<td>0.25</td>
<td>0.15</td>
<td>0.12</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
<td>FLAT</td>
<td>0.40</td>
<td>0.06</td>
<td>0.24</td>
<td>0.09</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>SES</td>
<td>-0.17</td>
<td>0.19</td>
<td>0.02</td>
<td>0.03</td>
<td>0.0</td>
<td>NA</td>
</tr>
<tr>
<td>MTPJ</td>
<td>PPP1</td>
<td>FRAG</td>
<td>0.03</td>
<td>0.08</td>
<td>0.17</td>
<td>0.04</td>
<td>-0.08</td>
</tr>
<tr>
<td></td>
<td>PDP1</td>
<td>FRAG</td>
<td>-0.06</td>
<td>0.06</td>
<td>0.16</td>
<td>0.24</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>ANO</td>
<td>SES</td>
<td>-0.09</td>
<td>0.02</td>
<td>0.16</td>
<td>0.09</td>
<td>0.27</td>
</tr>
<tr>
<td>TJ</td>
<td>ANY</td>
<td>MOD</td>
<td>0.23</td>
<td>-0.02</td>
<td>0.14</td>
<td>0.00</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>SPUR</td>
<td>0.10</td>
<td>0.01</td>
<td>0.07</td>
<td>-0.13</td>
<td>0.29</td>
<td>-0.11</td>
</tr>
<tr>
<td>FTPJ</td>
<td>LTRF</td>
<td>OCD</td>
<td>-0.04</td>
<td>0.09</td>
<td>-0.90*</td>
<td>0.10</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>SCL</td>
<td>0.21</td>
<td>0.09</td>
<td>0.27</td>
<td>0.26</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>FLAT</td>
<td>0.01</td>
<td>-0.14</td>
<td>0.23</td>
<td>0.02</td>
<td>0.20</td>
<td>-0.93*</td>
</tr>
<tr>
<td>ALL</td>
<td>OC</td>
<td>OC</td>
<td>0.09</td>
<td>0.09</td>
<td>0.19</td>
<td>0.04</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Key: CJ – carpal joints, MCPJ – metacarpophalangeal joints, MTPJ – metatarsophalangeal joints, TJ – tarsal joints, FTPJ – femorotibial and femoropatellar joints. Within CJ: ANY – any location within the carpal joints. Within MCPJ: SRMC3 – sagittal ridge of the third metacarpal bone, ANO – locations within the MCPJ other than SRMC3, PPP1, PDP1. Within MTPJ: PPP1 – proximal palmar/plantar to the first phalanx in the MTPJ, PDP1 – proximal dorsal to the first phalanx in the MTPJ, ANO – locations within the MTPJ other than SRMC3, PPP1, PDP1. Within TJ: ANO – any location other than LTRT, MTRT, DIRT or MM. Within FTPJ: LTRF – lateral trochlear ridge of the distal femur, MFC – medial femoral condyle.
EBV correlations between the same pairs of traits did not show the same patterns as phenotypic correlations between the same traits, but expanded upon the two groups identified in the previous analysis (i.e. the pooled OC lesion types at various anatomical sites). The first group of positively correlated traits included OCD at the LTRF, SCL at the MFC in the stifles, LYS occurring at the SRMC3 in the fore fetlocks and FRAG occurring PPP1 in the hind fetlocks (all of which were the dominant lesion type amongst the pooled OC lesion types at the sites considered above), with the addition of MOD and spurs in the front knees, and MOD in the hocks. The second group consisted of SES in the hind fetlocks, FRAG occurring PDP1 in the hind fetlocks, and spurs in the hocks. Non-OC traits first group showed positive EBV correlations with OC overall, while the second showed negative EBV correlations with OC overall.

Overall, these results supported those shown above for the pooled OC lesion types at OC and non-OC anatomical sites. The group consisting of OCD at the LTRF, SCL at the MFC in the stifles, LYS occurring at the SRMC3 in the fore fetlocks, FRAG occurring PPP1 in the hind fetlocks, MOD and spurs in the front knees, and MOD in the hocks is of particular interest as a target for genetic selection to minimise OC, because it contains multiple traits known to affect yearling price and race performance.

The *Horse* factor was significant for four OC super-set traits not shown in the tables: ALL lesion types at SRMC3 in the fore fetlocks (heritability of 0.15, *Horse* factor variance 0.67 ± 0.18), ALL lesion types at the MFC in the stifles (heritability of 0.12, *Horse* factor variance 0.48 ± 0.19), ALL lesion types at a pooled category of all OC sites (heritability of 0.09, *Horse* factor variance 0.35 ± 0.05), and the FRAG lesion type at all anatomical sites (pooling both OC and non-OC sites, heritability of 0.06, *Horse* factor variance 0.23 ± 0.06). For each of these super-set traits, the
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The majority of horses were affected by OC (i.e. while both OC and non-OC lesions were considered, the majority of lesions were OC).

Overall, the significance of the Horse factor provides an indicator that breeding values contribute to the occurrence of many OC traits and FRAG (and traits including FRAG) occurring PPP1 in the hind fetlocks. These traits are therefore likely to be amenable to minimisation via genetic selection. OC traits that did not pool lesion types or anatomical site categories often had higher heritability than those that did, although the heritability estimates were not necessarily significant for the more specific traits.

3.4.5. Homology between left and right

Table 3.7 shows the heritability of OC lesions occurring at OC lesion sites in the left and right fore fetlocks, hocks, stifles, and OC overall (a pooled category including these joints and the hind fetlocks). The heritability of OC lesions occurring in only the left joint(s) or only the right joint(s) was sometimes 0.00. Table 3.7 also shows the phenotypic correlation between left and right joints in each of these categories, and correlation between EBVs where genetic variation was identified as contributing to OC (i.e. heritability was > 0.00) in both the left and right joints, the number of affected horses in each category, and the number of bilaterally affected horses.

These results supported the pooling of observations between left and right pairs of joints. Phenotypic correlations for each pair of left and right joints were positive. There was very high correlation between OC in the left fore fetlock and OC in the right fore fetlock, and moderate correlation between OC in the left hock and OC in the right hock. Correlation between OC in the
left stifle and OC in the right stifle was low, presumably because of the predominance of lesions affecting the right stifle (see Chapter 2). Phenotypic correlation between OC lesion sites in the left joints and OC lesions in the right joints overall was moderate, reflecting the component joints of this category. EBV correlation for OC in left and right joints overall, and for OC in the left and right fore fetlocks, was moderately positive. The consistently positive phenotypic and EBV correlation between pairs of left and right joints indicates strong homology, and shows that it is appropriate to pool these joints together.

Table 3.7: Homology between left and right joints for the OC lesion types. NA: correlation between EBVs not estimated due to lack of genetic variation for one trait.

<table>
<thead>
<tr>
<th></th>
<th>( h^2 ) left</th>
<th>( h^2 ) right</th>
<th>Phenotypic correlation</th>
<th>EBV correlation</th>
<th>No. affected (left and/or right)</th>
<th>No. affected (both left and right)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.07</td>
<td>0.12</td>
<td>0.59</td>
<td>0.56</td>
<td>206</td>
<td>54</td>
</tr>
<tr>
<td>Fore fetlocks</td>
<td>0.13</td>
<td>0.16</td>
<td>0.90</td>
<td>0.69</td>
<td>60</td>
<td>27</td>
</tr>
<tr>
<td>Hocks</td>
<td>0.11</td>
<td>0.00</td>
<td>0.50</td>
<td>NA</td>
<td>72</td>
<td>11</td>
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<tr>
<td>Stifles</td>
<td>0.00</td>
<td>0.07</td>
<td>0.29</td>
<td>NA</td>
<td>87</td>
<td>11</td>
</tr>
</tbody>
</table>

3.5. Discussion

3.5.1. Methodology

Pre-sale radiograph reports, which can be obtained at no cost, provided the raw data source from which binary scores were derived for the presence of OC and other skeletal lesion traits in a sub-population of high-value Australian and New Zealand Thoroughbred yearlings. The contributions of both non-genetic factors and breeding values to OC and other skeletal lesion traits were successfully identified from these data. Ordinal or continuous scales (as opposed to binary scores) would increase the power of the analyses, and have been used in some studies of OC in other horse breeds.
where scoring of skeletal lesions and injuries was based on direct examination of radiographs (for example Wittwer and others 2007; van Grevenhof and others 2009b). The inclusion of data from Thoroughbred horses outside the high-value sub-population examined in the current study may also provide an indication of the extent to which findings from the current study apply to the entire Australasian Thoroughbred horse population. However, in the Australian and New Zealand Thoroughbred population, costs associated with acquiring sets of existing radiographs, or radiographing horses for which radiographs are not otherwise available, and subsequent interpretation by equine clinicians would need to be balanced against the potential to increase analytical power by simply collecting a greater proportion of the pre-sale radiograph reports.

3.5.2. Non-genetic factors affecting the prevalence of OC and other skeletal lesions

Changes in husbandry could be combined with genetic selection to target specific traits associated with negative sale and race outcomes. For example, a stud with a high rate of stifle OC (associated with poor race performance (Jackson and others 2009, Preston and others 2010)) may be able to minimise its occurrence by changing the diet of their young horses (based on research into dietary risk factors for OC), targeting at risk horses for earlier or later sales (depending on geographic region), or targeting earlier or later foaling times for mares with high risk offspring (again depending on region).

The impact of the non-genetic factors examined in this study varied between traits. Any changes implemented by stud managers seeking to minimise the occurrence of multiple skeletal lesion and injury traits may have conflicting effects on different traits. The effects of environmental factors or interactions that are partially or completely under the control of stud managers are discussed below,
these are Age × Region, DOB × Region, and Stud. Other factors that were considered in this analysis are then addressed.

Age × Region was significant for four OC traits, three non-OC traits and three OC super-set traits. The effect of age in each region differed for each trait (see Fig 3.4). From a profitability perspective, stud managers may often be in a situation where they must prioritise one trait over another to minimise the prevalence of the most important trait; for example, LYS at stifle OC sites is more likely to negatively affect race performance (and potentially also sale price) than LYS at the SRMC3 in the fore fetlocks, so stud managers in Region 2 may wish to sell their horses at a younger age when LYS at stifle OC sites is less common, despite the fact that the prevalence of LYS at OC sites in any joint and LYS at the SRMC3 in the fore fetlocks is higher at this time. Further research to identify any differing management techniques between horses younger or older at sale time could also provide insight into the environmental factors that can contribute to altered prevalence of skeletal lesions at sale time.

DOB × Region was significant for five OC traits, seven non-OC traits and two OC super-set traits. As was the case for Age × Region, the effect of DOB × Region differed for each trait (see Fig 3.3). Stud managers may often be in a situation where they must prioritise one trait over another to minimise the prevalence of the most important trait.

The traits for which the Stud factor was significant were OC traits, OC super-set traits, or were related to OC traits (i.e. FLAT at a pooled category of OC lesion sites, discussed below). This finding is consistent with diet being a factor affecting the prevalence of OC in this population, although biomechanical factors such as varied ground hardness and the type or amount of exercise...
undertaken by foals may also play a role. Further studies on diet and exercise may provide additional insight into potential OC minimisation strategies in this population.

Sex affects growth rates and behaviour in Thoroughbred horses, and therefore has the potential to play a role in the development of OC (Thompson 1995, Duberstein and Gilkeson 2010). Only four of the twenty OC traits examined in this study were affected by sex in this population (OC/LYS at the SRMC3, OC in the stifles, and LYS at any OC predilection site in any joint). Sex was found to be a significant factor for the prevalence of OC in studies of South German Coldblood and Dutch Warmblood horses, but not for Italian Maremmano horses (Pieramati and others 2003, Wittwer and others 2007, van Grevenhof, Schurink, and others 2009). The role of sex in the development of OC lesions is not clear-cut. However, where sex was a significant factor, lesions were always more prevalent in colts than in fillies.

The diagnosing veterinarian (Vet) was not found to be a significant factor for any skeletal lesion traits, indicating that different veterinarians provide diagnoses that are not significantly different from one another. The Vet factor came closest to significance for traits where the data collection method used in this study resulted in a lower reported prevalence than was seen in other similar studies that directly examined radiographs (see Chapter 2; for example FLAT at the SRMC3, all lesion types in the front knee, and MOD and spurs in the hock). This supports the suggestion of Jackson and others (2009) that more subtle lesions, such as FLAT, are more likely to be diagnosed differently between veterinarians.

Age when radiographed (Age) and date of birth (DOB) without Region interactions rarely affected the prevalence of skeletal lesion traits. In addition, the examination of the distribution of P-values
that was carried out to address the issue of multiple testing indicated that the frequency with which Age was found to be significant was similar to that which we would expect by chance (as was the case for the Vet factor). Thus, these factors (Age, DOB, and also Vet) should not be prioritised by stud managers seeking to minimise the prevalence of OC or other skeletal lesions through changes in husbandry practices.

3.5.3. The heritability of OC and other skeletal lesion and injury traits

The heritability of OC overall in this population was slightly lower than that found for other breeds using binary scoring methods, at 0.11 compared to 0.15 for Dutch Warmblood horses and 0.14 for Italian Maremmano horses (Pieramati and others 2003; van Grevenhof and others 2009b). The definition of OC used in these studies differed somewhat from that used in the current study: the study of Dutch Warmblood horses included a phenotype equivalent to the FLAT lesion type as well as the lesion types regarded as OC here, while only lesion types with bone chips or fragments (equivalent to OCD and FRAG in the current study) were examined in the study of Italian Maremmano horses.

Of the joints, (the fore fetlocks, hocks and stifles,) the highest heritability of OC was found in the fore fetlocks (0.15), where the single OC predilection site is the SRMC3. However, OC lesions at this site are likely to have been under-reported in this population (see Chapter 2). Severe lesions are more likely to be reported correctly, so the use of GLMM that assumes a threshold trait is still appropriate, although the trait itself may describe more severe OC lesions than the other OC traits examined in this analysis.
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The heritability of OC in both the hocks overall and the stifles overall was estimated at 0.10. Some component OC traits in these joints had the highest heritability estimates in this study (OCD at the LTRF (0.22) and SCL at the MFC (0.20) in the stifles, and OC at the MTRT in the hocks (0.17)), but in contrast to OC overall and OC in the fore fetlocks, the Horse factor (i.e. breeding values) was not found to be significant for any OC component traits in these joints. This result may change if these traits are revisited with additional data, or if the definition of OC is broadened to include FLAT at some specific anatomical sites, for example at the MFC in the stifle where OC and FLAT lesion types comprise the vast majority of lesions. Analyses of the OC lesion types and FLAT lesion type separately at this site did not find the Horse factor to be significant. In contrast, the Horse factor was significant (an indicator of the presence of significant additive genetic variation) and the heritability estimate was moderate (0.12) when the analysis examined all lesion types. This finding is consistent with the supposition that separating FLAT from OC lesion types at the MFC may result in poor modelling of both traits.

In contrast to findings from other horse breeds, heritability estimates for stifle OC traits were generally higher than for hock OC traits (for example Wittwer and others 2007; van Grevenhof and others 2009b; Lykkjen and others 2010). Unexpectedly, genetic variation was not found to be a contributing factor to OC occurring at the DIRT in the hocks, although OC at this site is heritable in other horse breeds that have significant genetic input from Thoroughbred horses, such as the Dutch Warmblood population, and Standardbred Trotters. It would be valuable to carry out further analyses, particularly regarding OC occurring at the DIRT (where genetic variation was not found to contribute to the occurrence of OC) with additional data and to revisit the definition of OC at this site, to ensure that this finding represents the true status of this trait in Thoroughbred horses.
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In addition to OC traits, and some traits with extensive overlap with OC traits, additive genetic variation was found to be significant for FRAG occurring PPP1 in the hind fetlocks. Positive heritability estimates have previously been obtained for an equivalent phenotype in the fetlocks of Hanoverian Warmblood horses (Stock and others 2005) The high prevalence of this trait and its association with impaired race performance indicate that it is a good target for further research with the aim of minimising its occurrence (Jackson and others 2009). As the lesion definition was broadened from FRAG to OC (FRAG + LYS + SCL + OCD) to ALL (OC + MOD, spurs, and injury lesion types) at this site, the significance of the Horse effect, an indicator of the presence of significant additive genetic variation, dropped. One explanation is that breeding values contribute specifically to the FRAG lesion type at this site.

