USING A MOUSE MODEL TO UNDERSTAND THE EFFECT OF HYBRIDIZATION ON SKELETAL AND PELAGE TRAIT VARIATION IN MAMMALIAN HYBRIDS

by

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Declaration

I, Robyn Humphreys, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university

This thesis has been submitted to the Turnitin module, and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

Robyn Humphreys

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Abstract

Hybridization is thought to have played an important role in human evolution, with hybridizing groups having significant differences in soft tissue trait variation. Ectodermal trait variation is of interest because primate hybrids show increased atypical non-metric dental and cranial trait variation thought to be the result of interactions between parental genomes which have diverged for ectodermal trait development (including hair and tooth development). There were also differences between hybridizing hominin groups for limb measurements which have changed significantly throughout human evolution. Here a mouse model is used to look at the effect of hybridization on coat morphology and long bone length. Using standardized photographs, the differences in mean RGB values for the dorsal and ventral coat were used to determine whether the hybrids were different from their parents for pelage colour of different regions of the body, dorsal ventral colour contrast, and levels of variation in coat colour. The sample is composed of parents from one specific and three sub-specific crosses, as well as F1, F2 and first generation backcrossed (B1) hybrids. Long bone measurements of the forelimbs and hind-limbs were collected from micro-CT scans of the sub-specific F1 hybrids and their parents. Previous data have shown that hybridization can have variable morphological outcomes: hybrids can look like one of the parents, they can be intermediate, or they can have extreme traits outside of the range of variation of the parents. Our results indicate that morphological outcomes for coat colour in F1 hybrids depends on factors such as genetic distance. However, the genetic background of one of the strains used for this experiment might contribute the transgressive phenotype of some of the F1 hybrids. Hybrid morphology also changes in subsequent generations (F2 and B1) as new recombinants formed, with transgressive coat colour phenotypes sometimes appearing even if they are not present in the F1 hybrid groups. Phenotypes produced in F1 hybrids are also seen in subsequent generations of hybrids. All sub-specific F1 hybrids were transgressive for long bone length. Compared to parental groups hybrids have a different relationship between the long bones of the forelimb (ratio of humerus to ulna). This is in line with previous data from primate hybrids, that shows that changes in the relationships between different regions of the body occurs in hybrids producing novel phenotypes. The inter-membral indices are not significantly different from one of the parents for two of the crosses. This data shows that hybridization can produce novel pelage phenotypes over multiple generations. There were many transitions in hair/skin morphology during human evolution and these tissue groups were and are under a great deal of selective pressure due to their direct interaction with the environment. Thus, understanding how these traits are impacted by hybridization will be important for disentangling how hybridization

affected our evolutionary trajectory and ability to occupy new regions of the world. Post cranial data, indicates that F1 hominin hybrids might have longer limbs in relation to parental populations, more work needs to be done on the post cranial remains of posited hominin hybrids as well as pedigreed mammalian hybrids to determine if this is a pattern which can be used to identify hybrids in the fossil record.

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model as a scientist but as a woman who actually does it all with a lot of love, kindness and a whole lot of grit and I am very proud to call you my supervisor.

Chapter 1: Introduction

For a long time, there was a debate about whether Neanderthals and modern humans interacted after the initial migration of modern humans out of Africa and about the nature of any possible interaction. Evidence from the archaeological and fossil records was used to argue for admixture between Neanderthals and modern humans (Bräuer, 1981; Duarte *et al*., 1999; Soficaru *et al*., 2007; Ackermann, 2010), and this admixture was confirmed by the sequencing of autosomal genes of ancient DNA (aDNA) from Neanderthals (Green *et al*., 2010). Specifically, a comparison of the Neanderthal and modern human genomes showed that modern humans outside of Africa have approximately 1 – 3% of their genomes derived from Neanderthals (Green *et al*., 2010). Ancient DNA has also been used to identify another group of hominins known as the Denisovans, which contributed to the genomes of South East Asians (Meyer *et al*., 2012). Ancient DNA has painted a picture of extensive gene flow between many hominin groups over an extended period and has shown that hybridization has had an impact on our own evolutionary trajectory. Hybridization between modern humans, Neanderthals and Denisovans is thought to have occurred around 50 – 100 thousand years ago (kya) there is also recent evidence that hybridization took place as far back as 270 kya (Posth *et al*., 2017). There are also indications from the analysis of modern genomes that extensive hybridization occurred in Africa more recently and in deeper time after the split between chimps, gorillas and the lineage which results in modern humans (Hammer *et al*., 2011; Lachance *et al*., 2012; Hsieh *et al*., 2016)

Ancient DNA provides snap shots into when hybridization occurred and how often in occurred. It does not assist us with identifying all hybrids in the fossil record because we don't have aDNA for all fossil material as it decays quickly in many environments. Understanding the morphological outcomes of hybridization will be important for identifying hybrids in the fossil record. This will be important for helping us understand where hybridization occurred and how hybridization impacted the morphology of hominins and interaction between them. To answer these questions, we need to be able to identify hybrids in the fossil record based on morphology, thus some work has been done on mammalian hybrids to determine if there are common patterns that can be used to identify hybrids in the fossil record. Patterns have already been identified with mammalian hybrids showing increased frequency of otherwise rare non-metric features (particularly in the dentition) and increased complexity in cranial sutures compared to parental groups (Cheverud, Jacobs and Moore, 1993; Ackermann, Rogers and Cheverud, 2006; Ackermann, 2007; Ackermann et al., 2010; Ackermann et al., 2014). Work also

needs to be done in modern mammals to understand the developmental underpinnings of these traits so that the genetic and morphological data can be combined to disentangle what happens/happened when hybridization events occurred.

Analyses of the crania of hybrids of yellow and olive baboon showed cranial measurements larger than the expected mid parental value for hybrids compared to parents but no extensive heterosis (Ackermann *et al*., 2006). However, the hybrids were transgressive for dental traits and had increased non-metric dental and sutural trait variation (Ackermann *et al*., 2006). It is thought that the dentition responded differently to hybridization when compared to the crania because they are derived from different germ layers (Ackermann, 2007). Teeth are derived from the ectoderm while the cranium is derived from the neural crest and the mesoderm (Pispa and Thesleff, 2003). It is hypothesized that divergence in ectodermal development genes is the cause of the transgressive phenotypes of the hybrid baboons (Ackermann, 2007). Along with teeth other appendages which arise from the ectoderm include hair, sweat glands and mammary glands (Pispa and Thesleff, 2003). These soft tissue traits are all very variable between mammals including primates and have undergone a great deal of evolutionary change throughout human evolution.

There have been major changes in the morphology and function of skin and its appendages during human evolution. These changes are important for adaptation to new environments, communication between primates, and are under a variety of selective pressures in primates and modern humans (Bradley and Mundy, 2008). In terms of human evolution first there was the transition to being functionally hairless, with modern humans having finer hair with a reduced morphology compared to other apes (Jablonski, 2004). Humans also have a thicker epidermis and higher levels of skin colour variation when compared to other primates because our skin is exposed different levels of UV in different regions of the world (Montagna, 1972; Jablonski, 2004). Humans also vary in how hair is distributed across the body, with differences in density of the number of follicles, the size of those follicles, and hair shaft diameter (Montagna, 1972).

Yellow and olive baboons are also described as having differences in their hair/pelage, including in texture, thickness and colour (Maples and McKern, 1967; Ito et al., 2001). Thus, the question arose as to whether changes in dentition in the hybrids (as described above) might result from the merger of genomes of hybridizing taxa that differ in pelage, with these pelage differences caused by divergence in the genes involved in ectodermal development (Ackermann, 2007). This might provide an important link between the external and skeletal morphology, and allow us to use skeletons to predict external phenotype and vice versa. However, the nature of this relationship is difficult to determine in primates because of their long reproductive periods, unknown pedigree of the hybrids

and not having skeletal, cranial and pelage data from the same specimens. Thus, mice serve as a great model animal for looking at this question because they breed quickly can be produced in large numbers, and data can be collected from bred mice for the coat as well as skeletal and cranial material.

There is currently very little work on pedigreed hybrid mammals looking at variation in coat colour. A lot of the work is available for mixed hybrid populations in wild hybrid zones; there is limited research on F1 hybrids and the samples sizes for these are small (Myers and Shafer, 1979; Hamada *et al*., 1988; Aguiar, Pie and Passos, 2008; Fuzessy *et al*., 2014). From the literature, we know that hybrids can vary in coat colour in relation to parental groups with some hybrids being cryptic (i.e. looking similar to a parent), other hybrids having transgressive traits, and still others with intermediate coat colour or mosaic with a combination of parental traits (Hamada *et al*., 1988; Aguiar *et al*., 2008; Fuzessy *et al*., 2014). We also see increased variation in primate hybrids in terms of facial coat patterning (Fuzessy *et al*., 2014). There has been considerable research on morphological variation in primates hybrids in the wild, however more work needs to be done on pedigreed hybrids (Hamada *et al*., 1988; Bynum, 2002; Aguiar *et al*., 2008; Fuzessy *et al*., 2014).

This study

The primary objective of this project is to quantify the phenotypic outcomes of hybridization in pelage and long bones in hybrid mice. These mice come from a Hybrid Mouse Project (Ackermann Lab) that generated one specific cross between *Mus musculus* and *Mus spretus,* and a series of sub-specific crosses of *Mus musculus* mice and their hybrids, to determine the morphological outcomes of hybridization in a model mammal. For the sub-specific crosses first generation (F1), second generation (F2) and first generation backcrossed hybrids (B1) were produced. The project aims to identify patterns that will make it easier to identify hybrids in the fossil record based on morphology. Thus this thesis forms part of an on-going project which aims to understand the developmental underpinnings of these patterns. In this regard understanding the effect of hybridization on coat colour will be important.