Overall, the positive heritability estimates and statistical significance of the Horse factor indicate the presence of significant additive genetic variation, suggesting that genetic selection could be used to reduce the occurrence of many OC traits, and FRAG occurring PPP1 in the hind fetlocks, in this sub-population of high-value Australian and New Zealand thoroughbred horses. If a program of genetic selection was to be implemented, quality assurance would be important to further improve the definition of some traits (the need for which is illustrated by the under-reporting of lesions at SRMC3) that may result from inconsistencies between individual veterinarians' diagnoses. Under-reporting of a trait is likely to reduce estimates of heritability.

3.5.4. Phenotypic and EBV correlations between skeletal lesion traits

OC occurs in various anatomical sites in multiple joints, and includes lesions with bone fragments and lesions without bone fragments. The examination of phenotypic and EBV correlations between
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OC component traits provided a foundation for comparing the results of this study with results from analyses of joint-wise OC status in Dutch Warmblood horses. These analyses and those between OC and other skeletal lesion traits also allowed examination of the definition of OC, with respect to the goal of controlling this disorder; for example, it may be appropriate for control strategies to define multiple separate OC traits, or to include additional skeletal lesion traits that are correlated with the occurrence of OC. The current study explored OC trait definition by determining phenotypic EBV correlations between OC overall and OC in specific joints; between individual lesion types at occurring at one or more OC lesion sites; for OC lesion types (pooled together) occurring at OC lesion sites and OC lesion types at other anatomical sites; and between single lesion types occurring at specific anatomical sites.

OC in the hocks and OC in the stifles appeared to be unrelated traits, based on slightly negative phenotypic and EBV correlations between these traits. Conflictingly, both OC in the hocks and OC in the stifles appeared to be somewhat related to OC in the fore fetlocks, as shown by slight positive phenotypic and EBV correlations for these traits. The examination of more specific OC traits, below, sheds more light on these between-joint relationships. An analysis of Dutch Warmblood horses found similarly slight relationships between joints, all of which were positive (van Grevenhof, Ducro, and others 2009).

OC is defined as including lesions with bone fragments (OCD and FRAG in the current study) and lesions without bone fragments (SCL and LYS in the current study), but phenotypic correlation and clustering analyses in a Dutch Warmblood population indicated that they should be considered as statistically separate traits (van Grevenhof, Ducro, and others 2009). Our analysis of the Australian and New Zealand Thoroughbred population was not clear-cut; the Warmblood-based hypothesis
was supported by a negative EBV correlation between the OCD and SCL lesion types, but there was no phenotypic correlation between the two lesion types with bone fragments (i.e. OCD and FRAG) and an EBV correlation could not be estimated for these two traits. Additionally, the LYS lesion type showed low positive phenotypic correlations with both the FRAG and SCL lesion types, and positive EBV correlations with both the OCD and SCL lesion types.

The FLAT lesion type, which was also regarded as an OC lesion type in the study of Dutch Warmbloods, had positive phenotypic correlations with both LYS and SCL as well as the pooled OC lesion types in this Thoroughbred population, supporting its interpretation as a mild OC phenotype. EBV correlations between FLAT and other lesion types could not be estimated. For analytical (if not clinical) purposes, the definition of OC could be extended to include the FLAT lesion type at some specific anatomical sites. As seen in Table 3.6, the FLAT phenotype is chiefly seen at the SRMC3 in the fore fetlocks and the SCL in the stifles. At the SRMC3 in the fore fetlocks, and the MFC in the stifles, the prevalence of the FLAT lesion type was affected by the same factors as the OC lesion types at the same sites. Together these factors support the use of the FLAT lesion type as an indicator of OC risk, and its interpretation in other studies (e.g. the study of Dutch Warmblood horses) as a phenotype representing a form of OC (van Grevenhof, Schurink, and others 2009).

The negative phenotypic and EBV correlations between the FRAG and SCL lesion types are related to the negative correlations between OC in the hock and OC in the stifles. The SCL lesion type was common in the stifle (at the MFC) but not the hocks, while the FRAG lesion type was more common in the hocks.
OC sites are often grouped by joint for analysis, but there was insufficient data to support such grouping in this population. The analysis of OC lesion types at individual OC sites and other anatomical sites showed that no yearlings were diagnosed at both common hock OC sites or both common stifle OC sites, but indicated a positive EBV correlation between OC at the two common stifle OC sites (the LTRF and SCL). EBV correlation between anatomical sites within the hock OC sites could not be estimated. In addition, no yearlings were affected by OC at either of the two common stifle OC sites as well as the MTRT in the hocks. This mutual exclusivity is likely to be the basis for negative phenotypic and EBV correlations between OC in the stifles and OC in the hocks. No similar analysis was carried out in the Dutch Warmblood population.

Groupings other than joint-wise groupings may be more effective in the management of OC. The analysis of OC lesion types at individual OC and non-OC anatomical sites suggested that there are clusters of OC traits with positive EBV correlations that may serve as good targets for genetic selection. The first cluster (OC at the SRMC3 in the fore fetlocks, and OC at the LTRF or MFC in the stifles), was also correlated with OC lesion types occurring PPP1 in the hind fetlocks, and contains multiple traits known to negatively affect race performance. The OC traits in this group reflect the sites most commonly affected in this population (see Chapter 2). The second cluster consists of OC lesion types occurring in the fore fetlocks at ANO, the hind fetlocks PDP1, and the MTRT in the hocks, which is the only typical OC lesion site in this cluster.

The analyses of single lesion types occurring at specific anatomical sites suggested that some non-OC traits could be grouped with OC traits for management. Positive correlations between EBVs allowed the refinement and expansion of the first cluster described above to: OCD at the LTRF, SCL at the MFC in the stifles, LYS occurring at the SRMC3 in the fore fetlocks, FRAG occurring
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PPPI in the hind fetlocks, MOD and spurs in the front knees, and MOD in the hocks. Since OC in the stifles (of which OCD at the LTRF and SCL at the MFC are major components) is known to negatively affect race performance, this particular cluster may be a good target for genetic selection to minimise OC. Not all traits in this cluster have been shown to affect race performance, so the most effective strategy may be to combine genetic selection with management of the environmental factors specifically to minimise stifle OC.

3.5.5. Homology between left and right joints

In this and other studies of OC and other skeletal lesions in horses, lesions occurring in homologous locations in the left and right joints are generally grouped together; for example, horses with OC lesions occurring at the DIRT in the left hock are pooled together with horses with OC lesions occurring in the DIRT in the right hock, and horses with lesions in the DIRT of both the left and right hocks are not differentiated from those with a lesion on just one side. In this study, the relationship between homologous joints in the left and right joints was explored by determining the phenotypic correlations between OC in the left and right fore fetlocks, hocks, and stifles, and the left joints and right joints overall. Correlations between EBVs were also estimated for OC in the left and right fore fetlocks and the left and right joints overall.

The grouping of homologous left and right joints in this population was supported by moderate to very high phenotypic correlations. Where correlations between EBVs could be estimated (i.e. in the left and right fore fetlocks and left and right joints overall), these also supported the grouping of homologous left and right joints. This finding was in agreement with the results presented for a Dutch Warmblood horse population, which found moderate phenotypic correlation between left and
right joints in the stifles and hocks (genetic correlation between OC in left and right joints was not investigated) (van Grevenhof, Ducro, and others 2009).

3.6. Conclusions

Within the sub-population of high-value Australasian Thoroughbred horses examined in the current study, breeding values contributed significantly to the occurrence of OC overall, to some OC component traits, and to FRAG occurring PPP1 in the hind fetlocks. From a genetic (if not clinical) perspective, OC is not a single disorder in this population, as lesions in the hocks and stifles are negatively correlated with one another, as are the SCL lesion type and lesion types that include bone fragments (i.e. OCD and FRAG). The current study also found that the FLAT lesion type should be included in the definition of OC, as it is positively correlated with the LYS, SCL and pooled OC lesion types.

Examination of correlations between EBVs for OC traits and other skeletal lesions indicate the presence of at least two groups of traits with positive EBV correlations. One group consisted of bone modelling (MOD) and spurs in the front knees, LYS and FLAT at the SRMC3 in the fore fetlocks (although under-reporting of lesions at SRMC3 is likely to have decreased estimates of correlation for these traits), FRAG at PPP1 in the hind fetlocks, MOD in the hocks, OCD at the MTRF, and SCL at the MFC in the stifles. This group includes multiple traits that have previously been shown to negatively affect race performance in Thoroughbred populations (e.g. OCD at the MTRF and FRAG at PPP1 in the hind fetlocks) and are among the most common traits in this population, and includes the most highly heritable traits in this study, making it an excellent target for genetic selection to minimise OC. Options such as scoring of severity, combined lesion scores or
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weighted lesion scores may also provide more powerful and accurate estimates of genetic status than the simple, binary traits examined here.

Three non-genetic factors or interactions that may be under the control of a stud manager (Stud, DOB × Region and Age × Region) were identified as affecting the prevalence of some OC, non-OC traits and OC super-set traits. The effects of these factors differed between different skeletal lesion traits, and not all factors were significant for all traits. Amongst the other non-genetic factors examined in this study, where Sex was significant, colts were always more likely to be affected than fillies. The diagnosing veterinarian (Vet) was not found to significantly affect the prevalence of any trait.

Based on identification of a group of economically important OC and related skeletal lesion traits with positive EBV correlations, it would be appropriate for future research to identify how selective breeding could be carried out on these traits in a cost-effective manner. Stud managers will need to prioritise particular traits, to reduce the chances that changes in husbandry (e.g. changes in the non-genetic factors affecting prevalence) may adversely affect the prevalence of these traits.

3.7. Acknowledgements

Sincere thanks are extended to the Australian Rural Industries Research and Development Corporation (RIRDC) for their financial support of the project, to the participating studs and veterinarians for providing their data, and to the statisticians in the Faculty of Veterinary Science for their vital input.
3.8. References


3.9. Supplementary material

Cells referring to OC traits at single anatomical sites have grey backgrounds; those referring to OC traits grouping multiple anatomical sites have heavy borders.

Lesion types: OCD – osteochondritis dissecans; LYS – marginal bone lysis; FRAG – bone chip(s) or fragment(s); SCL – subchondral cystic lesions; FLAT – flattened ridge; MOD – bone modelling; SES – sesamoiditis; Spurs – osteophytes or enthesiophytes; COLL – bone collapse or wedging; FRAC – fracture; EXS – exostosis; SC/PE – sclerosis or periostitis; OC – any of OCD, LYS, FRAG, or SCL; ALL – any lesion type.


Anatomical sites nested within CJ: ANY – any location within the carpal joints.

Nested within MCPJ: SRMC3 – sagittal ridge of the third metacarpal bone, PPP1 – proximal palmar/plantar to the first phalanx in the MCPJ, PDP1 – proximal dorsal to the first phalanx in the MCPJ, ANO – any location within the MCPJ not otherwise named.

Nested within MTPJ: SRMT3 – sagittal ridge of the third metatarsal bone, PPP1 – proximal palmar/plantar to the first phalanx in the MTPJ, PDP1 – proximal dorsal to the first phalanx in the MTPJ, ANO – any location within the MCPJ not otherwise named.

Nested within TJ: LTRT – lateral trochlear ridge of the talus, MTRT – medial trochlear ridge of the talus, DIRT – distal intermediate ridge of the tibia, MM – medial malleolus of the tibia, ANO – any location within the TJ not otherwise named, OC – any of LTRT, MTRT, DIRT or MM.

Nested within FTPJ: LTRF – lateral trochlear ridge of the femur, MTRF – medial trochlear ridge of the femur, MFC – medial femoral condyle, ANO - any location within the FTPJ not otherwise named, OC – any of LTRF, MTRF, or MFC.

Nested within ALL: OC – any of SMRC3, SRMT3, LTRT, MTRT, DIRT, MM, LTRF, MTRF, or MFC, ALL – any anatomical site.
Table S3.8: The results of GLMM analyses on skeletal lesion and injury traits occurring in 2% or more of the study population. P values are provided for fixed effects, covariates and covariates with random splines; z-ratios are provided for random effects. It is possible for the Vet and Stud variances to considerably increase the error variance of the heritability estimate, so the significance of the Horse factor is used as an indicator of the presence of significant additive genetic variation, rather than showing the standard error of heritability.

*: significance was calculated based on chi-squared; **: factor or interaction significantly affected prevalence for this trait.

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3.10. Appendix

The Materials and Methods section of Chapter 3 describes the data that were analysed. In fact, much more were collected but were not complete enough to be used.

Chapter 2 describes the Thoroughbred horse population analysed in this study: 1,004 yearlings and 299 weanlings, raised at seventeen collaborating studs. These horses were those that remained at the collaborating studs for the entire time from birth to sale; there were many more that moved to studs that weren't part of the study, and were lost from this research project. The total number of horses that were studied for at least part of their first year was actually 2,230, nearly twice the number that could be analysed at the end. While the loss of such a large proportion of the study population seems quite high, it's not really surprising. Many mares are boarded for the year at the stud of the stallion who serves them, so when they go to different stallions in successive years they generally move studs. One collaborating stud also shut down and liquidated stock while the project was going on.

Chapter 1 mentions that overfeeding is strongly associated with the occurrence of OC in horses, and that a protocol of overfeeding has been used to induce OC lesions in research horse populations. In light of this point, I personally assessed and recorded the body condition of each horse in the study at regular intervals (every six to eight weeks) from birth until sale time or its departure from the stud, as an indicator of their nutrition status. These data could have provided a fascinating addition to the by-stud variation demonstrated in Chapter 3 - can it be explained by overfeeding alone, or do other factors contribute too? Furthermore, confinement and the resultant lack of normal exercise is also associated with increased OC lesion severity, and anecdotally many vets associated with the study regarded confinement as a causative factor for OC lesions. Since each horse was seen
regularly, their locations were also recorded, for example that they were confined in a small yard due to illness, or were with others in a large paddock with free exercise and access to pasture.

Natural disasters interfered with data collection so that, in the end, these data on body condition and confinement were too patchy to be used. The first type of natural disaster was flooding, causing some collection visits to be missed in the geographical areas in Australia. The second disaster had far more impact: the Australian equine influenza (EI) outbreak. Complete travel restrictions on horses and property-by-property quarantine were used to control disease spread, so there was no travelling to see the horses in the project. Furthermore, I live about 1.5km from the Centennial Park stables where the outbreak was first diagnosed; this distance was within the range that EI was thought to be able to spread via aerosolised droplets on the wind. It would have been irresponsible to put myself in the position of being a possible vector for this disease.

Averaging the body condition data for each horse was considered as an option, to get indication of whether the horse was generally overweight or not. However, this didn't reflect the reality of the data: new-born foals are very consistent in condition, and they only start to differ from one another after a considerable period of time. The ages for which data were available would have a major impact on the average body condition for each horse and would have likely lead to erroneous conclusions.

Identifying each horse required the development of a shorthand describing their appearance: foal and dam colours, white markings and, if necessary, hair whorls (i.e. when the foal was dark brown with no white markings). This part was a little easier once the foals had been branded. For each visit to each stud, a data collection sheet was generated based on a web interface to the database created
Osteochondrosis and other common skeletal lesions in Australasian Thoroughbred yearlings: factors affecting prevalence, and genetic/phenotypic parameters

for this research project. These data collection sheets contained information I'd recorded previously regarding the stud itself and the names and expertise of stud staff, as well as a list of the horses seen previously at that stud with their physical description and age in days, with blank boxes to record body condition scores, locations, and notes. The notes field was used to record any noteworthy events, such as weaning or that X-rays had been carried out.

During the first stage of this research project, when studs were being recruited as collaborators, a short document was prepared describing the aim of the project and logistical aspects of how it was expected to play out. The following pages show this introductory document and an example data collection sheet.
Fig A3.5: An information sheet provided to studs participating in this research project. OC is referred to as 'OCD', since this is the name with which stud managers are familiar.
Equine OCD: Project Logistics

This RIRD-funded research project is investigating whether there is a genetic bias for equine osteochondrosis disseicans (OCD). The data collected during the project will also provide the opportunity to investigate whether any environmental management strategies (feed, exercise, or other management techniques) could be used to minimise rates of OCD in Australian and New Zealand Thoroughbred horses.

Heritability study

OCD in horses is thought to be a partially inherited disease, with both genetic and environmental contributing factors. This study aims to determine the relative contributions of genetic or environmental factors leading to OCD in Australian and New Zealand Thoroughbreds. In this project we will collect data regarding the pedigrees of all participating foals and any diagnoses that are made from survey or sale x-rays. We will also collect data on the environment and diet of participating foals.

Data will be collected by a researcher visiting the stud farm on a bi-monthly basis until participating foals are 12 months old.

The data collected on each visit will be:
1. The identity of any foals born, purchased, sold, or that have died since last visit
2. Where foals are being kept (boxes, or the size and type of paddock)
3. What the foals are eating (milk, pasture, supplements and hard feed)
4. Any weight or height measurements recorded by the farm
5. Whether the foal has been x-rayed (for surveys or sale)
6. The veterinarian’s diagnosis, based on the x-rays
7. The outcome of any treatment for OCD lesions

This data should be able to be collected using stud computer records and discussions with stud staff overseeing the daily management of the foals.

Experience with Australian studs suggests that the time required during each stud visit is about two hours per hundred foals per visit. The actual process will be negotiated with each stud farm depending on the stud’s own management processes.

Prior to the study, we will make a confidentiality agreement available to each stud. This agreement is to be signed by the stud and the researcher undertaking the work, Kao Castle. The agreement will protect both the identity of the studs who are participating in the project, and the identity of the individual horses.

Genotyping

The genotyping will be carried out on hair or blood samples. Samples would be taken when the foals have entered preparation for sale. Not all samples will necessarily be analysed.

Genotyping will be carried out in 2008 and 2009. The results of each genotyping analysis will be provided to the study if requested, but otherwise will remain confidential.

Maximising benefit to participating studs

Studs that participate in this study will have access to advice about both the environmental and genetic contributions to OCD in each of their own mares and foals. This information will be useful for revising husbandry techniques and strategies for buying healthy stock. The identity of all participating horses and participating studs will be protected with a bilateral confidentiality agreement with the University of Sydney, as would patient data in a medical trial. Participating in this research project will give studs a two-year advantage over non-participating competitors.

Fig A3.6: An information sheet describing project logistics provided to studs participating in this research project. OC is referred to as ‘OCD’ as this is how stud managers refer to the disorder.
4. Genome-wide association studies of skeletal lesion traits in Australasian Thoroughbred horses

4.1. Summary

*Reasons for performing study:* Genomic variation has not previously been reported for osteochondrosis (OC) or related traits in the Australasian Thoroughbred population.

*Objectives:* To identify genomic variation associated with the occurrence of OC overall, OC at specific anatomical sites, specific OC lesion types such as osteochondritis dissecans (OCD), and bone chip(s) or fragment(s) (FRAG) occurring proximal palmar/plantar to the first phalanx (PPP1) in the hind fetlocks, in a population of Australasian Thoroughbred horses.

*Methods:* Single Nucleotide Polymorphism (SNP)-based case-control genome-wide association studies (GWAS) were carried out for 11 OC traits, for a related trait, namely FRAG occurring PPP1 in the hind fetlocks, and for the chestnut coat colour as a positive control using the Illumina Equine SNP50 beadchip, in a sample of 140 Australasian Thoroughbred horses.

*Results:* A check for population stratification identified one large cluster comprising the majority of the population and two small outlier clusters, each comprising the offspring of a single Australian-born Thoroughbred stallion. Despite only a small number of cases being available for these analyses, genome-wide significant quantitative trait loci (QTLs) were found on chromosome 30 for lysis (LYS) at the sagittal ridge of the third metacarpal bone (SRMC3) in the fore fetlocks (within the large cluster only), and on chromosome 3 for the positive control chestnut coat colour (within all three clusters of this population, and within the large cluster only). The significance of the QTL for LYS at the SRMC3 was not as high when all three clusters were included in the analysis. QTLs that did not reach genome-wide significance level were identified for other OC traits, and for FRAG occurring PPP1 in the hind fetlocks.

*Conclusions:* The identification of a genome-wide significant QTL associated with an OC trait suggests that genotyping and genetic selection may be able to play a role in the control of OC in this population. Genotyping additional horses to enhance statistical power would allow validation of QTLs for OC traits, and FRAG occurring PPP1 in the hind fetlocks. The genomic homogeneity of the majority of the Australasian Thoroughbred population supports GWAS, even for complex traits such as OC.
Potential relevance: SNP-based case-control GWAS may be capable of identifying genomic variation associated with complex traits in the Thoroughbred horse breed, using low numbers of horses.
4.2. Introduction

Osteochondrosis (OC) has been shown to have a genetic component in horses. Heritability estimates for OC traits have ranged from approximately 0.1 to 0.5, varying by anatomical site and horse breed (for example Chapter 3; Philipsson and others 1993; Grondahl and Dolvik 1993; Pieramati and others 2003; Wittwer and others 2007a; van Grevenhof and others 2009). In the current study, heritability estimates ranged from 0.0 to 0.22 with a median value of 0.11 (see Chapter 3).

Several genome scans have been carried out to identify genomic sequence variants associated with OC traits in various horse breeds, using microsatellite markers and, more recently, single nucleotide polymorphisms (SNPs) on the Illumina Equine SNP50 beadchip. A recent study of Thoroughbred horses raised in Kentucky, USA, identified a region on Equus caballus chromosome (ECA) 3 associated with osteochondritis dissecans (OCD, the severe end-stage form of OC) in one or more of the fore fetlock, hind fetlock, hock, stifle and shoulder joints (Corbin and others 2012). Quantitative trait loci (QTLs) previously identified in other horse breeds (see below) were examined in the same population of USA-raised Thoroughbreds, and SNPs on ECA 4 and ECA 18 were also found to be significantly associated with OCD.

Microsatellite markers were used in a genome-wide linkage analysis of a Hanoverian Warmblood horse population to identify genome-wide significant QTLs for OC occurring at one or more anatomical sites in the fetlock or hock on ECA 2, 4, 5, 16, and 18 (Dierks and others 2007, Lampe and others 2009, Dierks and others 2010). A follow-up study of other Hanoverian Warmblood horses using the Illumina Equine SNP50 BeadChip (McCue and others 2012) confirmed QTLs on ECA 5 for OCD of the sagittal ridge of the third metacarpal bone (SRMC3) and/or sagittal ridge of
the third metatarsal bone (SRMT3) in the fore and hind fetlocks, and on ECA 16 and 21 for OCD in the hock, where lesions occurred at the distal intermediate ridge of the tibia (DIRT), the lateral trochlear ridge of the talus (LTRT) or the medial malleolus (MM) (Lampe 2009). Further studies in this breed identified a large number of QTLs on a range of chromosomes (ECA 1, 2, 5, 8, 9, 10, 12, 14, 16, 18, 20, 23 and 26) for various fetlock and hock OC traits (Distl and others 2012). These QTLs did not reach genome-wide significance.

In a South German Coldblood population, microsatellite markers were used in a linkage analysis to identify genome-wide significant QTLs on ECA 23 for OC occurring at one or more anatomical sites in the fetlock or hock, and on ECA 4 and 18 for OCD lesions occurring at the SRMC3 and/or SRMT3 in the fore and hind fetlocks (C. Wittwer, K. Löhring, C. Drögemüller, H. Hamann, and others 2007). The Illumina Equine SNP50 beadchip was used in a genome-wide association study (GWAS) to find genome-wide significant QTLs on ECA 5, 10, 27 and 28 for OCD at the DIRT in the hock in a population of Norwegian Standardbred trotters (S. Lykkjen and others 2010).

Despite the range of QTLs identified in these horse breeds, overlapping QTLs are relatively rare. So far, regions identified in more than one horse breed are on ECA 18 at around 36-39 Mb and ECA 4 at around 39Mb in Hanoverian Warmblood horses (for OC occurring at one or more anatomical sites in the fetlocks or hocks) and Thoroughbred horses (for OCD in one or more of a range of limb joints). The rarity of QTL validation between breeds may reflect one or more of the following issues: genuine differences in the underlying genetic contributions to OC in different horse breeds; differences in the anatomical distribution of lesions between breeds that can make comparison between breeds challenging; errors resulting from the unbalanced picking of affected
and unaffected individuals from genetically stratified populations; and the low power of these studies.

Candidate genes with a range of functions have been suggested based on the positions of QTLs identified in these studies. These candidates are involved in a variety of biological pathways, including response to loss of blood supply and stress, cellular differentiation, and cell signalling, and include structural molecules such as collagens. The diversity of these biological pathways reflect current thinking on the aetiopathogenesis of OC (Ytrehus and others 2007, Olstad and others 2008). However, none of these candidate genes has yet been confirmed as playing a role in the occurrence of OC.

Bone chip(s) or fragment(s) (FRAG) occurring proximal palmar/plantar to the first phalanx (PPP1) in the hind fetlocks are not thought to be OC, but occur at high frequency in the Thoroughbred horse population and some other horse breeds (Wittwer and others 2006, Jackson and others 2009, Preston and others 2010). Analysis in the Australasian Thoroughbred population showed a positive genetic association between these bone fragments and OC in the stifles and fetlocks (Chapter 3). A linkage analysis using microsatellite markers was carried out for FRAG occurring PPP1 in the front and hind fetlocks in South German Coldblood horses, where significant QTLs were located on ECA 1, 4, 8, 12 and 18 (C. Wittwer, K. Löhring, C. Drögemüller, H. Hamann, and others 2007).

SNP genotypes can serve as a data source for genomic testing and selection technologies, and the discovery of significant QTLs can help provide insight into the molecular pathways involved in disease pathogenesis. A technology called genomic selection utilises all available SNP genotypes, combined with phenotype and relationship data from pedigrees or the SNPs themselves, to produce
Genome-wide association studies of skeletal lesion traits in Australasian Thoroughbred horses

Genomic Estimated Breeding Values (GEBVs) to guide both disease prediction in non-breeding horses and genetic selection in the breeding population (Goddard and Hayes 2009). Genetic testing would be especially valuable for horses where the disease status of relatives is generally unknown, for example those imported from overseas.

In this study, Australasian Thoroughbred horses were genotyped using the Illumina Equine SNP50 beadchip. Analyses were carried out to identify genetic variation associated with several OC traits including OC overall, OC at specific anatomical sites, and specific OC lesion types; with FRAG occurring PPP1 in the hind fetlocks; and with the chestnut coat colour as a positive control. The discovery of QTLs in this study of Thoroughbred horses can be compared with those identified in other horse breeds, especially those with Thoroughbred admixture.

4.3. Methods

Tissue samples were obtained from 350 Australasian Thoroughbred horses with veterinary diagnostic reports describing skeletal lesions identified on weanling survey and/or yearling pre-sale radiographs, from the population described in Chapter 2. Diagnoses from weanling radiographs provide a very strong indication of yearling status: see also Chapter 2. Of the 350 tissue samples, 92 were whole blood, and 258 were mane hair samples. Ethical approval for the collection of these samples was granted by the University of Sydney Animal Ethics Committee (AEC reference number N00/7-2007/1/4667). The identity of all horses and studs participating in this research is protected by non-disclosure agreements.
4.3.1. Experimental design

Each analysis was carried out as a case-control GWAS; that is, each analysis compared allele frequencies between cases and controls, for each SNP that passed the quality control filters described below. GWAS carried out on case-control data sets such as these are susceptible to bias and confounding errors that result from the unbalanced selection of affected and unaffected individuals from genetically stratified populations. In such cases, differences in allele frequencies between groups of cases and controls reflect ancestry as well as any genetic variation that is truly associated with the trait of interest.

In this study, stratification was minimised by ensuring that cases and controls were drawn from the same genetic population(s): each affected horse was matched with one or more unaffected half-siblings or double cousins (where the sire of the unaffected horse was a half-sibling to one parent of the affected horse, and the dam was a half-sibling to the other parent), thus ensuring a high level of genetic similarity between each case group and control group (Boehnke and Langefeld 1998). Random selection of cases and controls was not an appropriate strategy because the high average co-ancestry of the population negated the assumption that individuals were unrelated (see Chapter 5). Family-based association analyses were not considered due to the absence of any parental genotypes. The experimental design was updated to utilise the entire pool of controls following an analysis for stratification within the population that could only be carried out subsequent to genotyping, described in the Results section of this chapter.

Eleven OC traits were identified where DNA samples were available for at least 10 cases and their controls. Sufficient samples (10 cases and their matched controls) were also available to analyse FRAG occurring PPP1 in the hind fetlocks and the positive control, the chestnut coat colour. With
the exception of the chestnut coat colour, each trait was as defined in Chapter 2. A total of 140 horses were selected for genotyping, consisting of 72 cases for various skeletal lesion traits, and 68 controls. Coat colour phenotypes were based on physical descriptions obtained during data collection (see Chapter 3).

Some radiographic abnormalities were accepted in control horses because they were not considered to be associated with the traits of interest. These were: fractures, collapse or wedging of the tarsal bones, exostoses, and periostitis. Osteophytes, enthesiophytes and sclerosis were also accepted in control animals, where they were reported as occurring at sites other than those considered for the traits under analysis.

4.3.2. DNA extraction and genotyping

DNA was extracted from all mane hair root and blood samples using QIAGEN DNeasy® Blood and Tissue kits. A user-developed protocol provided by QIAGEN was used to extract DNA from both blood (specifically the isolated nucleated cells, or 'buffy coat') and hair samples. The use of dithiothreitol (DTT) at the lysis stage reduced the time required for lysis from > 24 hours to 60 minutes (QIAGEN 2006). DNA extraction from hair used 15 to 30 mane hair roots, depending on the coarseness of the hair and size of the root bulb. The successful isolation of high quality DNA was verified by the presence of a strong band of ~30 kilobase fragments in an agarose gel for each sample. Samples were standardised at a concentration of 50 ng µL⁻¹ DNA in QIAGEN-supplied AE buffer (10mM Tris-Cl and 0.5mM EDTA) following isopropanol precipitation. Sample concentration was determined using a fluorometer with PicoGreen dye and the standard protocol for concentrated samples provided by the vendor.
Two service-provider laboratories carried out genotyping of the DNA samples using the Illumina Equine SNP50 beadchip, one receiving 48 samples and the other 96. Two of the samples sent to each service provider were from duplicate pairs (referred to henceforward as between-laboratory duplicates). Two additional duplicates were included in the 96-sample batch, providing within-laboratory duplicates for comparison. A total of 140 horses were genotyped.

4.3.3. Quality control and genome-wide association analysis

A check was carried out on all genotyped horses for sex discrepancies via X-chromosome inbreeding coefficients (F) using the whole-genome data analysis software PLINK (Purcell and others 2007). Samples with intermediate PLINK X-chromosome F (default threshold values are > 0.2, < 0.8) indicate indeterminate sex, as males with a single X chromosome are expected to be completely 'homozygous' (excluding genotyping or other errors), and females with two X chromosomes are expected to show heterozygosity in line with X-chromosome variation within the population. Some horses had intermediate PLINK X-chromosome F with values near the threshold of 0.2; however in all cases these were female horses with high inbreeding coefficients based on pedigree data. No samples were excluded on this basis of the check. The basis for the threshold of 0.2 is not explained in either Purcell and others (2007) or Purcell (2009).