This dissertation research aims to determine the following for F1 hybrids: the effect of hybridization on pelage colour and patterns in F1 hybrids and how they compare to parental groups; whether there are broad patterns in terms of the outcome of hybridization in coat colour in the F1 hybrids; how hybrids compare to parents for long bone measurements. For the subsequent generations of hybrids this research will determine if F2 hybrids tend to exhibit more transgressive traits, and whether they are more variable than their F1 parents as is expected, with new

recombinants forming in the F2 hybrids. With the B1 hybrids this research aims to determine if traits introduced in the F1 hybrid population carry through in backcrossed hybrids. Finally, it will focus on whether it is possible infer developmental changes from final pelage morphologies in hybrids.

This thesis is composed of seven chapters. There are two background chapters, the first (Chapter 2) focuses on general hybrid theory, and the second (Chapter 3) focuses mainly on the role of hybridization in human evolution. Chapter 2 provides background information regarding our current understanding of how hybridization affects evolutionary process for various organisms, including the phenotypic outcomes of hybridization and how this has affected the evolutionary trajectory of various organisms including mammals and primates. Developmental and molecular biology will be considered in order to understand what processes might be at play and acting to produce some of the common phenotypic outcomes of hybridization. Chapter 3 looks at the role of hybridization in human evolution specifically and what we know about the impact of hybridization on human evolution from both the fossil record as well as the available genomic data (aDNA from archaic hominins and genetic data from modern populations). This chapter will consider some of the theories regarding hybridization pre- and post- sequencing of Neanderthal aDNA, and the research possibilities which result from the constant generation of data regarding the genomic sequences from the past.

Chapter 4 is the Materials and Methods chapter. In this chapter the strains used for this project, as well as the different hybrid groups produced, are discussed. This chapter includes a description of data collection from digital photographs and Micro-CT scans, what statistical test were used to determine how hybrids were different from their parents and from each other, how hybridization affects variation in hybrids, and how hybrid morphology changes over time by looking at the pelage morphology of subsequent generations of hybrids, specifically F2 and B1 hybrids.

Chapter 5 is the results chapter and discusses the outcomes of hybridization in F1 mouse hybrids, focusing on the effect on pelage and long bone length. This is followed by analysis of the effect of hybridization on pelage variation in F2 and B1 hybrids. The results show that there are variable outcomes for F1 hybrids for pelage colour and patterning and factors such as genetic distance could play a role in determining phenotypic outcomes of hybridization. The F1 hybrids were also all transgressive for long bone length with all the hybrids having longer long bones than both parental groups. Transgressive phenotypes are not only produced in F1 hybrids, there are also transgressive traits occurring subsequent generation of hybrids (F2 and B1 hybrids), as well as the persistence of phenotypes introduced in the F1 hybrid groups.

Chapter 6 is the discussion and conclusion chapter, which compares the results from this project to some of the previous data we have for pelage colour in hybrids, and considers how factors such as genetic distance might affect the phenotypic outcomes of hybridization. This chapter also ties in some of the developmental biology introduced in the first chapter, showing how combining our current knowledge of developmental biology with morphological data can help us make insightful inferences about the consequences of hybridization in mammals.

The last chapter is the Conclusion (chapter 7). In this chapter I discuss how the results from this project might be applicable to human evolution and particular the effects of hybrization on soft tissue traits such as hair and skin/ hair colour and what this could mean for the role of hybridization in human evolution. I also discuss how the long bone results might be applicable to identifying hybrids in the fossil record. I end off this chapter with limitations to this study and future work.

Chapter 2: Hybridization an overview

Hybridization and its role in evolution

Hybridization occurs when two independently evolved lineages, that share a common ancestor, meet, mate and reproduce (Arnold and Hodges, 1995). It occurs across all life forms including plants, insects, birds and mammals (Grant and Grant, 1992; Rieseberg, 1997; Arnold and Meyer, 2006; Ackermann et al., 2006). It also occurs at different taxonomic levels including between sub-species, species and even between genera (Arnold, 1992). Hybridization occurs in captivity as well as in the wild. How successful hybridization is depends on many factors which will be further explored below.

Although hybridization is common, the impact of hybridization on evolution has been minimized historically. Reasons for minimizing its impact include the belief that hybridization is a rare process (Arnold, 1992; Mallet, 2007). However, there is a great deal of evidence that hybridization has occurred with some frequency (Mallet, 2007). It has been important for shaping the genomes and evolutionary trajectories of many mammals, including primates such as New World monkeys, Old World monkeys and apes (Arnold and Meyer, 2006; Ackermann, 2010; Zinner, Arnold and Roos, 2011, Fuzessy *et al*., 2014). Figure 2.1 highlights what we know about hybridization in hominins, old world monkeys and apes. Hybridization between primates has been recorded in natural hybrid zones and in captivity (Bynum, 2002, Myers and Shafer, 1979). Molecular data indicate that there is a great deal of phylogenetic discordance resulting from ancient hybridization events between primates (Arnold and Meyer, 2006). Recently there have also been many morphological studies of primate hybrid zones and on primate hybrid skeletal material (Bynum, 2002; Ackermann *et al*., 2006; Fuzessy *et al*., 2014).

Prevalent hybridization has also been recorded in other organisms, including birds, where around one in ten bird species is thought to hybridize (Grant and Grant, 2002). Hybridization is common among plant species and botanists have long known the importance of hybridization in evolution, with many plant species recognised as of hybrid origin (Rieseberg, 1997; Mallet, 2007). It is especially important in the agricultural industry where hybrids are produced because they often have higher yields, are more fertile and better able to reproduce (Yu *et al*., 1997).

In the sections that follow I will discuss what is known about the production of hybrids, their viability and their ability to reproduce, some of the phenotypic outcomes of hybridisation, and work on the genotype/phenotype and environment relationship and how this might affect the outcomes of

hybridization. After the general overview of hybrid theory and what is known from studies on the impact of hybridization on organisms, I will discuss some examples of mammal hybrids, focusing on our understanding of primate hybrids.

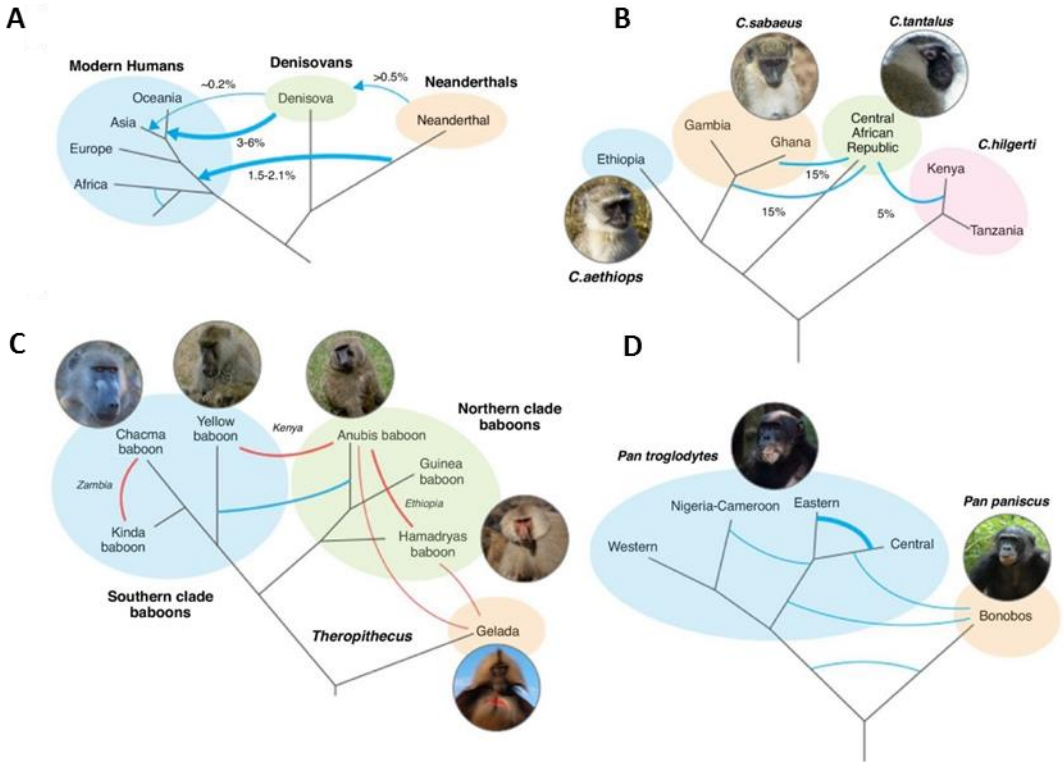

Figure 2.1: Image modified from Tung 2017 shows how extensive hybridization between different primates groups has been, it includes both ancient and on-going admixture events. Admixture and hybridization in (A) Hominins (modified (B) the vervet monkey genus Chlorocebus (C) baboons and geladas; and (D) chimpanzees and bonobos.