Analyses were carried out with a minimum minor allele frequency (MAF) of 0.175, a maximum missing rate per-SNP of 0.100, a maximum missing rate per individual of 0.200, and a Hardy-Weinberg equilibrium p-value (exact) filter of 0.001. The maximum missing rate per individual was increased to 0.200 from the default 0.100 to include genotypes from samples with low genotyping rates due to their chip position (see Results), while the maximum missing rate per-SNP was kept at the default 0.100 to exclude SNPs with poor genotyping rates overall. The minimum MAF was
increased from 0.01 to 0.175 to facilitate these analyses by reducing the number of SNPs. This change in MAF is unlikely to have a large effect on the results of these analyses because there is little power to detect the effect of rare SNPs in small sample sizes such as those in the current study (Spencer and others 2009).

Table 4.1 shows the number of cases and controls, the number of SNPs removed from the full set of 54,602 SNPs by the MAF filter and the total number of SNPs remaining in each analysis. No SNPs fell below the default maximum missing rate per-SNP of 0.100. The number of SNPs removed due to the Hardy-Weinberg equilibrium filter was 166 for OC traits and FRAG occurring PPP1 in the hind fetlocks where cases were found in all three population clusters (see below), 142 for sub-chondral cystic lesions (SCL) in the medial femoral condyles (MFC) in the stifles, and 224 for the chestnut coat colour trait.
Table 4.1: The number of cases and controls; SNPs removed by the MAF filter; and total SNPs analysed for OC traits, FRAG occurring PPP1 in the hind fetlocks, and chestnut coat colour.

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<td>23,422</td>
<td>29,849</td>
</tr>
<tr>
<td>Marginal bone lysis</td>
<td>Sagittal ridge of the third metacarpal bone</td>
<td>15 : 68</td>
<td>23,448</td>
<td>29,660</td>
</tr>
<tr>
<td>SCL</td>
<td>Medial femoral condyle</td>
<td>10 : 64</td>
<td>23,299</td>
<td>29,866</td>
</tr>
<tr>
<td>FRAG</td>
<td>Proximal palmar/plantar P1, metatarsophalangeal joint</td>
<td>16 : 68</td>
<td>23,472</td>
<td>29,636</td>
</tr>
<tr>
<td>Chestnut coat colour</td>
<td></td>
<td>29 : 72</td>
<td>23,243</td>
<td>30,245</td>
</tr>
</tbody>
</table>

Case-control association analyses for 11 OC traits, FRAG occurring PPP1 in the hind fetlocks, and the chestnut coat colour were carried out in PLINK using a Cochran-Mantel-Haenszel test for 2x2xK (where K = 3 is the number of clusters identified in an identical-by-state stratification analysis as per 'Population stratification' section below, and each 2x2 is a contingency table with the null hypothesis that case-control status is independent of genotype) tables and within-cluster permutation, to account for stratification between population clusters (Purcell and others 2007, Purcell 2009). Each analysis used all available controls in the clusters where cases occurred. The OC trait SCL in the MFC in the stifles and the chestnut coat colour had cases and controls in two
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clusters; all other traits had cases and controls in all three clusters. Analysis of the chestnut coat colour excluded horses with coat colours obscuring their chestnut status, such as grey. The chestnut coat colour trait is a recessive trait controlled by a single gene \( MC1R \) (Marklund and others 1996).

Empirical estimates of the significance were obtained based on 100,000 label-swapping permutations, where new data sets were created with case or control status randomly reassigned within each cluster. The \(-\log_{10}(p\text{ value based on }100K\text{ permutations})\) of a SNP, henceforward described as a score, is a statistic where values over 1.30 indicate genome-wide significance \((p < 0.05)\).

The genomic inflation factor was calculated for each trait. The genomic inflation factor quantifies the maximum inflation of significance that would result from the unbalanced selection of affected and unaffected individuals in a genetically stratified population (Devlin and Roeder 1999).

An attempt was made to estimate the phenotypic variance explained by all SNPs concurrently in the OC trait marginal bone lysis (LYS) at SRMC3, using the software GCTA (Yang and others 2011). The exclusion of closely related individuals, suggested in order to minimise the possibilities of genetic stratification and/or confounding with environmental effects, left too few individuals for a creditable analysis.
4.4. Results

4.4.1. Genotyping quality control

Table 4.2 shows summary statistics for within- and between-laboratory duplicate samples. One within-laboratory duplicate, with a sample run in position L, was found to have a high number of missing genotypes. Subsequent examination of other samples run in position L at the same laboratory showed consistently low genotyping rates for these samples. The proportion of bases called differently between the duplicates was not abnormal for the sample in position L, informing the GWAS strategy to include individuals with low genotyping rates while excluding SNPs with low genotyping rates. The average call rate for all 144 samples, including those with low genotyping rates, was high, at 97.6%.

Table 4.2: The number (%) of genotype calls differing in either sample because they were missing or called differently for within-laboratory and between-laboratory duplicates. * = sample was run in position L.

<table>
<thead>
<tr>
<th>Duplicate pair</th>
<th>Number (%) with one base missing</th>
<th>Number (%) with both bases missing</th>
<th>Number (%) of bases called differently</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Within-laboratory 1</td>
<td>401 (0.70%)</td>
<td>3283 (6.0%)</td>
<td>88 (0.16%)</td>
</tr>
<tr>
<td>Within-laboratory 2</td>
<td>27 (0.05%)</td>
<td>767 (1.4%)</td>
<td>110 (0.20%)</td>
</tr>
<tr>
<td>Between-laboratory 1</td>
<td>19 (0.03%)</td>
<td>1098 (2.0%)</td>
<td>16 (0.03%)</td>
</tr>
<tr>
<td>Between-laboratory 2</td>
<td>21 (0.04%)</td>
<td>1258 (2.0%)</td>
<td>11 (0.02%)</td>
</tr>
</tbody>
</table>

4.4.2. Population stratification

Fig 4.1 illustrates a check for population stratification carried out on the SNP genotypes using a two-dimensional scaling analysis of the NxN matrix of genome-wide identical-by-state (IBS)
Genome-wide association studies of skeletal lesion traits in Australasian Thoroughbred horses

pairwise distances (where \( N = 140 \), the number of genotyped horses) (Purcell and others 2007, Purcell 2009). It shows one large cluster of individuals representing the majority of the population and two small outlier clusters containing 13 and 15 individuals respectively. Each small cluster of genomic outliers comprised all the offspring of a single Australian-born Thoroughbred stallion. The genomic homogeneity of the horses within each cluster indicated that the GWAS could use all controls in each cluster where cases were present, thereby increasing the power of the analyses.

While the three clusters in this plot appear to be clearly separated, the process used to produce this plot amplifies any differences between sub-populations to fill the space available. If these horses were to be compared to horses from another breed, it is likely that they would be very closely grouped. The analysis excludes any SNPs that are not polymorphic, in this instance excluding SNPs that are monomorphic in the Thoroughbred breed but polymorphic in other horse breeds. In order to examine how different the two sires of the individual outlier clusters were to the remainder of the sire population, the average co-ancestry of each of these two sires and all other Australian sires with offspring born in 2005 was calculated in Chapter 5. The analysis showed similar levels of average co-ancestry between the sires of the two outlier groups and the remainder of the sire population, and within the remainder of the sire population itself, indicating that these sires do not have significantly different ancestry to sires of horses in the main cluster.
4.4.3. **Quantitative trait loci for skeletal lesions including osteochondrosis**

Table 4.3 summarises QTLs found by GWAS carried out for eleven OC traits, FRAG occurring PPP1 in the hind fetlocks, and the chestnut coat colour. QTLs are shown where the highest SNP score was greater than 0.10 and more than 1 SNP was involved in the QTL, thereby including QTLs reaching genome-wide significance (indicated by a maximum score of 1.30 or greater), and many that did not reach this threshold. QTLs below the genome-wide significance threshold were included for comparison with other studies. The genomic inflation factor (based on median chi-
squared) for each trait, and the location, raw P value for the peak SNP(s), and maximum score (based on permuted P value) are shown for each QTL. A genome-wide significant QTL was found for the positive control (chestnut coat colour), and the strongest QTL for OC traits was on ECA30 for LYS at the SRMC3 in the fore fetlocks (based on the highest score from permuted P values).
Table 4.3: Summary of QTLs above and below the significance threshold for OC traits, FRAG occurring PPP1 in the hind fetlocks, and the chestnut coat colour.

<table>
<thead>
<tr>
<th>Lesion type</th>
<th>Site</th>
<th>Genomic inflation</th>
<th>QTL chromosome: location(^8) (no. of SNP)</th>
<th>Raw P value</th>
<th>Score (from permuted P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>Any OC site</td>
<td>1.00</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>OC</td>
<td>Any hock OC site</td>
<td>1.05</td>
<td>ECA13: 11.0-12.2 (3) ECA8: 89.5-92.6 (4) ECA15: 72.1-75.3 (12)</td>
<td>1.10x10(^{-4}) 1.17x10(^{-4}) 1.60x10(^{-4})</td>
<td>0.18 0.17 0.12</td>
</tr>
<tr>
<td>OC</td>
<td>Any stifle OC site</td>
<td>1.00</td>
<td>ECA20: 56.6-57.4 (4) ECA26: 30.8-30.9 (3)</td>
<td>4.41x10(^{-5}) 1.20x10(^{-4})</td>
<td>0.48 0.18</td>
</tr>
<tr>
<td>OC</td>
<td>Distal intermediate ridge of the tibia</td>
<td>1.00</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>OC</td>
<td>Lateral trochlear ridge of the distal femur</td>
<td>1.00</td>
<td>ECA23: 7.4-13.0 (17)</td>
<td>3.37x10(^{-5})</td>
<td>0.52</td>
</tr>
<tr>
<td>OC</td>
<td>Medial femoral condyle</td>
<td>1.04</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>OCD</td>
<td>Any OC site</td>
<td>1.00</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>OCD</td>
<td>Lateral trochlear ridge of the distal femur</td>
<td>1.00</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LYS</td>
<td>Any OC site</td>
<td>1.02</td>
<td>ECA3: 28.7-29.5 (7) ECA30: 27.7-28.1 (10)</td>
<td>5.57x10(^{-5}) 1.01x10(^{-4})</td>
<td>0.41 0.23</td>
</tr>
<tr>
<td>LYS</td>
<td>Sagittal ridge of the third metacarpal bone</td>
<td>1.00</td>
<td>ECA30: 26.6-28.1 (8) ECA30: 10.4-10.4 (2)</td>
<td>1.49x10(^{-5}) 1.02x10(^{-4})</td>
<td>0.84 0.23</td>
</tr>
<tr>
<td>SCL</td>
<td>Medial femoral condyle</td>
<td>1.04</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>FRAG</td>
<td>Proximal palmar/plantar P1, metatarsophalangeal joint</td>
<td>1.05</td>
<td>ECA4: 88.8-89.0 (3)</td>
<td>8.29x10(^{-5})</td>
<td>0.26</td>
</tr>
<tr>
<td>Chestnut coat colour</td>
<td>1.57</td>
<td>ECA3: 26.6-49.0 (83) 13 other chromosomes to a maximum score of 1.59</td>
<td>1.06x10(^{-18})</td>
<td>5.00</td>
<td></td>
</tr>
</tbody>
</table>

---

\(^8\) Location is given with respect to the horse genome assembly EquCab2.0.
Fig 4.2 plots the nucleotide position of all examined SNPs on ECA 30 against their scores for the trait LYS at SRMC3 in the fore fetlocks. The QTL containing the highest scoring SNP, magnified for clarity in the figure, covers a region of approximately 1.5 Mb from 26.6-28.1 Mb, and peaked at BIEC2-828423, occurring at nucleotide position 27,985,647. Amongst the 15 cases 7 were homozygous G/G, 4 were heterozygous G/A and three were homozygous A/A at this SNP, while amongst the 68 controls 3 were homozygous G/G, 23 were heterozygous G/A, and 42 were homozygous A/A. Examination of this region showed five haplotypes for 12 SNPs covering this QTL and the region immediately downstream of it, and indicated that the most prevalent haplotype was associated with the absence of this trait. This haplotype comprised 33% of those seen in cases, and 71% of those seen in controls.
The distribution of haplotypes differed widely between the three population clusters. It was apparent that one sire represented by a small cluster was heterozygous for two haplotypes that were otherwise rare in this population, while the other was homozygous for the most prevalent haplotype. In both cases the frequency of the most prevalent haplotype in these clusters was altered so that there was little power to associate this haplotype with the absence (or indeed presence) of this trait within these clusters.

Fig 4.3 plots the nucleotide position of all examined SNPs on ECA30 against their scores for the trait LYS at SRMC3 in the fore fetlocks, where the analysis was carried out within the large cluster in this population. This cluster included 9 cases and 57 controls. The QTL within this cluster reached the genome-wide significance threshold, with the peak SNP BIEC2-828366 at nucleotide position 27,729,561 having a score of 1.33. Amongst the nine cases, six were homozygous G/G, three were heterozygous G/A, and one was homozygous A/A at this SNP, while amongst the 57 controls three were homozygous G/G, 19 were heterozygous G/A, 34 were homozygous A/A and one horse was missing genotypes.

This QTL consisted of the same SNPs found to be significantly associated with LYS at SRMC3 when all three population clusters were considered. The highest ranking SNP in the analysis of all three population clusters was found to be slightly less significant here, while the significance of five of the six SNPs upstream of this peak had increased.
Fig 4.3: The distribution and score (-log10[p value, 100K permutations]) of SNPs on ECA 30 showing association with LYS at the SRMC3 in the fore fetlocks, within the large population cluster.

Fig 4.4 shows quantile-quantile (Q-Q) plots ranking SNP significance for LYS at SRMC3 within the large cluster in this population against its expected values, given the assumption that the values have been obtained from a chi-squared distribution with one degree of freedom (the expected distribution under the null hypothesis). Deviation from the line of equality, shown as a dashed line, indicates the presence of true association between some SNP genotypes and the trait of interest. The distribution for LYS at SRMC3 supports the presence of one or more real QTLs comprising several SNPs, and the SNPs that deviate from the line of equality are those that comprise the QTL seen in Fig 4.3.
The highest scoring QTL for FRAG occurring PPP1 in the hind fetlocks was found on ECA4 but did not reach genome-wide significance. The highest scoring SNP at this QTL was BIEC2-872986, occurring at nucleotide position 88,756,341.

The highest scoring QTL for the chestnut coat colour was found on ECA 3. This QTL covered a region from 26.6-49.0 Mb, and the peak spanned from approximately 33Mb to 41Mb. The gene that is known to control this coat colour is $MC1R$, located within the peak of the QTL at approximately 36Mb. Chestnut cases in the main population cluster were heavily skewed toward one side of that cluster when plotted on the two-dimensional scaling analysis of the $NxN$ matrix of genome-wide IBS pairwise distances (not shown).
In light of the differences between the three population groups (highlighted by the wide variation in haplotype frequencies found at the QTL for LYS at SRMC3) further GWAS were carried out for the remaining traits within the main population cluster only. No additional genome-wide significant QTLs were found for skeletal lesion traits in these analyses. The highest scoring QTL for the chestnut coat colour was the same as that found when all three population groups were included in the analysis, and covered the same region of ECA 3. There were 26 cases and 59 controls in this analysis of the chestnut coat colour, and the genomic inflation factor increased slightly from 1.57 to 1.67.

4.5. Discussion

4.5.1. Population stratification

The check for population stratification revealed three distinct clusters (one large, two small) among the 140 horses in this study. The large cluster comprised the offspring of a number of stallions originating from Australia, New Zealand, the USA, and Europe. Each of the small clusters comprised all the offspring of a single Australian stallion. Future studies in this population should take into account the presence of some genomic diversity within the sub-population of high-value Australasian Thoroughbred horses used in the current study, and therefore also within the entire Australian and New Zealand Thoroughbred horses populations. Overall, the majority of Thoroughbred horses produced in Australia and New Zealand are one single genetic population, despite the diversity of geographic origins of the stallions and mares that produced these foals. Findings from this population may be applicable to Thoroughbred horses in the USA and Europe as well as Australia and New Zealand.
4.5.2. **Quantitative trait loci for skeletal lesions**

In the current study, population stratification was addressed by carrying out within-cluster analyses and permutation to obtain empirical estimates of significance for each trait. Since this analysis involved doing three sets (two in the case of the SCL at the MFC and chestnut traits) of 2x2 calculations, it is very similar to allocating two (or one) degrees of freedom for population stratification. This contrasts with the methodology carried out on samples from USA-raised Thoroughbred horses, described in Corbin and others (2011), that utilised a mixed linear model to control for population structure as well as non-genetic factors such as the presence of other skeletal lesions, and differences in husbandry methods between horse studs. A benefit of the method used in the current study is a relative increase in power, as there are fewer degrees of freedom in the analyses. Strengths of mixed linear models, such as the ability to adjust for other causes of variation and to take into account pedigree relatedness, either could not be utilised for the data set in the current study or were accounted for in other ways. Population stratification was compensated for adequately, as indicated by the lack of genomic inflation seen for traits other than the positive control chestnut coat colour. The genomic inflation factor for the chestnut coat colour was low (1.57) but higher than that seen for the skeletal lesion traits. This reflects the presence of some population stratification which likely occurred because matched cases and controls for this trait were not a consideration when horses were selected for genotyping. Non-genetic factors previously identified as having a significant effect on the skeletal lesion traits analysed here (see Chapter 3) were not addressed, as these factors had too many levels among the small number of cases examined in these analyses. The presence of skeletal lesions other than OC that may have positive genetic correlations with OC, which was a complicating factor for the USA-based cohort, was prevented by rigorous exclusion of these particular lesions in the control population.
A range of OC traits were examined, including OC overall, OC in the hocks, OC in the stifles, specific lesion types occurring at one or more anatomical sites (for example OCD occurring at any OC site), and OC or specific OC lesion types occurring at a single anatomical site (for example OC at the DIRT in the hocks). Small QTLs that did not reach the genome-wide significance threshold were discovered for many traits. Within the main population cluster, a single QTL for LYS at SRMC3 in the front fetlocks reached genome-wide significance and is discussed below. While lesions at SRMC3 were under-reported in this study population, severe lesions are likely to be correctly reported, indicating that the horses identified as affected for this trait may be more likely to have severe lesions than horses identified as affected for other traits (see Chapter 2 and Chapter 3). Genotyping additional horses may validate the smaller QTLs, and clarify the significance of the single QTL for LYS at SRMC3 in horses with lesions of various severities.

Some OC traits included multiple lesion types (e.g. OC includes both SCL and FRAG) or multiple lesion sites (e.g. OC overall), and in these instances the likelihood of QTL discovery was probably decreased by grouping together lesion types or sites with negative genetic associations, estimated as correlations between Estimated Breeding Values (EBVs). Chapter 3 showed both positive and negative EBV correlations between OC lesions occurring at different anatomical sites, and between different OC lesion types.

Despite the small number of cases available for these analyses, genome-wide significant QTLs were found for LYS at SRMC3 in the front fetlocks (within the large cluster of this population), and the positive control chestnut coat colour (within all three clusters of this population, and within the large cluster only). The identification of a genome-wide significant QTL for LYS at SRMC3 with a score of 1.33, just over the significance threshold of 1.30, apparent only in the main population
cluster highlights the large effects that can result from genomic variation between population sub-
groups, even when stratification is addressed in the analysis strategy.

Centrally located within the genome-wide significant QTL for LYS at SRMC3 is one known equine gene: the nuclear receptor subfamily 5, group A, member 2 (NR5A2) gene coding sequence, a constitutively active DNA-binding transcription factor covering approximately 130 kilobases (Ohno and others 2010). This gene has been shown to be involved in several biological pathways in different tissues, and at different developmental stages (Lee and Moore 2008). In zebrafish, blocking translation of the NR5A2 homologue (the ff1a gene) has been shown to prevent cartilage formation (Koskinen and others 2009). In humans, it was recently identified as a gene of interest in a GWAS focussing on osteoporosis (Zhang and others 2010). It is plausible that this gene could play a role in bone and cartilage development, or the inflammatory response, and thus play a role in the formation of OC lesions in horses.

The homologous region of the mouse genome contains five additional genes known to be active in one or more skeletal, angiogenesis, or growth pathways: PTPRV (protein tyrosine phosphatase, receptor type, V), ELF 3 (E74-like transcription factor 3), CSRPI (cysteine-rich protein), Tnnt2 (troponin T2, cardiac), and Cacna1s (calcium channel, voltage dependent, alpha 1S subunit). Among these genes PTPRV and ELF3 stand out as functional candidate genes. PTPRV is expressed in osteoblasts (bone cells that play a major role in the bone growth process), and has been shown to regulate energy metabolism in the skeleton (Lee and others 2007). PTPRV -/- mice show no skeletal abnormalities, which is in keeping with the normal skeletal morphology demonstrated by horses with OC (Dacquin and others 2004). ELF3 controls transcription of matrix metalloproteinase 13 (MMP13, essential to cartilage remodelling during bone growth due to its ability to cleave type II
collagens), and interacts with interleukin 1β, an indicator of inflammatory stress (Otero and others 2011).

In other horse breeds where genome scans have been carried out for fetlock OC traits, QTLs have been identified on ECA 18 (fetlock only), and ECA 2, 18 and 23 (fetlock and/or hock) (C. Wittwer, K. Löhring, C. Drögemüller, H. Hamann, and others 2007, Lampe 2009, Dierks and others 2010). No QTLs were identified on these chromosomes for fetlock OC in this study, although a QTL at a different location on ECA 23 (7.4-13Mb in the current study vs. 37.4Mb) was found for OC at the lateral trochlear ridge of the distal femur (LTRF) in the stifles. The number of horses examined in the present study may be too small to allow the identification of QTLs in these locations with small effects in the Thoroughbred breed. Alternatively, this may reflect genuine differences in the genetic basis of OC lesions at this location in different horse breeds, or in different environments, since there is a substantial proportion of Thoroughbred lineage in Hanoverian Warmblood horses (Hamann and Distl 2008).

The recent study of Thoroughbred horses from Kentucky, USA, that examined OCD occurring in one or more of the fore fetlocks, hind fetlocks, hocks, stifles and shoulders found a single significant SNP on ECA 3 (Corbin and others 2012). The current study of Australasian Thoroughbred horses found no QTLs were identified for the equivalent trait (OCD at one or more of the same joints, excluding the shoulders, which represented just 0.6% of the USA-born cohort). In addition, no QTLs were identified on ECA 3 for other OC traits. The absence of validation may reflect genuine genetic differences between the two populations, particularly if the Kentucky cohort included dirt-racing Thoroughbred horses (these are not used in Australia and New Zealand).
In the recent study of Norwegian Standardbred horses that focussed on OC lesions occurring at the DIRT, QTLs were identified on ECA 5, 10, 27 and 28 (Sigrid Lykkjen and others 2010). In the current study, no QTLs were identified for this trait.

The QTL for FRAG occurring PPP1 in the hind fetlocks, although not reaching genome-wide significance, occurred on the same chromosome as one of the QTLs identified for a similar trait (FRAG occurring PPP1 in both the front and hind fetlocks) in South German Coldblood horses (C. Wittwer, K. Löhring, C. Drögemüller, H. Hamann, and others 2007). However, on further examination these QTLs did not overlap; the South German Coldblood QTL was located around 33Mb, while the Thoroughbred QTL was located at 89Mb. Despite the lack of overlap between these QTLs, this trait is both highly prevalent and has the highest heritability estimate (0.18, see Chapter 3) of the traits examined in the GWAS analyses of the current study, and it would be valuable to carry out SNP genotyping of additional horses to determine whether this QTL contains genetic variation that contributes significantly to this trait.

Selection against OC in Thoroughbred horses has the potential to reduce the prevalence of this disorder, but would not appeal to horse breeders if the selection process could negatively affect athletic performance. A recent study on positive selection in the Thoroughbred horse genome found the most significant indicators of either positive or balancing selection occurred on ECA 4, 5, 6, 11, 17, 18, 25 and 27 (Gu and others 2009). None of the QTLs for OC identified in this study occurred on these chromosomes, so it is therefore unlikely that selection against OC would affect athletic performance in this population. The QTL for FRAG occurring PPP1 in the hind fetlocks on ECA 4 at 89 Mb does not overlap with the region on ECA 4 identified as exhibiting positive selection in the Thoroughbred breed, which was located at approximately 38Mb. Therefore, selection against
Genome-wide association studies of skeletal lesion traits in Australasian Thoroughbred horses

FRAG occurring PPP1 in the hind fetlocks would also be unlikely to have significant negative effects on athletic performance.

The Thoroughbred horse breed is a good candidate for genomic selection using the SNP marker set that has been implemented in the Illumina Equine SNP50 beadchip due to its small effective population size and extensive linkage disequilibrium (Corbin and others 2010). The creation of predictions based on genome-wide SNP data would require phenotypic records from several thousand horses to achieve moderate accuracy for traits with heritability between 0.1 and 0.2, such as the OC traits examined here (Goddard and Hayes 2009). While the Australian and New Zealand Thoroughbred populations are large enough to produce a reasonable data set in a few years, it may only be cost effective if research into performance traits, reproduction traits or other diseases was carried on concurrently.

The QTL for the single gene controlling the chestnut coat colour trait in this population was clear, based on 29 chestnut and 72 non-chestnut horses. The genomic inflation factor for this trait was higher than for the skeletal lesion traits (1.57 versus 1.00 - 1.05), supporting the presence of some unaccounted-for population stratification in this analysis. The effect of this population stratification can be seen in the identification of many other QTLs for this trait, including some reaching genome-wide significance, reflecting regions of the genome other than that surrounding the MC1R gene likely to be identical by descent amongst a disproportionate number of cases and/or amongst a disproportionate number of controls. A separate analysis of the same trait in a smaller number of Thoroughbred horses (11 cases and 26 controls) also successfully identified a tighter genomic region housing this gene, and also identified regions on other chromosomes with high scoring SNPs
(McCue and others 2012). While some single gene traits in this population could be identified with a very small number of cases and controls, it is clear that these should be selected carefully.

4.6. Conclusions

A genome-wide significant QTL was identified on ECA 30 for the OC trait LYS at SRMC3 in a homogenous population consisting of high-value Australasian Thoroughbred horses. A DNA-binding transcription factor with roles in cartilage and bone development is located in this QTL: NR5A2. Two additional candidate genes (ELF3 and PTPRV) were found in the homologous region of the mouse genome. Additional genotyping may help identify QTLs for additional OC traits or FRAG occurring PPP1 in the hind fetlocks in this Thoroughbred horse population.

QTLs for OC traits that have been identified in other horse breeds were not supported by the results of this study. This follows the trend seen so far of different QTLs being identified in different breeds. The reasons underlying these between-breed differences are not clear, but may involve genuine differences in the underlying genetic contributors to OC. Genetic drift can lead different populations to fix different risk or protective alleles, while other populations remain polymorphic, leading to the identification of different QTLs for alterations occurring in the same complex biological pathway(s). Alternatively, experimental design flaws where genetic stratification within a breed may have resulted in false identification of QTLs.

The majority of the population sampled for this analysis was genomically homogenous. However, the offspring of two Australian stallions were sufficiently different from the main population that they were apparent as genomic outliers. Future studies utilising the Illumina Equine SNP50
Genome-wide association studies of skeletal lesion traits in Australasian Thoroughbred horses

BeadChip in the Australian and New Zealand Thoroughbred population should try to account for the presence of some genomic diversity within both the high-value sub-population examined in the current study, and the Australasian Thoroughbred horse population overall.

4.7. Acknowledgements

Many thanks to the Australian Rural Industries Research and Development Corporation (RIRDC) for their financial support of this project, and to the participating studs and veterinarians that participated in the collection of samples.

4.8. References


Genome-wide association studies of skeletal lesion traits in Australasian Thoroughbred horses


Genome-wide association analysis of osteochondrosis of the tibiotarsal joint in Norwegian Standardbred trotters. *Animal Genetics* 41 (Suppl 2), 111–120


5. A pedigree analysis of Australian Thoroughbred horses

5.1. Summary

*Reasons for performing study:* There are no published studies to date that explore the population structure, use of breeding stock or rate of loss of genetic variability in the Australian Thoroughbred population.

*Objectives:* To explore past changes in breeding practice in the Australian Thoroughbred population (e.g. changes in sire usage and the origins of imported breeding stock) and their impact, as illustrated by trends in 1) rate of inbreeding as indicated by average inbreeding coefficient; and 2) loss of genetic variability due to unequal use of founders, population bottlenecks and genetic drift over time as indicated by the effective numbers of founders (f_e), ancestors (f_a), and founder genomes (N_g).

*Methods:* The Australian Stud Book provided their entire pedigree data file, extending from horses born in 2005 back to the founding sires and dams of the Thoroughbred breed in England. Inbreeding coefficients (F) were calculated for every registered Australian Thoroughbred horse in the pedigree, and their ancestors. Year of birth and country of origin data were used to group the population into cohorts for the calculation of average co-ancestry (mean kinship), f_e, f_a, and N_g.

*Results:* The rate of inbreeding has remained low since 1973, with the period between 2001 and 2005 showing the highest rate (ΔF = 4.63x10^{-4} per year, or 0.47% per generation). The unequal use of founder animals, an early bottleneck and recent genetic drift were identified as reducing genetic variability in the breed, but not to an alarming extent. The importation of breeding stock from traditional sources (New Zealand, Europe and North America) is no longer increasing genetic variability. The number of sires is decreasing and their co-ancestry is increasing.

*Conclusions:* There has been a low rate of loss of genetic variability in the Australian Thoroughbred population since 1973. This rate of loss is now increasing and is likely to increase further in coming decades.

*Potential relevance:* It may become reasonable to treat Thoroughbred horses in Australia, New Zealand, Europe, and North America as one single population in the future.
5.2. Introduction

The Thoroughbred horse breed was founded in England around the turn of the 18th century. It is best known for its use in horse racing. The first Thoroughbred horse was brought to Australia in 1799, just eleven years after the arrival of the first English settlers. The first recorded Australian Thoroughbred foal was born in 1818. The Australian Stud Book, as the official record of Australian Thoroughbred parentage, was founded in its modern form in 1878, by Mr. William C. Yuille. While the majority of horses in these early Australian records trace their ancestry to mares in the General Stud Book of England, sixteen mares of unknown pedigree were accepted as ‘colonial’ taproot or founder mares due to the high standard of performance of their progeny (Ford 2006).

The Thoroughbred breed (world-wide) is essentially a closed population. Over the past three hundred years genetic drift and selection for athletic traits have occurred, and genetic variation within the breed has decreased (Cunningham and others 2001; Gu and others 2009). Quantitative measures of the expected loss of genetic variation include the inbreeding coefficient, F, that estimates the loss of heterozygosity for an individual due to alleles inherited from each parent being identical by descent, relative to a base population (Wright 1922). Previous pedigree-based analyses of inbreeding in Thoroughbreds bred in the United Kingdom found the average inbreeding coefficient (average F) to be 0.125 for a group of 6,550 Thoroughbred mares at stud in 1964, and 0.130 ± 0.014 for all horses in the 1987-96 population in the UK General Stud Book (Mahon and Cunningham 1982, Cunningham and others 2001). Both studies utilised pedigree information extending back to the foundation sires and dams of the Thoroughbred breed, as recorded by the General Stud Book of the United Kingdom. Despite its public reputation for high levels of inbreeding, the Thoroughbred breed has in fact experienced very low levels of inbreeding,
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averaging around 0.62% per generation (an average F of 0.125 was found for 21.5 generations by Mahon and Cunningham (1982)).