Hybrid viability

One reason hybridization has been overlooked as an important mechanism for driving evolutionary diversification is due to the belief that it is often unsuccessful due to pre- and post-zygotic barriers (Arnold *et al*., 1999; Mallet, 2007). There is a great deal of focus on biological changes that occur during speciation resulting in barriers to gene flow between two closely related lineages (Burke and Arnold, 2001). Many mechanisms have been identified which explain some of the post-zygotic barriers to hybridization, which are often caused by Dobzansky Muller incompatibilities (Burke and Arnold, 2001). These incompatibilities result in inviable hybrids due to genes which have functionally diverged in the two parental populations being unable to interact in the hybrid offspring

(Burke and Arnold, 2001). This results in the disrupted development of the hybrid or no development at all. However, there is variability in the viability of hybrid offspring (Arnold, 1992; Burke and Arnold, 2001). A small proportion of the hybrid offspring may be viable along with variability in the ability of F1 hybrids to reproduce. One would expect that due to this variability, and only a small proportion of offspring being viable, hybridization might not have a great impact on parental populations. However, in these scenarios hybridization can still have an impact if there are many opportunities for hybridization to occur, even if only a few viable offspring are produced and go on to reproduce (Arnold *et al*., 1999). Excellent examples of this are the three hybrid species which are the result of hybridization between the sunflowers *Helianthus annuus* and *H. petiolaris* (Rieseberg, Carter and Zona, 1990; Lexer et al., 2003; Rieseberg et al., 2003). Hybridization between these two species resulted in F1 hybrids that have pollen fertility of around 14% on average with some having 0% pollen fertility, and the F2 and B1 hybrids result in a seed set of 1% and 2% respectively, and yet three hybrid species have been identified as a result of this cross (Arnold and Hodges, 1995; Arnold et al., 1999). Thus, even though the first few generations might be unfit they can nevertheless have an impact on subsequent generations if there are many opportunities for hybridization to occur (Arnold *et al*., 1999).

Hybrid fitness

Even without barriers to reproduction hybrids are often still not perceived to have a substantial impact on diversification. This is because they are generally considered to be less fit than parental groups and thus limited in their impact on parental populations. However, how fit a hybrid is relative to the parental group depends on many factors. In a study looking at the fitness of hybrids relative to their parental groups across plant and animal species it was shown that hybrids are often as fit as one of the parents, or more fit; only 16 out of 44 crosses were less fit than both their parents (Arnold and Hodges, 1995; Arnold and Martin, 2010). Hybrids can be less fit than their parents due to endogenous factors, which will result in hybrids being less fit regardless of the environment (Burke and Arnold, 2001). This is most likely due to incompatibilities which arise as result of the two co-adapted genomes of the parents coming together in the hybrid; even though they might be readily produced the hybrids might still be less fit than the parents (Parris, 2001). They can also be less fit due to exogenous factors such as the environment (Burke and Arnold, 2001). The phenotype environment interaction can be an important factor for determining hybrid fitness and the fitness of their descendants. Hybrids might be more fit than their parents in novel or intermediate environments at the edges of the ranges of the

parental groups (Burke and Arnold, 2001; Arnold and Martin, 2010). Good examples of phenotype-environment interaction are Louisiana iris hybrids which are the result of a cross between *Iris fulva* and *I. brevicaulis* (Arnold and Martin, 2010). Fitness of the hybrid is dependent on the hybrid phenotype as well as the environment. Hybrids more similar to one or the other parents thrived in the native environment of the parent they looked like, while intermediate hybrids were less fit in parental environments (Johnston et al., 2001; Arnold and Martin, 2010). In another study, it was shown that stickleback fish F1 and F2 hybrids were fitter than their parents in laboratory conditions, but when placed in the parental environments they were less fit (Hatfield and Schluter, 1999). Thus, most of the selection against the hybrids was due to their fitness in the environmental context and not due to their inherent/endogenous unfitness (Hatfield and Schluter, 1999). There is also variation in the fitness of the hybrid in relation to their parents (Arnold and Hodges, 1995). Some hybrids might be fitter than their parents while others are of intermediate fitness or are less fit (Arnold and Hodges, 1995; Arnold and Martin, 2010). Thus, when determining the fitness of hybrids it might be best to group the hybrids and determine how fitness varies across groups, because the average fitness of the hybrid group might be low in relation to the parental group when in fact there are hybrids fitter than the parents.

When hybrids are less fit than their parents this is known as hybrid inferiority. We know a lot about hybrid inferiority due to endogenous factors and hybrid incompatibility. Hybrid incompatibility is the result of two co-adapted genomes which have evolved independently coming together, with alleles that have evolved independently in the parental populations interacting in the hybrid resulting in hybrid breakdown (Burke and Arnold, 2001). Negative epistasis between genes is one of the primary reasons for hybrid inferiority (Burke and Arnold, 2001). Most work on hybrid inferiority and its genetic underpinnings comes from studies of hybrid flies and plants (Burke and Arnold, 2001). These have shown that the effects of hybrid inferiority may not always show up in the F1 hybrids but may show up in F2 hybrids as is the case with hybrids produced from crosses of genetically differentiated populations of copepods *Tigriopus californicus* (Burton, 1990; Burke and Arnold, 2001)

At the other end of the spectrum we have hybrids which are fitter than their parents and display hybrid superiority. Hybrid superiority can be the result of new phenotypic traits outside of the range of variation of the parents. The novel genetic combinations and phenotypes may provide new opportunities for different evolutionary trajectories (Rieseberg, Archer and Wayne, 1999). When F1 hybrids have phenotypic traits outside of the range of variation seen in either parental group these are known as transgressive traits (Rieseberg *et al*., 1999). Transgressive traits can lead to hybrid superiority resulting in the hybrids being fitter than their parental groups. However, it is important

that this fitness be passed down to subsequent generations in order for it to have an impact on the evolutionary trajectory of the hybrids. Hybrid vigour can also arise in later generations of hybrids, when new genomic combinations arise (Burke and Arnold, 2001). This can be due to new favourable epistatic interactions as has been recorded in sunflower *H. annuus* and *H. petiolaris* hybrids (Rieseberg et al., 1996; Burke and Arnold, 2001). However, establishing the relationship between extreme phenotypes and increased fitness of the hybrids has not been done and thus this is not a proven mechanism of increased fitness of hybrids in relation to their parental groups (Burke and Arnold, 2001). It's been suggested that hybridization may be important in cases where one or both parental populations are small and have accumulated deleterious genes (or inbreeding depression). Hybridization ameliorates this situation by introducing new genes into the parental population (Ellstrand and Schierenbeck, 2000; Burke and Arnold, 2001)

From the above it is clear that we have some insight into the fitness of hybrids but for understanding this in context, and determining the true effect of hybridization on fitness in relation to parents, it is the long term studies of hybrids that have been the most illuminating. These studies offer us the ability to look at the interaction between genotype/phenotype and the environment, what factors influence the fitness of hybrids and how hybridization plays out over time. Some of the best characterised long-term studies of hybridization include those on Louisiana irises, sunflowers and Darwin's finches (Arnold and Hodges, 1995; Arnold and Martin, 2010).

Long-term studies have been conducted on sunflowers because their evolutionary history indicates that there has been reticulation between diverging groups over time (Rieseberg, 1997; Rieseberg *et al*., 1999). One of the hybrid species produced is *H. paradoxus*, is more fit than its parents in extreme environments (Rieseberg, 1997; Rieseberg *et al*., 1999). Its ability to inhabit these environments is a result of its hybrid origin, which produced a transgressive phenotype. Genes associated with this phenotype have different gene expression patterns when compared to parental groups (Rieseberg et al., 1999; Arnold and Martin, 2010). The formation of the *H. paradoxus* hybrid specieis, along with the data showing the low viability of initial sunflower hybrids, shows the impact of hybridization over an extended period of time when many opportunities for hybridization are coupled with transgressive phenotypes in a novel environment. Long terms studies have also been done with the Louisiana irises to look at the role of genotype-environment interactions that occur in order to produce hybrid lineages. These have consisted of multi-generational studies of hybrids. Here, specific quantitative trait loci were identified which were associated with hybrids being more fit than parents as measured by survivorship. Hybrids could again be grouped by fitness, with some being

fitter, some less fit and others of intermediate fitness in relation to the parents (Arnold and Meyer, 2006).

There are fewer long-term studies looking at the fitness of hybrid animals. One such study included research on hybridization in Darwin's finches on the Galapagos Island of Daphne Major (Grant and Grant, 2002). Hybrid finches are variable in beak size and shape, which are important determinants of fitness (Grant and Grant, 1992; Grant and Grant, 1994; Grant and Grant, 1996). Some hybrids were more fit than their parents during periods of perturbation because they could exploit a wider variety of seeds whereas their parents' beaks were more specialised (Grant and Grant, 1992; Grant and Grant, 1996). The hybrids did not have a difference in fitness in terms of reproduction when compared to conspecifics, had longer survivorship than their conspecifics and were able to backcross into parental populations during the period of observation on the island (Grant and Grant, 1992). Recently, a new hybrid lineage formed on the island of Daphne Major, this hybrid lineage is the result of a cross between *Geospiza fortis* and a *G. conirostris*, which migrated to the island (Lamichhaney *et al*., 2017). The F1 hybrid produced from this cross backcrossed into the native *G. fortis* population on the island, while the B1 hybrids produced from this cross sib-mated and all subsequent generations (4-6 generations) are derived from this cross (Lamichhaney *et al*., 2017). These hybrid finches are larger, have larger bills and a distinctive song, thus they don't breed with the *G. fortis* finches which are smaller (Lamichhaney *et al*., 2017). The morphological traits of the hybrid lineage, along with isolated breeding are thought to be the cause of their ecological success (Lamichhaney *et al*., 2017).

Thus, overall the picture painted above is that hybrid viability and success does not fit into a neat box. Instead we see variability in hybrid offspring in terms of fitness; we also see an important genotype/phenotype-environment component. The effect of hybridization also changes over time as new recombinants are formed and parental populations are impacted by back crossing and introgression of new genetic material.

Hybrid Zones

Hybrid zones are regions of contact between two lineages that are genetically distinct (Barton and Gale, 1993; Jiggins and Mallet, 2000). In these regions they meet, mate and reproduce hybrids that are viable (Barton and Gale, 1993). The size and the extent of the hybrid zone can vary; they can be narrow or span over many kilometres depending on different factors (Barton and Gale, 1993; Jiggins and Mallet, 2000). Multi-generational hybrids are present, with increased variation within the hybrid

zone both morphologically and in terms of fitness due to new genetic combinations. Hybrid zones are important for understanding the formation and maintenance of species boundaries and thus have been well studied in that regard (Barton and Gale, 1993; Jiggins and Mallet, 2000).