The Thoroughbred horse breed is unusual amongst commercial animal breeds in not allowing any form of artificial breeding (Australian Stud Book 2010). Thoroughbred mares rarely have more than one foal per year, and individual stallions can sire at most 400 foals per year, if they are highly fertile and used in both the Northern and Southern hemisphere breeding seasons. The loss of genetic variation in the Thoroughbred breed may have been limited by breeders forgoing the use of artificial breeding technologies. Quantitative measures of whole-population genetic diversity include fe, fa, and Ng (Lacy 1989, Boichard and others 1997).

The fe statistic represents the number of founding animals that, if they contributed exactly equally, would produce the same genetic diversity as the population under study. The more unequal the contribution of the actual founder animals, the greater the difference between fe and the number of actual founders in the pedigree (Lacy 1989). The fa statistic is conceptually similar, and represents the number of ancestors (including founders) that, if they contributed equally and independently, would produce the same genetic diversity as the study cohort. It seeks to take account of population bottlenecks subsequent to foundation in addition to the unequal contribution of founder animals. The greater the fe:fa ratio, the more severe the bottleneck (Boichard and others 1997). The Ng statistic seeks to take account of the unequal contribution of founders, population bottlenecks, and the loss of alleles over time due to genetic drift, and can be estimated via gene-dropping replicates or calculated exactly as half the inverse of the average co-ancestry for the group considered (Caballero and Toro 2000). All three statistics are expected to decrease over time in a closed population, and fe > fa > Ng.
This analysis of pedigree data from the Australian Thoroughbred horse population aims to explore past changes in breeding practice (e.g. changes in sire usage and the origins of imported breeding stock) and their impact, as illustrated by trends in average $F$, the effective numbers of founders ($f_e$), ancestors ($f_a$), and founder genomes ($N_g$) over time.

5.3. Materials and methods

The Australian Stud Book supplied their entire pedigree data set in a single electronic file. These data describe 819,642 individual horses, extending back to the founding sires and dams of the Thoroughbred breed in England. The file includes all Thoroughbred horses born in or brought to Australia from 1973 to 2006, and some part-Thoroughbred horses. Horses born prior to 1972 with no descendants born after 1972 may not necessarily be recorded in the pedigree file, although stakes-winners, their ancestors, and close relatives are generally included.

5.3.1. The pedigree data

A sub-pedigree containing only registered Australian Thoroughbred horses and their ancestors was extracted, excluding horses with no ancestral information and no progeny. This sub-pedigree contains 606,092 individual horses. Table 5.1 provides summary statistics for this sub-pedigree, which was used as the basis for all analyses. Analyses were restricted to foal cohorts born after 1973 (that is, in the period for which data were available for all horses) except where earlier cohorts were required to provide historical context.
Table 5.1: Summary statistics for the pedigree of Australian Thoroughbred horses used in the analyses. Founders are ancestors with unknown parents. Discrete generation equivalents indicate pedigree depth by averaging the number of generations between each individual and their most distant known ancestors.

<table>
<thead>
<tr>
<th>Group</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total pedigree</td>
<td>606,092</td>
</tr>
<tr>
<td>Sires</td>
<td>19,322</td>
</tr>
<tr>
<td>Dams</td>
<td>184,159</td>
</tr>
<tr>
<td>Both parents known</td>
<td>601,886</td>
</tr>
<tr>
<td>One parent known</td>
<td>225</td>
</tr>
<tr>
<td>Founders with progeny</td>
<td>3,981</td>
</tr>
<tr>
<td>Average discrete generation equivalents (whole pop.)</td>
<td>22.63 generations</td>
</tr>
</tbody>
</table>

5.3.2. **Inbreeding coefficients and other parameters**

Inbreeding coefficients were calculated for all horses in the pedigree file, using the inverted matrix algorithm of Colleau as further developed and implemented in CFC 1.0\(^9\) by Sargolzaei (Colleau 2002, Sargolzaei and others 2006b). The average of the inbreeding coefficients of horses born in each year was calculated using Python programs written for that purpose (Python version 2.4.1, March 30 2005). \(\Delta F\) was calculated as change in F over a period divided by the length of that period. For low rates of inbreeding such as those expected in the current study, the difference is small between this simple method and the more complex \(\frac{(1 – F_{t1})(1 – F_{t2})/(1 – \Delta F)^a}{n}\) (where \(t_1\) is the earliest point, \(t_2\) is the latest point, and \(n\) is the number of years or generations in a given period).

CFC 1.0 was also used to calculate \(f_e\) and \(Ng\) for year cohorts at five-year intervals from 1975 to 2005 (Boichard and others 1997). Pedig2007\(^10\) was used to calculate \(fa\) for the same cohorts (Boichard 2002). For each of the fifth year cohorts, generation intervals were calculated for the

\(^9\) CFC 1.0 is specialised pedigree analysis software. The use of an inverted matrix algorithm allows CFC 1.0 to efficiently calculate F and co-ancestry coefficients in many-generation pedigrees.

\(^10\) Like CFC 1.0, Pedig2007 is specialised pedigree analysis software.
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pathways sire to son, sire to daughter, dam to daughter, and dam to son; and were then averaged across offspring and across parents.

In addition to calculating the number of sires producing Australian Thoroughbred foals in particular years, the effective number of sires (\(NeffS\)) in 1987 (when sire numbers peaked) and in 2005 (the most recent cohort included in analyses) was calculated as \(NeffS = 1/\sum s_i^2\) where \(s_i\) is the proportion of offspring from sire \(i\) in that year's foals (Leroy and Baumung 2011).

5.3.3. Identifying population trends resulting from horse importation

Data describing the country of registration of each horse were utilised to examine the use of imported sires and dams in Australian Thoroughbred breeding.

Since the mid-1980s, the practice of using shuttle stallions has become popular in Australia. Shuttle stallions are flown between the Northern and Southern hemispheres, for use in both breeding seasons. For the purposes of this analysis, shuttle stallions were not differentiated from other imported stallions. The overall number of sires and the average sire co-ancestry were summarised from 1985-2005 by the foal's year of birth. The average co-ancestry of sires with offspring in the year of interest was calculated using the indirect method of Colleau (2002), as implemented in CFC 1.0 (Sargolzaei and others 2006b, Sargolzaei and Colleau 2006). By default this algorithm includes self-relationships in calculation of average relationship, although their inclusion in CFC is not specified (Sargolzaei and others 2006a). Additionally, average between-group co-ancestry was calculated between each of the two sires whose offspring formed an outlier group in Chapter 4 and a group consisting of all other sires with offspring born in 2005, in order to determine whether these two stallions can be differentiated from the remainder of their cohort.
The F of an offspring is equal to the co-ancestry of its two parents. In addition to the average co-ancestry of sires described above, conclusions were drawn regarding the average co-ancestry of sire and dam pairs where one or both was imported, and separately of Australian sires and dams (for matings that took place, not all possible matings) based on the average F of the relevant foal cohorts.

5.3.4. Determining the gene contribution of high-impact sires

The total gene contributions of all ancestors were calculated for Australian Thoroughbred horse cohorts born every fifth year between 1945 and 2005 using a modified version of Pedig2007's prob_orig routine (Boichard 2002). The top ten 20th century stallions were selected for further examination, based on a ranking of the highest total gene contribution achieved by each sire within this period. Calculating total gene contribution (as opposed to overall relatedness or marginal gene contributions) allows conclusions to be drawn regarding proliferation of the specific alleles carried by those stallions. Overall relatedness includes genes transferred through collateral relatives, while marginal contributions may discount a sire's contribution for genes passed on by a descendant, but still deriving from the sire in question. In the Thoroughbred breed, where many popular stallions are closely related, the iterative approach used in the calculation of marginal gene contribution prevents valid comparison of different time points. The order in which marginal gene contributions are calculated changes at each time point, and this causes dramatic fluctuations in marginal gene contributions for non-founding sires.
5.4. Results

5.4.1. Inbreeding in Australian Thoroughbred horses

The average F of Australian Thoroughbreds born in 2005 was 0.143 ± 0.010. The average F of all Australian horses born from 1987-96 was somewhat greater than that calculated by Cunningham and others (2001) for the UK Thoroughbred population in the same period, at 0.140 compared to 0.130.

Fig 5.1 shows the average and standard deviation of F for Australian Thoroughbred horses born between 1818 and 2005, and the number of registered Thoroughbred horse births recorded in each year. The plot of average F between 1973 and 2005 is additionally shown in a magnified box with the co-ordinate axes stretched so that trends can be seen more clearly. Average co-ancestry was consistently slightly higher than average F (data not shown), a finding in accordance with F being equal to the co-ancestry of the individual's parents and the upward trend of average F over time in this population.
Fig 5.1: Top – average F (with standard deviation) for registered Australian Thoroughbred horses born between 1818 and 2005. Bottom – the number of registered Australian Thoroughbred horses over the same period. Magnified box – average F between 1973 and 2005. The year 1973 is the point from which data are available for all registered Australian Thoroughbred horses.

Visually, it can be argued that $\Delta F$ differed between three periods: 1973-1986, 1987-2000, and 2001-2005. From 1973 to 1986, $\Delta F$ was 0.000339 (0.34% per generation), while from 1987-2000, $\Delta F$ was 0.000116, or 0.12% per generation. In recent years (2001-2005) $\Delta F$ increased to 0.000463 per year, equivalent to 0.47% per generation.
5.4.2. Generation intervals

As can be seen in Table 5.2, generation intervals in the Australian Thoroughbred population increased after 1975 and then have decreased in the two most recent cohorts. The average generation interval across the cohorts (weighting each horse equally) was 10.1 years. The average age of dams and sires ranged from 9.7 to 10.4 years, and 10.4 to 11.6 years, respectively. There were no consistent differences in generation intervals to sons and to daughters.

Table 5.2: Generation intervals of Australian Thoroughbred horses, calculated from the fifth-year cohorts between 1975 and 2005.

<table>
<thead>
<tr>
<th>Year of birth</th>
<th>Dam to daughter</th>
<th>Dam to son</th>
<th>Dam average</th>
<th>Sire to daughter</th>
<th>Sire to son</th>
<th>Sire average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>9.8</td>
<td>9.9</td>
<td>9.8</td>
<td>10.4</td>
<td>10.4</td>
<td>10.4</td>
</tr>
<tr>
<td>1980</td>
<td>9.7</td>
<td>9.7</td>
<td>9.7</td>
<td>10.4</td>
<td>10.4</td>
<td>10.4</td>
</tr>
<tr>
<td>1985</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.8</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>1990</td>
<td>10.1</td>
<td>10.2</td>
<td>10.1</td>
<td>11.1</td>
<td>11.1</td>
<td>11.1</td>
</tr>
<tr>
<td>1995</td>
<td>10.3</td>
<td>10.4</td>
<td>10.4</td>
<td>11.6</td>
<td>11.6</td>
<td>11.6</td>
</tr>
<tr>
<td>2000</td>
<td>10.4</td>
<td>10.5</td>
<td>10.4</td>
<td>11.4</td>
<td>11.4</td>
<td>11.4</td>
</tr>
<tr>
<td>2005</td>
<td>10.3</td>
<td>10.2</td>
<td>10.2</td>
<td>10.6</td>
<td>10.6</td>
<td>10.6</td>
</tr>
</tbody>
</table>

5.4.3. Effective numbers of founders, ancestors, and founder genomes

Fig 5.2 shows values of $f_e$, $f_a$ and $N_g$ for Australian Thoroughbreds born in the fifth-year cohorts since 1975. Reflecting the known history of the breed, $f_e$ was found to be significantly smaller than the number of founders in the pedigree (3,981), and to decrease over time. The number of founders in this pedigree is far higher than that identified by Cunningham and others 2001, and is likely to reflect missing information in the Australian pedigree file concerning the distant ancestry of horses imported from countries other than the UK. The overall estimate of $f_e$ for Australian Thoroughbreds
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born in 2005 was 29.8, very similar to the estimate for English Thoroughbreds (28.15), and lower than (but not greatly dissimilar to) those published for the Andalusian horse (39) and the Spanish Arab horse (38.6), breeds that also have deep pedigrees and closed populations (Cunningham and others 2001, Valera and others 2005, Cervantes and others 2008). The \( f_a \) statistic decreased in a similar manner to \( f_e \), and the ratio \( f_e:f_a \) decreased from 2.33 to 2.27. The decrease of \( N_g \) over time shows the largest drops occurring in the most recent decade.

![Graph](image.png)

Fig 5.2: Top – effective number of founders (\( f_e \)) for fifth-year cohorts of Australian Thoroughbred horses; Middle – effective number of ancestors (\( f_a \)); Bottom – effective number of founder genomes (\( N_g \)).

5.4.4. Proportion of horses selected for breeding

The period from 1973 to 2005 exhibited large increases in annual production of foals prior to 1988, a short slump coinciding with the aftermath of the 1988 stock market crash, and then stabilisation
(see Fig 5.1). The peak in annual production in the late 1980s was achieved using a greater proportion of contemporary females as breeding stock: 62% of Australian fillies born in 1980 and 1981 became dams, compared to only 36% of fillies born in 1988.

5.4.5. The use of imported horses in breeding

Australian Thoroughbred breeders have always imported both stallions and mares for breeding purposes. The origin of imported horses has changed over the last 60 years. Fig 5.3 shows the origins of Australian Thoroughbred sires and dams at four time points from 1955 to 2005. The distribution for 1955 is included for comparison, but is based on incomplete data. The proportion of imported mares has stayed steady at around 15%, while the proportion of imported stallions has ranged from more than 60% to less than 40%.

![Fig 5.3: The proportion of sires (left) and dams (right) sourced from Australia, New Zealand, North America, Europe, and elsewhere.](image-url)
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In 1955, almost all imported sires and dams were obtained from Europe and New Zealand. From 1975, the number of mares and stallions imported from Canada and the USA increased steadily, at the expense of importation from Europe. Since 1995, an increasing number of horses have been imported from South America, Japan, and the rest of the world. However, by 2005, these horses represented just 1% of sires and 0.1% of dams in the Australian breeding population.

From 1985 to 2005, the proportion of registered Australian Thoroughbred horses with an imported sire and/or dam has remained constant, at around 60% of the total population. As can be seen in Fig 5.4, the average F of the progeny of imported sires and/or dams has been consistently lower than the average F of progeny of Australian-born Thoroughbreds. However, as also illustrated in Fig 5.4, the gap between the two groups has narrowed significantly, from more than 0.01 in 1985 to 0.000603 in 2005. Australian-born sires and dams are now almost as closely related to imported horses as they are to one another.
5.4.6. **Trends in the use of sires**

Sires have become more closely related over the past 20 years, and the rate of increase in average sire co-ancestry has accelerated since the year 2000 (see Fig 5.5). The number of sires (of both local and international origin) of Australian Thoroughbreds has decreased significantly over the past 20 years. Sire numbers peaked at 2,138 in 1987 (producing 17,305 registered foals), but had dropped to 835 by 2005 (producing 17,287 registered foals). The effective numbers of sires in these years were 809 and 275, respectively.
The average within-group sire co-ancestry of all sires with offspring born in 2005 was 0.1303. The average between-group sire co-ancestries calculated between each individual sire of the two outlier groups seen in Chapter 4 and a group comprising all other sires with offspring born in 2005 were 0.1326 and 0.1308. The average within-group sire co-ancestry for the group of all other sires with offspring born in 2005 was still 0.1303. Differentiation between the two outlier groups seen in Chapter 4 and the remainder of the genotyped population therefore appears to be based on relatively small genetic differences between sires.

Fig 5.6 shows the total gene contribution (average direct relationship) of the top ten 20th century stallions contributing to Australian Thoroughbred foal cohorts born each fifth year between 1945
and 2005. The stallions are: Son In Law (GB, 1911) Phalaris (GB, 1913), Gainsborough (GB, 1915), Blandford (IRE, 1919), Nearco (ITY, 135), Nasrullah (IRE, 1940), Star Kingdom (IRE, 1946), Northern Dancer (CAN, 1961), Mr. Prospector (USA, 1970) and Danehill (USA, 1986). Four of these stallions are related by direct descent in the male line: Phalaris is a grand-sire of Nearco; Nearco is a grand-sire of Northern Dancer; and Northern Dancer is a grand-sire of Danehill. Additionally, Nasrullah is a son of Nearco, and Star Kingdom is a great-grand-son of Gainsborough.

![Figure 5.6](image)

*Fig 5.6: The total gene contributions of ten Thoroughbred stallions born in the 20th century.*

In 2005, Northern Dancer provided 8.4% of the Australian foal gene pool. The contributions of both Northern Dancer and Danehill are increasing at a far greater rate than those of stallions born earlier.
in the century. Descendants contribute to the total gene contribution of their ancestors, and the steep rise of Northern Dancer's gene contribution is due in part to the popularity of Danehill.

5.5. Discussion

Reflecting widespread consensus, the Food and Agriculture Organisation of the United Nations suggests a rate of inbreeding (ΔF) of 1% per generation as the maximum permissible rate of inbreeding for farm animals (FAO 1998). While ΔF has remained well below 1% per generation in the Australian Thoroughbred horse population since 1973, the magnitude of increases that have been seen (i.e. from 0.12% per generation between 1987 and 2000, to 0.47% per generation between 2001 and 2005) indicate that this threshold could be reached in the future. An examination of single nucleotide polymorphism (SNP) data in the UK Thoroughbred population indicated that F estimated from observed and expected heterozygosity is increasing at a still higher rate in that population, and estimated that the figure of 1% per generation has almost certainly already been surpassed (Binns and others 2012).

Effective population size (Ne) is the number of individuals in an idealised population that would result in the same ΔF as seen in the actual population (Wright 1931). An examination of linkage disequilibrium among SNPs in a Thoroughbred population estimated Ne ranging between 120 and 200 in the four most recent generations (Corbin and others 2010). The estimate of inbreeding from Binns and others (2012) indicates a much lower Ne, perhaps less than 30 (Ne = 1/2ΔF), although the actual value is unclear. The most recent rate of inbreeding found in the current study (0.47% per generation) would indicate an Ne of 107. This value is approximately twice that thought to be required to retain 90% of variation over 100 years, assuming a generation interval of ten years
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(Frankham and others 2010). The period immediately prior to 2001, where $\Delta F$ was 0.12% per generation, indicates a much higher $N_e$ of approximately 415. Both studies of UK Thoroughbreds also found that $N_e$ decreased (or that $\Delta F$ increased) in a time period equivalent to the most recent generation, indicating that similar trends in effective population size may be occurring in both the UK and Australia.

The effective number of founders ($f_e$) in the Australian Thoroughbred horse population (29.8) is slightly more than that previously reported by Cunningham and others (2001) for Thoroughbred horses in England (28.2), potentially reflecting the contribution of the sixteen Australian mares promoted to Thoroughbred status by the Australian Stud Book. These $f_e$ values are much lower than the actual number of founders, reflecting an early bottleneck where now-dominant sire lines were reduced to single stallions (Cunningham and others 2001).

The Australian Thoroughbred horse population (in common with other horse populations) has a combination of features that influence the actions of horse breeders, and the breed's response to those actions. Firstly, the average generation interval in horse breeds is often more than 10 years (for example Biedermann and others 2005; Valera and others 2005; Poncet and others 2006; Teegen and others 2008; Thiruvenkadan and others 2009). The genetic effects of changes in breeding practices are therefore slow to appear and difficult to perceive on a year-to-year time-scale. Secondly, since most mares have only one foal per year, the use of mares is governed by overall demand for foals, and breeders aiming to genetically diversify or consolidate their stock therefore focus on stallion choice, where greater flexibility is possible. Thirdly, advances in equine reproductive care and dietary knowledge have accrued at far greater rates in recent decades than our genetic knowledge of horses, providing incentive for horse breeders to focus on improved nutrition.
and manipulation of fertility as ways to invest in the efficient production of strong, healthy foals (Rogers and others 2007, Nath and others 2010).

The current study shows long-term changes in the Australian Thoroughbred population that are consistent with past changes in breeding practices. The first example is the introduction of North American Thoroughbred sires from the 1970s (partially replacing the use of European sires), and their continued use to the present day (see Fig 5.5, and also apparent in Fig 5.2). This introduction of new blood-lines was accompanied by a reduction in the rate of inbreeding, apparent from the mid-1970s and with greatest effect from 1987 to 2001, when average $\Delta F$ was just 0.12% per generation (see Fig 5.4). From 1985 to 2005, average F for foals with both Australian parents fell while average F for progeny of imported parents rose. Presumably this reflects the incorporation of new genes into the Australian parent population, so that they became less related to each other, but became more closely related to imported parents, than had previously been the case.

The second example is the practice of having significantly greater numbers of foals sired by popular stallions or stallion families. This is illustrated by the drop in the number of unique sires of Australian Thoroughbreds, which has fallen by almost two thirds since 1987, while the number of foals produced each year has remained approximately steady (Fig 5.4, Fig 5.1). During this period, the effective number of sires dropped from 809 (38% of total sire numbers) to 275 (33% of total sire numbers), indicating that sire use in the remaining sire pool has also become slightly more unbalanced.

The same time period has seen the rapid rise of Northern Dancer and the even-more-rapid rise of his grandson Danehill (Fig 5.6). Northern Dancer contributed 8.4% of the gene pool of Australian
Thoroughbreds born in 2005, more than nine of the top ten founders of the Thoroughbred breed (Cunningham and others 2001). The impact of Northern Dancer is still rising, so the frequency of any rare alleles carried by Northern Dancer will be increasing, especially if they were also carried by his descendant Danehill.

The current study also found that decreases in genetic diversity in Australian Thoroughbred foals occurred concurrently with the decrease in stallion numbers and the rise of Northern Dancer and Danehill. Fig 5.2 shows recent (since 1995) loss of genetic diversity in Australian Thoroughbred foals due specifically to genetic drift, as indicated by the increased deviation of $N_g$ from $f_e$ and $f_a$. The average co-ancestry of all sires of Australian Thoroughbreds has also begun to rise more rapidly since 1995 (Fig 5.5). Overall, the genetic diversity of sires has not been maintained as their numbers have dropped, and there has been a decrease in the genetic diversity of the foals they have produced.

The third example of a change in breeding practices is the use of overseas shuttle stallions, beginning in the mid-1980s. Shuttle stallions have popular bloodlines, and produce offspring in more than one continent, increasing the likelihood that horses subsequently imported to Australia will have close relatives already present here. The converging average F of Australian offspring of imported or shuttle stallions and/or imported mares, and offspring of Australian stallions and mares (Fig 5.4) indicates that as of 2005, matings involving shuttle or imported horses no longer brought additional genetic diversity to this population. It is plausible that the genetic diversity gained by introducing North American blood-lines has been exhausted after 35 years of increasing use, and that the continual movement of Thoroughbreds between Australia, New Zealand, Europe, and North America has resulted in blood-lines once typical of a particular continent being spread almost
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uniformly between these countries. That said, the Australian Thoroughbred population is not yet (and may never be) completely homogenous, as was demonstrated by the differentiation of the offspring of two Australian stallions in the genomic analysis undertaken in Chapter 4.

5.6. Conclusions

The low rate of inbreeding since 1973 indicates that breeding practices in that time have not posed a threat to the genetic well-being of the Australian Thoroughbred. Whilst the rate of inbreeding has increased since 2001, it remains low overall.

The recent (since 1990) decreases in sire numbers and increases in average co-ancestry of sires will probably lead to an increase in the rate of inbreeding in Australian Thoroughbreds over the next few decades. There will be some lag before the effect can be seen, because of the ten-year generation interval in this population. Future increases in the rate of inbreeding will not be able to be slowed by the use of imported horses from traditional sources (popular families from New Zealand, Europe and north America), because Australian Thoroughbreds are already almost as closely related to these imported horses as they are to one another.

It seems likely that the continual churn of Thoroughbred horses around the globe, including the use of shuttle stallions, has greatly increased the average relatedness of Thoroughbred horses particularly throughout Europe, North America, Australia and New Zealand. If the flow of genetic material is maintained between these regions at the levels of the last three decades, it may become reasonable to treat these populations as one single population in the future.
5.7. Acknowledgements

Many thanks to the Australian Stud Book for providing the data for this study, and to Suzanne Lemon and Luke Harper for the temporary use of their Windows PC.

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June 24, 2010


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*Livestock Production Science* 95, 57–66


Wright, S. (1931) Evolution in Mendelian populations. *Genetics* 16, 97–159
6. General discussion and conclusions

6.1. The prevalence of osteochondrosis and other skeletal lesions and injuries

Osteochondrosis (OC) occurs when there is a localised failure in the biological process that replaces the soft, articular growth cartilage with hard bone tissue. This process is called endochondral ossification. OC lesions develop in young horses in articular cartilage and may extend into the underlying subchondral bone. These lesions are usually identified by radiography, but since cartilage is relatively translucent to X-rays, only those lesions that include altered bone morphology will be seen. OC diagnosis must be based on veterinary interpretation, the location of the lesion and its appearance on the radiograph.

The diagnosis of OC from radiographs alone is therefore not consistent between studies of equine OC. For example, some studies have limited their analyses to osteochondritis dissecans (OCD, the end-stage form of OC) occurring at some or all OC predilection sites (e.g. Pieramati and others 2003; Stock and Distl, 2005; Corbin and others 2012). In contrast, a recent study of OC in Dutch Warmblood horses examined smoothly and roughly flattened bone contours, subchondral cystic lesions, bone fragments and OCD at OC predilection sites in the fore and hind fetlocks, hocks and stifles (van Grevenhof and others 2009). In the current study, the definition of OC was similar to that used in the study of Dutch Warmblood horses, consisting of roughly flattened bone contours, subchondral cystic lesions, bone fragments and OCD (but excluding smoothly flattened bone contours) at OC predilection sites in the same joints (i.e. the fore and hind fetlocks, hocks and stifles). This definition was based on veterinary advice and commonly accepted definitions of OC phenotypes, and is consistent with the definition of OC used in other previously published...
radiographic surveys of Thoroughbred yearlings in Australia and New Zealand (Jeffcott 1991, Oliver and others 2008, Jackson and others 2009). Smoothly flattened bone contours were excluded on the basis that they may not necessarily be indicative of OC.

A total of 20.5% of the Thoroughbred yearlings in this study were found to have OC lesions at one or more anatomical sites. In the fore and hind fetlocks, 6.0% and 1.3% of yearlings had OC lesions, respectively. In the hocks, 7.2% of yearlings had OC lesions, while 8.7% were had OC lesions in the stifles. The anatomical sites most commonly affected were the sagittal ridge of the third metacarpal bone (SRMC3) in the fore fetlocks (6.0% affected), the distal intermediate ridge of the tibia (DIRT) in the hocks (3.1% affected), the lateral trochlear ridge of the femur (LTRF) in the stifles (3.7% affected), and the medial femoral condyle (MFC), also in the stifles (4.3% affected).

The identification of these sites as the primary locations for OC in Thoroughbred horses in Australia and New Zealand was in agreement with other published radiographic surveys of other Australian and New Zealand Thoroughbred yearling populations (Oliver and others 2008, Jackson and others 2009). Some horses were diagnosed with OC at more than one OC predilection site (e.g. at the MFC in the stifle and the SRMC3 in the fore fetlocks). A study of Dutch Warmblood horses that examined the same range of joints found that many horses in that breed were affected in more than one joint, with an overall prevalence of 35% encompassing a prevalence of 21% in the stifles, 19% in the hocks, and 18% in the fore and/or hind fetlocks (based on OC phenotypes described as ≥ C grade, equivalent to the definition of OC used in the current study) (van Grevenhof and others 2009). A study of Norwegian Standardbred Trotters that examined the fore and hind fetlocks and hocks (but not the stifles) also found that some horses were diagnosed with OC at more than one OC predilection site (Lykkjen and others 2012).
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Apart from OC, the most common skeletal lesions diagnosed in the Thoroughbred yearling population in the current study were bone modelling (MOD) in the front knees (5.4%), spurs in the front knees (5.7%), sesamoiditis (broadly defined as changes or vascular channels in the sesamoid bones in the current study) in the fore fetlocks (14.0%) and hind fetlocks (7.9%), bone fragments (FRAG) occurring proximal palmar/plantar to the first phalanx (PPP1) in the hind fetlocks (6.5%), MOD in the hocks (3.9%) and spurs in the hocks (13.5%). These findings were also in agreement with the findings of the previous surveys of radiographic lesions in Australian and New Zealand Thoroughbred yearling populations.

Some of the skeletal lesions identified as occurring frequently in the study population have been associated with lower sale price or decreased race performance. OC at any anatomical site in the stifle, and bone fragments either PPP1 or proximal dorsal to the first phalanx (PDP1) in both the fore and hind fetlocks have been associated with lower sales price in the USA (Preston and others 2010). In Australia, OCD and SCL in the stifles, OC at the sagittal ridge of the third metatarsal bone (SRMT3) in the hind fetlocks, and some lesions in the sesamoid bones have been associated with decreased race performance at two and three years of age, but their effect on price is unknown (Jackson and others 2009). Lower sales prices have a direct effect on a stud's profitability, and poorer race performance can have the same effect indirectly, by harming a stud's reputation. These particular skeletal lesions may therefore be of special interest to Thoroughbred stud managers in Australia and New Zealand.

The data used in the present study were derived from written reports obtained by stud managers to assist them in the preparation and sale of high-value Thoroughbred weanlings and yearlings raised in Australia and New Zealand. There was good agreement between the prevalence and distribution
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of lesions reported in the current study, and those previously reported in related populations of
Australian and New Zealand Thoroughbred yearlings based on direct examination of radiographs
(Oliver and others 2008, Jackson and others 2009). This supports the use of these reports as an
effective data source for the study of OC in this population, although OC occurring at the SRMC3
and SRMT3 may be under-reported. Similarities in the reported distribution and prevalence of
FRAG in the fore and hind fetlocks also support the use of pre-sale reports for FRAG in the
fetlocks.

6.2. The use of information from weanlings and yearlings

Skeletal lesion findings from weanling radiographs provided a strong indication of findings from
yearling radiographs. Among the 84 horses with two written reports based on radiographs taken at
two different times (i.e. weanling age and yearling age), none developed new lesions when they
were found to be unaffected as weanlings. However, in a few instances lesions present in weanlings
had resolved without surgical intervention by the time the horses were re-radiographed as yearlings.

The strong associations between findings from weanling and yearling radiographs support the use
of findings from weanling radiographs as a data source when yearling radiographs are not available.
Amongst those horses with radiographs taken at both weanling and yearling ages, the identification
of OC lesions that resolve without surgical intervention may provide the opportunity to gain
additional insight into the mechanisms underlying recovery of OC lesions in the Thoroughbred
breed. Weanling radiographs are taken to allow the identification of lesions that can be treated with
corrective surgery prior to the horse's sale as a yearling.
A study of Dutch Warmblood foals found that after the age of 8 months, OC lesions were permanent and did not spontaneously resolve (Dik and others 1999). The current study showed that this was not the case in the Australian and New Zealand Thoroughbred population, because there were some instances of OC lesions healing spontaneously subsequent to weanling radiographs being taken at an average age of 10.3 months.

6.3. Defining osteochondrosis traits as targets for management

The current study estimated the heritability of OC overall in a sub-population of high-value Australian and New Zealand Thoroughbred horses as 0.11. Many OC component traits with more specific definitions (e.g. occurring at a specific anatomical site) had higher estimates of heritability, with the highest being OCD at the LTRF, at 0.22. The heritability of OC at other commonly affected anatomical sites was generally greater than zero (0.15 at the SRMC3 in the fore fetlocks, and 0.17 at the MFC in the stifles), although surprisingly genetic variance was not found to contribute to OC at the DIRT. The heritability of OC in the hocks was estimated to be 0.10, as was the heritability of OC in the stifles.