Cline theory has been important for understanding the dynamics of hybrid zones (Barton and Gale, 1993). Hybrid zones often produce clines based on changes in frequencies of alleles or morphological traits contributed by either parental group across the hybrid zone (Barton and Gale, 1993; Gay et al., 2008) . Clines are maintained by intrinsic factors in which case the fitness of the hybrid regardless of the environment will play a big role in the cline, or exogenous factors in which case the environment will play a big role in the distribution of traits or alleles (Jiggins and Mallet, 2000). Clines can be maintained by a balance between the dispersal of the hybrids from the hybrid zone and selection against the hybrids outside of the hybrid zone. On some occasions this could be due to heterozygote disadvantage of the hybrid with very little difference in the environment (Jiggins and Mallet, 2000). Hybrids might also be more fit than their parental groups in the hybrid zone, known as bounded hybrid superiority; here the difference in environment maintains the cline and the range of the hybrids (Barton and Hewitt, 1985).

Using allele frequencies to determine which alleles have crossed hybrid zones and moved from one parental population into the other can be useful for understanding which regions of the genome have diverged between the parental groups (Barton and Gale, 1993; Jiggins and Mallet, 2000). Cline theory has also been useful for understanding the movement of hybrid zones and how parental populations are affected (Jiggins and Mallet, 2000; Buggs, 2007). Hybridization might have a greater impact on one parental population resulting in asymmetrical introgression (Buggs, 2007). Movement of the hybrid zone can be seen by the introgression of neutral markers from one parental population into another. In this situation one population will have higher levels of introgression than the other due to the advance of a parental group (Buggs, 2007), potentially resulting in introgression into what might be considered “pure” parental populations. This was shown to occur in a mouse hybrid zone with allozyme markers indicating that there was asymmetrical introgression from *Mus. musculus domesticus* into *M. m. musculus* (Buggs, 2007). Mate preference can also affect movement of the hybrid zone. Male dominance can play a role, resulting in only males from one parental group producing hybrids, moving the hybrid zone into the range of the other parental group (Barton and Hewitt, 1985; Jiggins and Mallet, 2000; Buggs, 2007). If there is a mate preference in one group but not in the other this could also result in the hybrid zone moving in favour of the group where there is mate preference for conspecifics. Hybrid zones can also move due to changes in environment, or climate (Buggs, 2007).

Though our understanding of hybrid zones has somewhat improved there is still a need to understand how factors such as population size and adaptation affect hybrid zones. There is also a need to do more long-term studies of hybrid zones in order to understand how they move, and what factors affect their movement. Hybrid zones are dynamic and they have been important for understanding how hybridization plays out in natural environments. Much of the morphological data that we have for primate hybrids come from hybrid zones and thus it is especially important to understand their dynamics when considering the effects of hybridization on primate morphology and human evolution.

Phenotypic outcomes of hybridization

The phenotypic outcomes of hybridization can be quite variable. A hybrid's phenotype is dependent on many factors such as what generation of hybrid they are (e.g. F1 hybrids can be very different from backcrossed hybrids). It also depends on the lineages hybridizing. Even within the F1 hybrids there could be a great deal of phenotypic variability (Grant and Grant, 1994; Arnold and Hodges, 1995). What is expected when hybridization does occur is that hybrids will have intermediate trait values when compared to the parental groups, close to the mid-parental range in an additive genetic model. However, when hybridization occurs there are often deviations from this expectation (Grant and Grant, 1994; Arnold and Hodges, 1995).

Hybrids can be cryptic, looking like one of the parents and only identifiable through molecular techniques, they can have an intermediate phenotype, or they can have mosaic phenotypes which result from having a combination of parental traits (Rieseberg *et al*., 2003; Ackermann *et al*., 2006). In some instance hybrids can be heterotic, i.e. outside of the range of parental phenotypes. Heterotic traits were first identified in agricultural contexts (Lippman and Zamir, 2007). For agricultural crops heterotic traits are traits that are larger or allow the hybrid to be more productive than the parents (Lippman and Zamir, 2007). These are recognized in many agricultural crops with a large literature regarding heterosis focused on understanding heterosis in maize and rice hybrids. Considerable research has been done to determine which genomic factors and patterns are common among crosses that have phenotypic superiority in relation to their parents (Lippman and Zamir, 2007).

Since heterosis was first identified there have been many attempts to understand the underlying causes. Quantitative genetics was used to explain some of these traits and heterosis was initially

attributed to dominance, and overdominance (Lippman and Zamir, 2007; Hochholdinger and Hoecker, 2007). In terms of dominance producing heterosis in hybrids the theory is that recessive alleles accumulate in parental groups but are masked in the hybrid by alleles from the other parent (Lippman and Zamir, 2007; Hochholdinger and Hoecker, 2007). Overdominance results in hybrids being superior to the parental groups because they are have higher levels of heterozygosity than the parents, resulting in a phenotype which is superior to the homozygous state found in either parent (Lippman and Zamir, 2007; Hochholdinger and Hoecker, 2007). Many genes associated with heterotic traits have been identified using QTL analysis (Hochholdinger and Hoecker, 2007; Lippman and Zamir, 2007; Chen, 2013). More recently with advancements in molecular techniques it has been easier to identify some of the mechanisms responsible for heterosis (Birchler *et al*., 2010; Chen, 2013). The consensus is that there are allelic interactions in the hybrids which result in altered gene expression causing differences in trait means for growth, stress tolerance and fitness of hybrids (Chen, 2013). Though the dominance hypothesis has been used to explain heterosis in hybrids, what we see is that genes which result in heterosis may not necessarily be deleterious in parental populations so there is no unmasking in the hybrid offspring (Birchler *et al*., 2010; Chen, 2013). Heterosis is not the opposite of inbreeding depression; it has been suggested that the terms overdominance and dominance should be abandoned because they don't adequately explain how heterosis for traits occurs in hybrids (Hochholdinger and Hoecker, 2007; Chen, 2013).

When looking at patterns of gene expression, genome wide changes in gene expression of intraspecific hybrids have been noted (Chen, 2013). This includes rice, maize, wheat and *Arabidopsis* hybrids. Gene expression changes in hybrids are also correlated with changes in gene expression networks resulting in heterosis in metabolic processes and development (Hochholdinger and Hoecker, 2007; Chen, 2013). Research investigating gene expression patterns of hybrids compared to parental groups is most common. Differences in gene expression are considered additive in which case the gene expression is similar to the expected mid-parental mean, or non-additive, which means that the expression is smaller or larger than the expected mid parental mean, and can be smaller than the parent with the smallest level of expression or larger than the parent with the largest level of gene expression (Swanson-Wagner et al., 2006; Hochholdinger and Hoecker, 2007; Chen, 2013). There might be a relationship between how many genes have non–additive gene expression and the heterotic effect on hybrid offspring (Chen, 2013). Though a lot of work has been done, it has been done at different stages of development and on different tissue groups and there are no common genes which have been identified; instead heterosis can be attributed to changes in global gene expression in the hybrids (Hochholdinger and Hoecker, 2007).

F1 hybrids can also have transgressive traits; in this case the hybrid has trait values outside of the range of variation seen in either parental group (Rieseberg et al., 1999; Dittrich-Reed and Fitzpatrick, 2013). Transgressive traits can also be a novel combination of traits which provide an adaptive advantage to the hybrids (Dittrich-Reed and Fitzpatrick, 2013). Transgressive phenotypes can be in both a positive or negative direction relative to both parents (Rieseberg et al., 1999; Dittrich-Reed and Fitzpatrick, 2013). Transgressive traits in hybrids are thought to be common; in a meta-analysis of plant and animal hybrids 58% of traits from 113 studies of plants hybrids had transgressive phenotypes (Rieseberg *et al*., 1999). Crosses from inbred lines were also more likely to result in the production of transgressive traits when compared to wild outcrossed hybrids (Rieseberg *et al*., 1999). Less transgressive traits were reported in animal hybrids with only 31% of traits reported as transgressive in the 58 animal studies (Rieseberg *et al*., 1999). In animals, most of the traits which showed transgressive segregation were morphological traits, life history traits, behavioural traits and other qualitative traits (Rieseberg *et al*., 1999). The types of traits which can be transgressive include morphological traits, the biochemical composition of tissues, physiology, life history and tolerance to biotic and abiotic factors (Rieseberg *et al*., 1999). Transgressiveness in tolerance traits is important for hybrids as it can contribute to higher fitness relative to their parental groups in new environments (Rieseberg *et al*., 1999). Hybrid ability to tolerate different pathogens better than parents has also contributed to increased hybrid fitness in certain environments. This appears to have been a crucial factor in human evolution with many genes related to immunity introgressed into the genomes of modern humans, allowing them to expand their range (Dannemann, Andrés and Kelso, 2016).

Transgressive segregation occurs when hybrids with transgressive traits go on to form their own population and possibly hybrid species (Rieseberg et al., 1999; Albertson and Kocher, 2005). Differences in life histories can cause transgressive segregation to occur because parental groups and hybrids have differences in mate/pollination timing which results in backcrossing and cross fertilization not occurring (Rieseberg *et al*., 1999; Amaral *et al*., 2014). It can also occur because the transgressive phenotypes allow the hybrids to occupy novel environments which their parents cannot (Rieseberg *et al*., 1999; Dannemann *et al*., 2016). There are many theories regarding the expression of transgressive traits. These are similar to those used to explain heterosis and these terms have often been used interchangeably. One theory is that hybrids are transgressive for certain traits as a result of expression of rare recessive alleles or complementary gene action (Rieseberg, Archer, and Wayne 1999, 363-372). Expression of rare recessive alleles is however thought to account for a small proportion of traits which are expressed trangressively (Rieseberg *et al*., 1999). QTL analysis has shown that complementary gene action can explain many of the transgressive phenotypes in plant hybrids, with alleles fixed in opposite directions in the parental groups being one of the primary causes of

transgressive segregation in hybrid plants (Rieseberg, Archer, and Wayne 1999, 363-372). Based on the idea that transgressive segregation is the result of complementary gene action it is predicted that transgression will most likely occur in hybrids which result from crosses between more distantly related organisms as a result of more alleles being fixed over a longer period of time (Rieseberg, Archer, and Wayne 1999, 363-372). However, there were mixed results in support of this, in some cases genetic distance has resulted in transgressive segregation while in others there was no correlation between transgressive segregation and genetic distance (Rieseberg, Archer, and Wayne 1999, 363-372). Another prediction is that the more similar two parents are phenotypically the more likely it is that the F2 hybrids will have transgressive phenotypes (Rieseberg et al., 1999; Albertson and Kocher, 2005). This hypothesis has a great deal of support from experimental crosses (Rieseberg *et al*., 1999). The theory is that genetic differences accumulate between two groups while the phenotype remains the same due to stabilizing selection which will be discussed further below. This results in the accumulation of alleles in opposite directions in the parental groups, with transgressive traits being more likely for traits which are similar in the parental groups (Rieseberg, Archer, and Wayne 1999, 363-372). The third prediction is that traits which are under directional selection and not under stabilizing selection or genetic drift are less likely to be transgressive because directional selection is thought to fix alleles which act in the same direction as the phenotype seen in the parental groups, thus it expected that hybrids will be produced which are at the mid-parental ranges (Rieseberg et al., 1999; Albertson and Kocher, 2005; Amaral et al., 2014). These traits are often also heritable and are less likely to result in developmental instability. Often transgressive phenotypes will also appear in generations subsequent to the F1 hybrid population, when new recombinants are formed (Abi-Rached *et al.,* 2011, Dannemann *et al*., 2016).

A good example of transgressive segregation in birds comes from the hybrid finch lineage on Daphne Major; this lineage has transgressive bill morphology (Lamichhaney *et al*., 2017). The hybrid lineage also has a different mating song than parental groups. This, along with morphological differences, results in breeding isolation from parental groups producing transgressive segregation (Lamichhaney *et al*., 2017). In mammals an example of transgressive segregation would be that of the clymene dolphin (*Stenella clymene*) which is thought to be a hybrid species resulting from a cross between the striped dolphin (*S. coeruleoalba*) and the spinner dolphin (*S. longisrostris*) (Amaral *et al*., 2014). This was initially suspected because the clymene dolphins have the skull shape similar to striped dolphin but the external appearance and behaviour of the spinner dolphin (Amaral *et al*., 2014). Genomic analysis of mitochondrial (mtDNA) sequences indicates that there is a closer relationship between *S. clymene* and *S. coeruleoalba* while autosomal data indicates that there is a closer relationship between *S. clymene* and *S. longisrostris* (Amaral *et al*., 2014). This phylogenetic

discordance along with the morphological data has led to the suggestion that *S. clymene* has hybrid origins. *S. clymene* is thought to have transgressively segregated from its parental group because it has a range of variation outside of what we see in the parental groups (Amaral *et al*., 2014). There is also evidence of recent hybridization between this group and the parental groups. The hybrid origin of *S. clymene* is thought to be the result of sexual selection and assortative mating as opposed to hybrids occupying new environments because *S. clymene* exploits the same resources as *S. longisrostris* (Amaral *et al*., 2014).

Phenotypic traits in F2 hybrids

It has been suggested that in F2 hybrids there is a release from constraints that cause traits to covary (Rieseberg et al., 1999; Albertson and Kocher, 2005; Renaud, Alibert and Auffray, 2012). F2 hybrids like their F1 parents are expected to have intermediate phenotypes between the original parental populations in terms of morphology. In a study of cichlid fish, in which F2 hybrids result from crosses between *Labeotropheus fuelleborni* and *Metriaclima zebra*, traits which were under directional selection had intermediate trait values overlapping with the F1 hybrids, while traits which were not under directional selection, but instead were under another selective force (possibly stabilizing selection), resulted in transgressive phenotypes outside of the range of variation seen in the parental groups (Albertson and Kocher, 2005). In these crosses the F2 hybrids occupied a greater region of the PC1 and had more variation than that seen in the F1 hybrids (Albertson and Kocher, 2005). F2 hybrids were also often more transgressive than their parental groups; the F1 parents had transgressive phenotypes resulting in F1 hybrids exceeding the phenotypic range of the parents by 14% while the F2 hybrids exceeded this space by 22% (Albertson and Kocher, 2005). Thus F2 hybrids are expected to have transgressive phenotypes and to be more transgressive than their F1 parents.

F2 hybrids are also thought to be more variable because of new genomic combinations present in the hybrids (Albertson and Kocher, 2005; Renaud et al., 2012). A study investigated how the relationship between different modules of the mandible in hybrids compared to their parents (Renaud *et al*., 2012). Modules have some genetic independence from each other and might respond to hybridization differently even though they form part of the whole of the mandible and will determine its morphology (Renaud *et al*., 2012). Traits composed of many modules, such as the mandible, had slightly higher levels of transgression (>15% of transgressive traits in F2 hybrids) when compared to traits with only one module which had lower levels of transgression (5-10% depending on the trait in F2 hybrids.) (Renaud *et al*., 2012). New mandible shapes were produced and composed of new combinations of modules with varied sizes and shapes not seen in the parental groups; this was the

case for in both the F1 and F2 hybrid (Renaud *et al*., 2012). This is because modules are independent groups or tissues which have different genetic underpinnings. Because of this module can diverge independently and thus might respond to hybridiziation differently though they form part of the same whole (Renaud *et al*., 2012). It was also thought that the number new combinations would increase in the second generation, thus producing more transgressive phenotypes in the F2 generation than the F1 generation; this was the case across all traits measured in the mandible. (Renaud *et al*., 2012). The new genetic combinations forming in the hybrids are thought to loosen constraints producing these transgressive traits in the F2 hybrids (Renaud *et al*., 2012). Not only did different modules have different responses to hybridization with some being transgressive while others were not, there was also less integration in the F2 hybrids. There was a relationship between transgresisveness and integration; the more transgressive an F2 hybrid was the less integrated different regions of the mandible were (Renaud *et al*., 2012). A similar explanation was offered for the range of variation seen in a study of marmoset hybrids, where the hybrids had facial patterns which did not co-vary with the morphometric measurements (Fuzessy *et al*., 2014); this will be discussed further below.

To sum, F2 hybrids thus are expected to have lower levels of integration, be more transgressive, and have larger amounts of variance when compared to their F1 parents and the original parental populations. This is due to the new genomic combinations present in the F2 hybrids (Parsons, Son and Albertson, 2011).

Backcrossed hybrids

B1 hybrids are expected to be intermediate between the two parental groups under an additive genetic model (thus intermediate between the F1 hybrids and the parent being back crossed into) (Grant and Grant, 1996). They are thus expected to be more similar to the original parental (non-hybrid) species that they are being backcrossed into. There is very little work on pedigreed backcrossed hybrids and their morphology. One exception is the finches from Daphne Major (Grant and Grant, 1996). The B1 hybrids, resulting from the *G.fuliginosa* and *G. fortis* crosses, were different from their F1 parents and the original parental population in terms of overall morphology (based on a MANOVA), but they were not significantly different for individual traits (Grant and Grant, 1996). *G. scandens x G. fortis* F1 backcrossed into *G. scandens* was not significantly different from the parents, while the backcross into *G. fortis* produced B1 hybrids which were significantly different from the parent for certain traits such as bill depth (Grant and Grant, 1996). Thus, in the backcrossed hybrids there appears to be some variation, but we don't expect to see as many traits significantly different

from the parental groups as we do in the F1 and F2 hybrids. However, B1 hybrids can have new combinations of traits which might not be in the parents thus being distinct in terms of their overall morphology (Grant and Grant, 1996).

Previous work looking at the heritability of transgressive traits in F1 hybrids showed that these traits can be found in descendent backcrossed populations but not at the same frequencies as in the parental populations (Grant and Grant, 1996; Ackermann et al., 2014). This was the case with non-metric dental trait variation which is thought to be an indication of heterosis in F1 hybrid baboons, along with non-metric trait variation in the sutural morphology (Ackermann *et al*., 2014). Similarly, studies looking at the heritability of hybrid traits in back crossed hybrid finches also indicated that they tended to have high heritabilities for traits (Grant and Grant, 1996; Ackermann et al., 2014).

Thus we expect backcrossed hybrids be intermediate between the two parental groups for measurements, however we also see transgressive traits in backcrossed hybrids which were introduced in the F1 parental population. Many other hybrids from previously studied natural hybrid zones come from multigenerational hybrid groups in which there are backcrossed hybrids but their pedigree is unknown; these will be discussed further below when looking at primate and mammal hybrids.