Following the definition of OC, the current study explored grouping OC component traits with the aim of controlling this disorder. From a genetic perspective, it found that OC is not a single disorder in this population, as lesions in the hocks and stifles are negatively correlated with one another, as are the SCL lesion type and lesion types that include bone fragments (i.e. OCD and FRAG). The current study also found that flattened bone contours (FLAT) could be included in the definition of OC, as the FLAT lesion type was found to have positive phenotypic correlations with the LYS, SCL and pooled OC lesion types, in a pooled category of all OC lesion sites. Unfortunately, EBV
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correlations (a proxy for genetic correlations) could not be determined between FLAT and the other lesion types. Three non-genetic factors or interactions that may be under the control of a stud manager (Stud, DOB × Region and Age × Region) were identified as being associated with the prevalence of some OC traits. The effects of these factors differed between different skeletal lesion traits, and not all factors were significant for all traits.

The effect of OC lesions on athletic performance and monetary value differs depending on the type of lesion and where it is located. For example, both price and performance are negatively affected by stifle OC, while there are no significant negative effects associated with OC at the SRMC3 in the fore fetlocks (Jackson and others 2009, Preston and others 2010). Stud managers will therefore want to minimise OC traits known to have negative effects, and those with high or above-average prevalence at their stud.

The identification of groups of traits with positive genetic associations may allow stud managers to minimise several traits simultaneously via genetic selection. Examination of correlations between EBVs for OC traits and other skeletal lesions indicates the presence of at least two groups of traits with positive EBV correlations. One group consisted of bone modelling (MOD) and spurs in the knees, LYS and FLAT at the SRMC3 in the fore fetlocks, FRAG occurring PPP1 in the hind fetlocks, MOD in the hocks, OCD at the MTRF, and SCL at the MFC in the stifles. This group includes multiple traits that have previously been shown to negatively affect race performance in Thoroughbred populations (e.g. OCD at the MTRF and FRAG occurring PPP1 in the hind fetlocks), and includes the most highly heritable traits in this study, making it an excellent target for genetic selection to minimise OC. Selection against both this group of traits and the other group identified in the current study (which has negative genetic correlations with the group described here) could
be carried out, although progress would not be as fast as it would if the between-group correlations were positive. Modeling may be able to provide some estimation of what degree of genetic progress could be made in this circumstance.

The manipulation of non-genetic factors may also be a useful tool for the minimisation of particular OC component traits. The current study found that the effect of non-genetic factors differed between traits, and in many cases was dependent on the geographic region in which the horse was raised. For example, a stud manager in one of the regions may find that yearlings that are older at sale time are less likely to be diagnosed with LYS at SRMC3 but more likely to be diagnosed with MOD in the knees, while foals born late in the season are less likely to be diagnosed with FRAG occurring PPP1 in the hind fetlocks. In this situation, genetic selection may aid in reducing the prevalence of all traits in the cluster, but the stud manager would also need to decide whether to prioritise the minimisation of LYS at SRMC3, which is high in prevalence but not associated with poorer race performance, or MOD in the knees and FRAG occurring PPP1 in the hind fetlocks (where foals born late in the season are less likely to be affected, and will generally be younger at sale time), the second of which has lower prevalence but is associated with poor race performance.

6.4. Selective breeding to manage osteochondrosis and other skeletal lesions

This study found that breeding values contributed significantly to the prevalence of OC overall. When component OC traits and other skeletal lesion traits were examined in more detail, additive genetic variance was found to be a significant source of variation in the occurrence of most OC component traits, some traits with significant overlap with OC traits, and FRAG occurring PPP1 in the hind fetlocks. Genetic selection could therefore be used to reduce the prevalence of these traits.
In recent decades, breeding programs in first world agricultural animal species have generally implemented selection based on Estimated Breeding Values (EBVs). EBVs quantify the genetic merit of an animal with respect to a single trait of interest. The genetic merit of the animal refers to its value as judged by the average status of its offspring, compared with the average performance of the offspring of other horses in the population. The computation of EBVs relies on accurate pedigree information (easily available for Thoroughbred horses) and data describing the occurrence of the trait of interest in ancestors and relatives, as well as the horse itself. EBVs increase in accuracy as the amount of data regarding that trait increases, and for traits with heritability between 0.1 and 0.2, such as those examined in this study, reach a high level of accuracy when they are based on several- to tens-of-thousands of records. Useful levels of accuracy (i.e. where selection based on EBVs can produce substantial genetic gain for the trait of interest) can be achieved with far fewer records, particularly in instances where the status of the horse itself is known. A horse's own phenotype provides valuable data contributing to the prediction of its own genetic merit and that of its offspring because it includes the sum of the effects of the genetic loci carried by that specific animal (rather than the sums of genetic loci carried by other, less closely related, individuals). The value of data describing a horse's own phenotype depends on the heritability of the trait in question, and for traits with low heritability there will be low correlation between a horse's phenotype and its breeding value (i.e. the correlation is the square root of the heritability of that trait).

More recently, techniques have been developed to include single nucleotide polymorphism (SNP) data in the calculation of EBVs, resulting in Genomic Estimated Breeding Values (GEBVs) that can be substantially more accurate than EBVs (Goddard and Hayes 2009). The calculation of GEBVs would require phenotypic records and SNP genotypes from a training set of several thousand horses.
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to achieve moderate accuracy for traits with heritability between 0.1 and 0.2, such as the OC traits examined here. The SNP effects estimated from the training set are then applied to the SNP genotypes of the horse for which a GEBV is being calculated, along with phenotypic data from that horse and relatives, if available. The monetary value of individual Thoroughbred yearlings varies far more widely than that of most other production animals at the start of their productive life, with some individuals selling for over AUD $2 million. This will create strong impetus to ensure that selection is particularly accurate and successful for high value individuals in any Thoroughbred herd. The use of GEBVs may be appropriate to this situation. For traits where additive genetic variation is due in part to loci of large effect, the accuracy of GEBVs can potentially be higher than for traits with no loci of large effect (Hayes and others 2010).

No matter what selection technology was implemented, stud managers would need to submit written reports such as those used as a data source in this study, or alternative data sources such as radiographs, as a basis for the determination of OC or other skeletal lesion phenotypes on an ongoing basis. Funding would be required to provide for data entry, radiograph interpretation (if required), tissue sample collection and genotyping (if required), calculation of EBVs or GEBVs, and personnel to carry out ongoing liaison and dialogue with participating studs.

6.5. The role of genetic testing

Genetic testing via SNP genotyping (and potentially other technologies) enables the use of relatively accurate genetic selection technologies such as GEBVs (described above). In the Australian and New Zealand Thoroughbred population, genetic testing would have multiple purposes. The first is to create GEBVs to guide genetic selection to minimise OC, specific OC
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component traits, or other skeletal lesions such as FRAG occurring PPP1 in the hind fetlocks. In this instance, breeders choosing new stallions and mares to become part of their breeding herd would use GEBVs to choose horses whose offspring are relatively unlikely to develop OC, particular OC component traits, or other skeletal lesions.

A second purpose would be to help stud managers identify current or future foals whose genomic profiles indicate an increased risk of developing OC, so that these horses can be prioritised when non-genetic factors contributing to the likelihood of an OC diagnosis cannot be minimised for the entire herd. For example, given that pregnancies in the Thoroughbred horse breed must result from natural matings, it is impossible for all foals from a popular stallion (producing 120 offspring) to be born at just one end of the breeding season. When being born early in the breeding season is important in reducing the prevalence of the particular lesion type under selection, stud managers could focus on ensuring that those mares whose offspring are relatively high risk are mated early to produce early foals. This would minimise the impact on the workload of the stallion and stud staff, while removing one important risk factor for those foals that are most susceptible.

A third purpose would be to provide some insight into the genetic merit of horses imported from overseas, whose performance in Australian or New Zealand conditions is unknown. While there is likely to be a relative scarcity of phenotypic data from the ancestors and relatives of these horses, prediction of genetic merit via GEBVs based primarily on SNP genotypes would provide insight into the likelihood of skeletal lesions in the offspring of these horses (depending on the heritability of the relevant lesion trait), when no other guide is available. In order to effectively utilise GEBVs on imported horses, these imports must be from the same genetic population as those horses from which the GEBVs were developed (i.e. the Australasian Thoroughbred population). Fortunately, the
pedigree analysis of Australian horses in the current study concluded that Thoroughbred horses in Australia, New Zealand, Europe and North America may now be regarded as one single genetic population. The analysis of population structure in the GWAS component of the current study also supported this conclusion (although it also found that the offspring of two Australian stallions were genomically distinct from the main international population and one another, indicating that the Australian Thoroughbred population is not completely homogenous).

A fourth purpose is to provide ongoing insights into the molecular mechanisms underlying the pathogenesis of OC and other skeletal lesions. As the number of horses with SNP genotypes and phenotypic data increases, it is likely that genome-wide association studies would identify further quantitative trait loci (QTL) beyond those identified in this and other studies (see Chapter 4). This may help identify genes and molecular pathways involved in these diseases, potentially allowing the development of treatments or preventive measures to further reduce the impact of these disorders. For example, in the current study, SNP-based case-control genome-wide association studies (GWAS) were carried out for 11 OC traits, FRAG occurring PPP1 in the hind fetlocks, and for the chestnut coat colour as a positive control using the Illumina Equine SNP50 beadchip, in a group of 140 horses. A check for population stratification identified one large cluster comprising the majority of the population and two small outlier clusters, each comprising the offspring of a single Australian-born Thoroughbred stallion. Despite only a small number of cases being available for these analyses, genome-wide significant quantitative trait loci were found on equine chromosome 30 for LYS at the SRMC3 in the fore fetlocks (within the large cluster only), and on chromosome 3 for the positive control chestnut coat colour (within all three clusters of this population, and within the large cluster only). Some plausible candidate genes that may be involved with OC were found in and around the QTL for LYS at SRMC3 on equine chromosome 30.
6.6. Managing osteochondrosis and other skeletal lesions in a changing population

The pedigree analysis of Australian Thoroughbred horses indicated that breeding practices in this population have undergone changes in the most recent decades. Some of these changes, such as the use of North American bloodlines, have already had effects that can be seen in the Australian Thoroughbred population. Other changes, such as recent increases in sire co-ancestry and decreases in sire numbers, are yet to have clear effects on the genetic makeup of this population, but are likely to lead to increases in the rate of inbreeding.

Changes in the genetic makeup of the Australian Thoroughbred population are likely to alter its response to selection to minimise the occurrence of OC. When genomic selection is utilised (i.e. the use of GEBVs based on the SNP effects estimated from a training set), the response to selection generally decreases over subsequent generations. The effects of these changes over time can be minimised by regarding selection as a dynamic process, where SNP effects are re-calculated on an ongoing basis to utilise the most recent genotypic and phenotypic data. It may also become necessary to place a high priority on minimising the loss of genetic variation in this population (i.e. minimising the rate of inbreeding). The goal of minimising the rate of inbreeding should be integrated into any genomic selection program from the outset, especially given that SNP data can provide a more accurate indication of inbreeding than pedigree data alone (Goddard and others 2010). This and other needs, such as selection for athletic performance, will always need to be considered in balance with selection to minimise OC in this population.
6.7. Other research targets for equine osteochondrosis in Australasian Thoroughbred horses

Many of conclusions about equine OC discussed here are based on research carried out on non-Thoroughbred horse breeds, in the northern hemisphere (the exceptions are studies of copper supplementation in New Zealand Thoroughbreds, (Gee and others 2005, Gee and others 2007) and dietary energy, protein, calcium and phosphorus in Australian mixed breed foals (C. J Savage and others 1993, C. J. Savage and others 1993)). In order to be able to confidently apply the results of northern hemisphere research on non-dietary contributors to OC, it would be valuable to validate these studies in the local Australian and New Zealand population. Research into the progression and healing of OC lesions and the role of genetic factors contributing to OC in this population would allow the further development and refinement of strategies to reduce its occurrence in this population.

The combined body of research for equine OC provides insights that may be applicable to the Thoroughbred horse populations of Australia and New Zealand. For example, some nutritional factors are associated with increased OC occurrence. Many components of a young horse's diet are under the control of the stud manager, particularly once that horse has been weaned from its dam. Stud managers aiming to minimise the occurrence of OC could ensure that their horses are not exposed to dietary factors that are known to cause or exacerbate the occurrence of OC.

6.8. Conclusions

This research project indicated that OC is a common disorder within the sub-population of high-value Australian and New Zealand Thoroughbred weanlings and yearlings. The heritability of OC
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Overall in this population was estimated to be 0.11 on the underlying scale, indicating that breeding programs intended to minimise the occurrence of OC could be successful. Individual OC traits with higher heritability estimates, such as OCD at the LTRF where heritability was twice that of OC overall, may show a stronger response to selection. Response to selection is proportional to the product of heritability and the phenotypic standard deviation; in this instance the phenotypic standard deviation on the underlying scale for OCD at the LTRF is approximately 1.5 times that of OC overall, so that selection against OCD at the LTRF would be expected to be approximately three times stronger than against OC overall given the same selection intensity. Considering both the prevalence and effects of various OC component traits, the stifle OC traits OCD at the LTRF and SCL at the MFC should be prioritised for genetic selection. These traits (OCD at the LTRF and SCL at the MFC) have positive genetic associations with another OC trait, LYS at the SRMC3 in the fore fetlocks, and with FRAG occurring PPP1 in the hind fetlocks, which is also linked to poor race performance. Together, this cluster of traits is a good target for genetic selection to minimise OC in this population, and effective selection would result in financial benefit to breeders as well as improving the welfare of the horses. The data required in order to implement genetic selection in this population is easily available, but the creation and maintenance of such a program would require ongoing financial investment. This investment could come from either the industry as a whole, or individual breeders who are open to embracing selective breeding technologies (e.g. the use of EBVs or GEBVs). There is also the potential to extend any genetic selection program to include selection for particular athletic traits, or selection against other disorders with a genetic component.

Genetic testing technologies such as SNP genotyping, which would be required for the use of breeding programs utilising GEBVs, could be used for purposes other than genomic selection
within the Australian and New Zealand Thoroughbred population. In some instances, genotypic data could guide the selection of horses to be imported from overseas, by providing some insight into the probable OC status of any future offspring when no other guide is available. These data also have the potential to provide ongoing insights into the molecular mechanisms underlying the pathogenesis of OC and other skeletal lesions. For example, the current study identified a genome-wide significant QTL for LYS on equine chromosome 30. From this QTL, candidate genes were identified that could be part of the biochemical pathways underlying the pathogenesis of OC.

Some non-genetic factors or interactions were identified as affecting the prevalence of some OC traits, non-OC traits, and OC super-set traits (where both OC lesions and non-OC lesions are encompassed by the trait definition). Three of these factors have the potential to be under the control of stud managers: firstly, aspects of stud management such as diet, that may contribute to the occurrence of OC; secondly, the age at which a yearling is sold, by region; and thirdly, the time at which a foal is born (which can be altered if the mare is not already pregnant), by region. The effect of these factors differed between traits, and they were not significant for all traits. Therefore, changes to husbandry methods undertaken with the aim of minimising OC are likely to alter the prevalence of some, but not all, OC component traits.

The current study also included a pedigree analysis of the Australian Thoroughbred population, using data provided by the Australian Stud Book. The impact of past changes in breeding practice, including changes in sire usage and the origins of imported breeding stock, were examined via trends in the rate of inbreeding and loss of genetic variability due to unequal use of founders, population bottlenecks and genetic drift over time. There has been a low rate of loss of genetic variability in the Australian Thoroughbred population since 1973. This rate of loss is now increasing.
and is likely to increase further in coming decades. The importation of breeding stock from traditional sources (New Zealand, Europe and North America) is no longer increasing genetic variability. The number of sires is decreasing and their co-ancestry is increasing. Any strategies undertaken to minimise OC in this population over the coming decades will need to take into account these likely changes to the Australian Thoroughbred population.

6.9. References


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