Hybridization in mammals

Hybridization has been recorded in many mammals. We have learnt a lot of about hybridization from attempts to cross domestic stock, including about hybrid inviability, and good examples of Haldene's rule come from these domesticated crosses as will be discussed further below (Gustafson et al., 1993; Allen and Short, 1997). Haldene's rule states that when two lineages hybridize and one sex is more often infertile or inviable, it is likely to be the heterogrametic sex; in the case of mammals these would be the males (Turelli and Orr 1995). For example, hybridization between domesticated animals has been used to produce offspring with desirable traits, such as the hardiness of the mule (Allen and Short, 1997), and cattle with higher yields of meat, tolerance to different weather conditions, and new (prettier) coats with unique traits (Peters and Slen, 1964; Koch et al., 1995; Wheeler, Russel and Redden, 1995; Ward et al., 1999). Hybridization is also common in the wild in both land mammals and water mammals. For example, hybridization has been documented extensively at *Canis* hybrid zones; this will be discussed in detail below as they have been so informative regarding the outcomes of mammalian hybridization. As another example, hybridization has been extensive between different dolphin species, contributing to our understanding of the

morphological outcomes of hybridisation, and the formation of hybrid species by transgressive segregation (Zornetzer and Duffield, 2003; Amaral et al., 2014). Anthropogenic activity has played a role in mammalian hybridization (beyond the types of domestic stock crossing mentioned above), with groups bought into contact because of movement of domesticated animals such as cats and dogs into the ranges of their wild counterparts (Daniels *et al*., 1998; Hindrikson *et al*., 2012). These examples along with others will be discussed below.

Mammalian hybrid production and viability of hybrids

As mentioned above, many mammals hybridize. In many cases, this hybridization is not particularly successful from a reproductive point of view. For example, goat (*Capra hircus*) and sheep (*Ovis aries*) can mate but don't produce viable hybrids; hybrids are not thought to survive past three weeks of gestation (McGovern, 1973), however there are exceptions to this rule. There have also been attempts to produce camel (*Camelus dromedaries*) x llama (*Lama guanicoe*) hybrids which have the same diploid chromosome number of 74,and diverged approximately 11 million years ago (Skidmore *et al*., 1999). Crosses between male camels and female llamas and the reciprocal cross were on most occasions unsuccessful, resulting in foetus reabsorption, miscarriage or still births (Skidmore *et al*., 1999). Only one male hybrid was born and described as phenotypically intermediate however most of the hybrids produced were female (Skidmore *et al*., 1999). Though the genus cross is not as successful, the subspecies crosses of Old World and New World camellideas can successfully produce fertile F1 hybrids (Skidmore *et al*., 1999). Extensive hybridization has been noted between alpacas and llamas, with 80% of alpacas and 40% of llamas showing evidence of hybridization (Skidmore *et al*., 1999). Hybrids are recognized by Quechua herders who rear them as*llamawari* or llama-like and *paqowari* or alpaca-like (Wheeler *et al*., 1995; Wheeler, 2012). Hybridization has also occurred between wild South American Camillidae's and their domesticated counterparts (Wheeler *et al*., 1995; Wheeler, 2012). Horses and donkeys have the ability to interbreed and produce F1 hybrids despite the fact that they have different chromosomal numbers as do most equids (Allen and Short, 1997). Most of the hybrids from these crosses are infertile and unable to reproduce, with only a few reports of hybrids producing viable offspring (Allen and Short, 1997). Even in these situations the subsequent generations are sterile (Allen and Short, 1997). Haldene's rule also applies for horse donkey hybrids with more female hybrids produced than males (the heterogametic sex) (Allen and Short, 1997). There are also differences in the success between different crosses with, female horse (mare) x male donkey (jack) hybridization more successful than the reciprocal cross (Allen and Short, 1997). Other equids are able to produce fertile hybrids, hybridization occurs between the Grevy zebra (*Equus grevyi*) and plain zebras (*E. burchelli*), this is due to a skew sex ratio with more male Grevy's zebras in the Grevy's

zebra population as well as the larger population on Plain zebras at the hybrid zones where they meet (Cordingley *et al*., 2009). The F1 female hybrids are able to successfully backcross into the parental populations, though the male hybrids have been observed breeding this has not resulted in offspring and thus male hybrid infertility is suspected (Cordingley *et al*., 2009).

However, hybridization has also been very successful for some mammalian groups. For example, hybridization has been documented between wolves (*Canis lupus*) and dogs (*C. familiaris),* sister groups in the genus *Canis,* across Europe, Africa and North America (Hindrikson *et al*., 2012). In Europe the hybridization is usually sexually asymmetrical occurring between female wolves and male dogs (Hindrikson *et al*., 2012). However, there are exceptions to this rule (Hindrikson *et al*. 2012, e46465). Hybridization between *C. lupus* the grey wolf and domesticated dogs result in the production of fertile offspring (Hindrikson *et al*., 2012). Hybridization is also known to occur between grey wolves, eastern wolves (*C. lycaon*) and coyotes (*C. latrans*) which diverged from a common ancestor 1-2 million years ago. Their ranges overlap in North America (Rutledge *et al*., 2010). In regions where the three groups overlap three species hybrid swarms occur, however in regions where grey wolves and coyotes overlap there is no hybridization between the two groups. It is thought that the intermediate sized eastern wolf mediates gene flow between grey wolves and coyotes (Rutledge *et al*., 2010). Again, hybridization occurs in a sex biased manner as shown by genomic analysis; with large grey wolf Y-chromosomes predominating in hybrids along with female mtDNA from smaller coyotes (Kays, Curtis and Kirchman, 2010). These *Canis* hybridization events have great impact on the parental groups, and hybrids are able to successfully reproduce as will be discussed below. Thus reproduction between species to produce viable hybrids can have variable outcomes. Those discussed above are but a few well-studied examples of the outcomes of mammalian hybridization.

The phenotypic and evolutionary outcomes of hybridization in mammalian hybrids

Canis hybrids provide a great example of the effects of hybridization, including how it allows for introgression of beneficial variation which allows parental populations to adapt to new environments. The introduction of domesticated dogs into many regions has resulted in dog-wolf hybrids being produced. Dog-wolf hybrids are thought to differ behaviourally from their wolf parents, with hybridization resulting in the loss of many adaptive traits wolves need. Hybridization with domesticated dogs, due to anthropogenic activity, is thus seen as a threat to these wolf populations (Hindrikson *et al*., 2012). Wolf and dog populations have remained genetically distinct in spite of the

large amount of hybridization; this could be due to selection against hybrids, with very little introgression (Hindrikson *et al*., 2012).

In some cases hybridization may have proven beneficial for wolves adapting to new anthropogenic environments, through introgression of beneficial genes from dogs. Introgression of a variant of the *K locus* which results in wolves having a black pelage is an example of this. This gene rose to a high frequency in wolves due to the immune benefits provided by the production of high levels of melanin (Anderson *et al*., 2009). It has also been suggested that the expansion in range of coyotes into new regions has been aided by hybridization (Lalueza-Fox *et al*., 2007), with coyotes with hybrid ancestry thought to be larger as a result of admixture with wolves. These larger coyotes could exploit and colonize new regions faster than the smaller non hybrid counterparts (Kays *et al*., 2010).

Canid hybrids also demonstrate considerable phenotype environment interaction which determines phenotypic outcomes for hybrids. Three species hybrids resulting from crosses between grey wolves, eastern wolves (*C. lycaon*) and coyotes (*C. latrans*) in regions where they overlap have morphologies thought to be the result of selection for those traits which were favourable in the environment in which they were found (Rutledge *et al*., 2010). This is similar to what we see with the phenotype environment interaction seen in some plants – i.e. Louisiana iris hybrids. Though there have been repeated hybridization events in the region which might have had a homogonizing effect, it is clear that the three parental groups have remained phenotypically distinct (Rutledge *et al*., 2010). When looking at another coyote-wolf (*C. latrans* × *C. lupus*) hybrid zone the hybrids have intermediate measures for body size when compared to the coyotes and wolves (Sears *et al*., 2003). However, the hybrids found in areas that were more forested with low road density were larger and had more wolf-like body morphology along with a more wolf-like diet (Sears *et al*., 2003). Hybrids which were found in fragmented forest regions with many roads had smaller bodies as well as body and skull morphology more similar to coyotes along with a similar diet of smaller animals (Sears *et al*., 2003).

Finally, canids have provided us with considerable information about the morphology of hybrids. Coyote-wolf hybrids from a mixed hybrid zone, where the pedigree of the hybrids was unknown, were intermediate for morphometric measurements, being smaller than the larger wolf parent *(C. lycaon)* while being larger than the coyote (*C. latrans*) parent (Sears *et al*., 2003). These include measurements of forelimb and hind limb length as well as total length of the hybrids (Sears *et al*., 2003). Even though the hybrid morphology varied by environment with larger hybrids in forested environments the largest of these hybrids were still smaller and significantly different from the larger wolf parent (Sears *et al*., 2003). The only exception was in terms of the difference in forelimb length which was not significantly different (Sears *et al*., 2003). Thus though we see that the environment can affect variation within the

hybrid group, with different size hybrids thriving in different environments, they are still large only in relation to the other hybrids but not transgressive when compared to the larger parent.

Some mammalian hybrids do exhibit transgressive body size, such as the genus cross between gelada and hamadryas baboons which diverged ~4.5 Ma, where the F1 hybrids were larger than their parents even though they were sub-adult at the time of data collection (Jolly *et al*., 1997; Zinner *et al*., 2013). Ligers and tigons, which are the result of a cross between a male lion and a female tiger and the reciprocal cross, are often on average larger than both their parents, this is also a genus cross with the divergence time between the lions and tigers occurring ~ 3.9 Ma (Gray, 1972; Davis, Li and Murphy, 2010). Sheep x goat hybrids have also been produced; a natural hybrid between a Tswana goat and sheep was transgressive for body size and morphometric measurements, being larger than its medium sized parents and being similar to a larger breed of goat (Mine *et al*., 2000). Many of the crosses which produce hybrids which are transgressive for body size are crosses at the genus level; however inter-generic crosses don't always have to produce transgressive phenotypes. For example, intergeneric hybridization has been recorded between captive bottlenose dolphins (*Tursiops truncates*) and the common dolphin (*Delphinus capensis*). Hybrids were generally intermediate for various morphometric measurements when compared to their parental groups (Zornetzer and Duffield, 2003). They were also generally intermediate for colour when compared to the parental groups (Zornetzer and Duffield, 2003). Though the hybrids were intermediate between their parents they were well within the range of the *T. truncates* group which is very variable. Here we also see the hybrid having morphometric measurements in the range of the *T. truncates* while having patterning similar to the *D. capensis* and colour phenotype and morphometric traits not co-segregating in the hybrid (Zornetzer and Duffield, 2003). Transgressive phenotypes have also been recorded between sub-specific crosses, including mouse hybrids (mandibles) and saddle back tamarins (cranial size) (Cheverud *et al*., 1993; Renaud *et al*., 2012).

Hybrids can also be more similar to one parent morphologically while having the behavior of the other parent. Hybrids between Grevy's Zebras and plain zebras are an example of this. Plain zebra's stripes are thick and extend to the dorsal region while Grevy's zebra stripes end above the ventral region and do not touch at the belly (Cordingley *et al*., 2009). The hybrids are similar to their larger Grevy's zebra parents in size, also have stripes which end above the ventral region, but are broader than the stripes of the Grevy's zebra parent (Cordingley *et al*., 2009). Thus overall the F1 hybrids are more Grevy like morphologically (Cordingley *et al*., 2009). Behaviourally the F1 hybrids are similar to their plain zebra parents, and integrate with the plain zebra herds (Cordingley *et al*., 2009). Sometimes hybridization can make parental groups indistinguishable. Hybridization between wild and

domesticated cats is also common and occurs in many regions. In Scotland it is difficult to distinguish between a feral cat and a wild cat based on pelage morphology alone (Daniels *et al*., 1998). Only limb and gut length could be used distinguish wild and domesticated cats as two separate groups with the hybrid cats having a wide range of morphology (Daniels *et al*., 1998).

Domesticated hybrids have also often been produced because they possess unique traits. Members of the bovine family are a great example of this. For example, bison x domesticated cattle hybrids have denser coats compared to parents and finer and smaller hairs similar to bison, allowing them to better tolerate grazing in colder temperatures (Peters and Slen, 1964; Ward et al., 1999). Bison and *Bos taurus* hybrids were also shown to be slightly larger than *B. taurus* but this difference was not significant; though the hybrids were born larger they consumed and gained less mass over the same period than their *B. taurus* parents. Other differences included having more red muscle fibre than parental groups (Koch *et al*., 1995). New World Camelidae hybrids are also common and some are produced because of the desirable hair morphology. In the case of a cross between the wild vicuña (*Vicugna vicugna*) and domesticated alpaca (*V. pacos*) the hybrids have finer hair fibres, with more hybrids being produced for this particular coat (Wheeler *et al*., 1995; Wheeler, 2012). In another cross between guanaco (*Lama guanicoe*) and llama (*L. glama*) the hybrids were described as guanaco-like morphologically and behaviourally and thus are not ideal for domestic purposes (Wheeler, 2012).

Thus, from this section we see many of the same outcomes in mammalian hybrids as we do in other organisms which hybridize and have been studied in greater depth, and the same rules apply in terms of the outcomes of hybridization. We see similar outcomes in terms of hybrid viability and phenotypic outcomes. Hybridization results in intermediate phenotypes as well as transgressive phenotypes. It serves as a source of new variation resulting in parental populations being better adapted to novel environments and expanding into new environments. We also see that there is a clear relationship between the environment and the phenotype, which plays a role in the morphology and survival of the hybrids. However, more quantitative work needs to be done with mammal hybrids that systematically look at the effects of hybridization on the phenotype. This might be harder due to ethical issues. However, great work has been done looking at the phenotype environment relationship in hybrids. This work done in conjunction with more genetic work can be very powerful as has been done with some of the primate examples discussed below.

Pelage in primates and the effects of hybridation on pelage variation

There is a considerable amount of phenotypic variation in pelage colour and patterning in primates, with primates being one of the most diverse mammals in terms of pelage patterning (Bradley and Mundy, 2008). Yet relatively little is known about the development of coat colour patterning in primates. We know that changes in pelage occur during development with some primates having a juvenile coat which changes as they mature (Bradley and Mundy, 2008). However, there is very little information about prenatal coat colour development. Studies looking at the molecular basis of variation in coat colour in macaques attempted to identify the genes which underlie black and white colour variation and none of the candidates genes were shown to be differentially expressed in different coloured macaques,(Bradley and Mundy, 2008; Bradley et al., 2013), mutations in candidate genes such as *ASIP* and *MC1R* don't always produce the expected phenotypes (Mundy and Kelly, 2006; Haitina et al., 2007). We do however know why there is this a high level of variability in coat colour and what causes these changes in coat colour. Reasons for change in coat colour include differences in facial patterning for conspecific recognition, especially when different groups are found within the same region; body colour patterning to evade predation in the case of small primates dorsal ventral patterning is important; there is also disruptive colouring, pattern blending and background matching for camouflage. Changes also occur due to sexual dimorphism as well as due to sexual selection (Bradley and Mundy, 2008).

The coat and skin are important interfaces with the environment and are under a great deal of selective pressure. In mice, variation in coat colour has been associated with the colour of the substrate they live on, with darker mice found in regions with darker soils, and lighter coat colours associated with light sand; this aids in protection from predation (Mikkola, 2007). Dorsal ventral colour differences are also common in primates; this is known as counter shading (Kamilar and Bradley, 2011). Counter shading is only present in primates which have banded hairs, with coat colour determined by expression of the agouti gene (Bradley and Mundy, 2008). Counter shading might involve similar developmental pathways in primates as it does in mice. Other primates have a bipartite coat colour pattern as well as more intricate colour patterning with different regions of the body being different colours (Bradley and Mundy, 2008).

Research on primate pelage indicates that there is considerable variation in pelage colour, length, texture and density on the surface on the skin (Bradley and Mundy, 2008). Humans are unique because we are functionally hairless with much reduced hair morphology and a unique distribution of hair

(Montagna, 1972; Jablonski, 2004). Humans also display a great deal of variation in hair colour, texture and distribution along the body. Primates don't only differ in terms of pelage but also the morphology of the skin, with humans having a very thick epidermis, while other primates with thicker pelage have very thin smooth skin epidermis (Montagna, 1972). We also differ in the appendages, such as glands, which can be quite variable and specialised among primates, with humans having more eccrine glands which unlike the sebaceous glands that produces an oily liquid produces a watery liquid which allows for better dissipation of heat and thermoregulation (Montagna, 1972). Skin and pelage have gone through many evolutionary changes throughout human and primate evolution. They are also under a great deal of selective pressure. Though we cannot know what Neanderthal skin and hair morphology exactly looked like, what we know from aDNA is that they had variability in skin colour with lighter and darker skin tones within Neanderthals. A variant of the *BNC2* gene which is thought to be the result of introgression is associated with the formation of pigmentation spots with aging and found at high frequencies in modern Europeans (Vernot and Akey, 2014). Modern humans have many other genetic variants derived from Neanderthals which result in variation in skin colour which will be discussed more in the next chapter (Culotta, 2007; Lalueza-Fox *et al*., 2007; Meyer *et al*., 2012).

Impact of hybridization on primate coat colour

Most research looking at variation in the coat colour of hybrid primates has been in living populations at hybrid zones with the purpose of determining the morphological features of hybrids (Kelaita and Cortés-Ortiz, 2013; Fuzessy et al., 2014). In most cases, hybrid primates show a larger range of variation in coat colour and patterning when compared to parental species (Peres, Patton and da Silva, Maria Nazareth F, 1996; Fuzessy et al., 2014). However, some cryptic look like either one of the parental groups (Jolly *et al*., 1997; Bynum, 2002; Fuzessy *et al*., 2014, Malukiewicz, *et al*. 2014 and 2015). In many of these studies other metric trait data was collected including crown rump length, body mass, limb measurements and relative limb length and the relationship between these traits and variation in pelage colour and patterning has been analysed. Though hybrids may group with one parent in terms of pelage colour and patterning they may group more closely with the other parent for traits such as body size or limb proportions (Bynum, 2002; Fuzessy *et al*., 2014). This has resulted in the hypothesis that hybridization results in loosening of evolutionary constraints with hybrids being less integrated than their parents (Fuzessy *et al*., 2014). There might be a lessening of integration in hybrids, however when considering the pleiotropic effects of a gene or a set of genes in a pathway, which could cause co-variation between certain traits, it is important to consider traits that are related to each other (Wagner and Zhang, 2011). Examples of this covarying traits would be skin and teeth or

dorsal ventral patterning and skeletal body patterning. When determining whether pleiotropy is the cause of co-variation or if co-variation changes between developmentally integrated traits it is important to measure traits which are associated via a pleiotropic gene or developmental pathway (Wagner and Zhang, 2011). If traits which do no co-vary are measured it might appear as if there is a lessening of integration in the hybrids. However, you are considering traits which might not be particularly integrated. In many cases, the relationship might not be recognized because related traits were not measured.

Phenotypic variation in primate hybrids

As previously mentioned, hybridization is common among primates. Many primate hybrids and primate hybrid zones have been identified due to mosaic or intermediate coat colour patterns. Primates have a great deal of variation in coat colour, with different species/sub-species having different coat colours, patterns, and textures. Primate hybrid zones occur in Africa, Asia and the Americas. Many of the dynamics discussed above have been seen in primate hybrid zones, including increased levels of variation, novel phenotypes and new combinations of traits resulting in novel phenotypes. These dynamics will alsobe discussed below in the context of coat colour and morphometric measurements, as well as the relationship between the two phenotypes where relevant.

In terms of coat colour, in natural hybrid populations we see hybrids that have intermediate coat colour as well as some who look similar to their parents; we also see increased levels of variation. For example, macaque *Macaca tonkeana* and *M. hecki* hybrids in Indonesia display intermediate and mosaic phenotypes composed of "pure" features of either parental group. There was a great deal of variation observed in the hybrid zone; some hybrids were more like *M. hecki* in one region while in other regions they were more like *M. tonkeana* (Bynum, 2002). Though this is a multigenerational hybrid group which has been there for an extended period of time we still see traits segregating together in the hybrid population. This could be due to pleiotropic effects, natural selection or linkage between genes (Bynum, 2002). We see the same pattern in terms of coat colour variation in a wild marmoset hybrid population (*Callithrix geoffroyi* x *C. penicillate)*, a multigenerational hybrid group with hybrids of unknown pedigrees who were most likely backcrossed. Hybridization resulted in an increase in facial coat colour patterns in the hybrid zone (Fuzessy *et al*., 2014). Hybrids range from intermediate phenotypes for facial coat colour patterning, to those that look more to similar to the parental groups (Fuzessy *et al*., 2014). Morphometric measurements were also taken for this group. An interesting result was that hybrids with a parental-like facial pattern did not always have the

expected corresponding morphometric measurements; this will be discussed further below. This could indicate that there was dissociation between morphometric measurements and facial patterning (Jolly *et al*., 1997; Fuzessy *et al*., 2014). Thus, we see increased variation in coat colour in hybrid zones, with hybrids having phenotypes ranging between the two parental groups. In one group we see traits co-segregating, while in the other group this is not the case even though they are both multigenerational hybrid groups and there would have been many opportunities for recombination and new genomic combinations to arise in the hybrid zone. Factors such as how much back crossing occurs and how much breeding between hybrids occurs might play a role in determining whether there will be dissociation between traits as discussed below.

The pattern of mosaic and intermediate morphology is also seen in other primate hybrids including those between gelada and hamadryas baboons (Jolly *et al*., 1997). Some hybrids have mosaic phenotypes while others have an intermediate morphology. For these hybrids, we have information on dentition as well. Measurements of the dental arcade of the hybrids indicate that they had longer dental arcades than either parental group, and that the elongation occurs mainly in the pre-maxilla (Jolly *et al*., 1997). The hybrids had larger teeth than both parental groups on most occasions with the females having larger cheek teeth than both the geladas and hamadryas females (Jolly *et al*., 1997). We also know that these hybrids were transgressive in terms of size when compared to the parental groups. This corresponds to data presented above showing that baboon hybrids have transgressive dental morphologies (Jolly *et al*., 1997).

New variation in coat colour can also be introduced through hybridization resulting in increased levels of variation. As an example of such variation, in a cross between howler monkeys *Alouatta caraya* and *A. clamitans*, female hybrid monkeys had new coat colour patterns not seen in either parental group (Aguiar *et al*., 2008). The parental groups are monocolour with the *A. caraya* females having a golden coat colour and the *A. clamitans* females being dark brown in colour (Aguiar *et al*., 2008). Some hybrids had intermediate coat colours with others having new coat colour patterns, with some regions of the body being dark brown while other regions of the body were golden in colour (Aguiar *et al*., 2008).

Hybrids can also be cryptic, i.e. they look like one of the parental groups. Cryptic hybrids are usually only identified through genomic analysis. Although work has been done to look at the relationship between the genotype and the phenotype in plant hybrids, less work has been done in mammalian hybrids. Hybrids from multigenerational howler monkey hybrid group consisting of many backcrossed hybrids were genotyped and phenotypic data was also collected (Kelaita and Cortés-Ortiz, 2013). The relationship between genotype and phenotype was investigated. Hybrids were most like

the parents with whom they shared the highest proportion of their genome (Kelaita and Cortés-Ortiz, 2013). In many cases the hybrids were not statistically significantly different in terms of morphometric measurements from the parent with whom they shared most of their genome. Intermediate genotypic hybrids had higher levels of morphological variability when compared to those hybrids which had higher proportions of their genome accounted for by one parent (Kelaita and Cortés-Ortiz, 2013). In this study, they also used non-metric traits such as pelage patterns to identify hybrids. Dissociation between non-metric traits such as coat colour and metric measurements occurs in the group classified as intermediate genotypically. This intermediate genotype group was classified as more *A. pigra*-like for their non-metric traits while their metric measurements grouped them with *A. palliata* in a PCA analysis (Kelaita and Cortés-Ortiz, 2013). This is a similar pattern to what was observed in the marmosets. Thus morphological variation might vary in the backcrossed hybrids depending on the amount of backcrossing and how much interbreeding occurs between the hybrids themselves (Kelaita and Cortés-Ortiz, 2013). This might be why we have different outcomes in the macaque hybrid zone, where we see co-segregation of traits, while in the howler monkey and the marmoset hybrid zones we see dissociation between traits for some hybrids (Kelaita and Cortés-Ortiz, 2013).

Hybridization in captivity has occurred and these events provide unique opportunities to observe the development of hybrids from birth. This was the case with hybridization between between siamangs (*Hylobates syndactylus*) and gibbons (*H. muelleri abbotti*) (Myers and Shafer, 1979; Wolkin and Myers, 1980). Two hybrids were produced in captivity and their development was recorded. Developmental differences in coat growth were noted in the hybrids, with one hybrid born without hair and developing a think shiny black coat over a three month period, while the other hybrid was born with a brown coat (Wolkin and Myers, 1980). The hybrids had coat similar in texture and colour to their siamang mother, including a similarly distinctive middle hair part, while having a white ring around the face characteristic of gibbons (Wolkin and Myers, 1980). Gibbons have thick coats with a high level of hair density, with on average 1834 hairs per cm^2 while siamangs have 429 hairs/cm^2; the hybrids had on average 893 hairs/cm^2 (Wolkin and Myers, 1980). The hybrids did not have throat sacs which are typical of siamangs and usually hairless. Instead, the hybrids had hair in the throat region similar to that of gibbons (Wolkin and Myers, 1980). Thus we don't only see changes in coat colour patterning, but also the distribution of hair in the hybrids; in this case they have a hair density much lower than the mid-parental value (apx. 1131 hairs/cm^2).

In sum, hybrid primates have similar outcomes for coat colour patterning as other traits. Interesting work can also be done looking at other pelage traits which can be informative such as hair

thickness, density, and patterning on the body if there are differences between primates for these aspects.

Effect of hybridization on limb lengths in primate hybrids

In primates the effects of hybridization can vary across traits, with a range of phenotypes including intermediate as well as parental- like phenotypes based on data from multigenerational hybrid zones (Bynum, 2002; Kelaita and Cortés-Ortiz, 2013; Fuzessy et al., 2014). Comparisons are made between parents and hybrids for individual morphometric measurements (i.e. comparison of measurements such as body and hind limb length). Relationships between traits (i.e. the length of the forelimb in relation to the length of the body) are also compared. Finally limb data has also been used to to determine which traits account for most of the variation when comparing hybrids to their parents using methods such as PCA analysis, which will be discussed in this section (Wolkin and Myers, 1980; Jolly et al., 1997; Fuzessy et al., 2014). In hybrids, we see new relationships forming between different regions of the body resulting in transgressive body shapes even if individual measurements might not be transgressive, in other instances they might be similar to a parental groups (Wolkin and Myers, 1980; Jolly et al., 1997; Bynum, 2002; Fuzessy et al., 2014). We have information from primate hybrid zones for baboon, macaque, marmoset as well as gibbon-siamang hybrids which are included in this section dedicated to variation in limb measurements in hybrids. Once again, there isn't a lot information on F1 hybrids, this is mostly based on work with multigenerational natural hybrid populations.

In some cases, the hybrids might be more similar to a parental group for relationships between traits. In a cross between *Papio* and *Theropithecus,* the male hybrid had a *Papio*-like relationship between the forelimb and the hindlimb while the female hybrid had intermediate proportions (Jolly *et al*., 1997). In the marmoset hybrid population which was discussed above between *C. geoffroyi* x *C. penicillata* morphometric measurements were collected including femur lengths (Fuzessy *et al*., 2014). The hybrids did not have any transgressive morphometric traits and were larger than the smaller parent, while being smaller than the larger parent. Though the hybrids did not have transgressive traits they were not strictly similar to the expected mid-parental range being more similar to *C. penicillata* for some traits and more similar to *C. geoffroyi* for other traits including femur and hand length (Fuzessy *et al*. 2014). In general, the hybrids were significantly different from the parental group for average morphometric measurements. PCA analysis indicated that overall the combination of morphometric traits resulted in some hybrids being transgressive in terms of body

shape (i.e. having different relationships between different regions of the body) (Fuzessy *et al*., 2014).The hybrids fell outside of the shape space seen in either parental group (Fuzessy *et al*. 2014). There is also a dissociation between facial colour patterning and body measurements in this hybrid populations. The hybrids with facial patterns similar to *C. penicillata* where transgressive in the PCA analysis, having PCA scores outside of the range of variation of either parental group; we see a similar pattern for those who have a facial pattern similar to *C. geoffroyi* (Fuzessy *et al*., 2014). Regardless of facial patterns the hybrids were within the *C. geoffroyi* range of variation for body size. The hybrids with intermediate facial patterns were the only ones that did not fit in with this pattern and fell within the range of variation seen in *C. penicillata.* Thus, facial pattern was not a good predictor of morphometric measurements (Fuzessy *et al*., 2014).

When comparing morphometric measurements of a hypothesized hybrid population between Chinese and Indian rhesus macaques (*M. mulatta*) and long-tailed macaques (*M. fascicularis*), the hybrids were on average smaller than the larger parental group (*M. mulatta)* and larger than the smaller parental group (*M. fascicularis*) (Bynum, 2002). For morphometric measurements including upper arm length, forearm, thigh and leg length, they were significantly different from the parental groups for all but leg length (Bynum, 2002). The hybrids were close to the mid parental range for leg length measurements. However, the hybrids tended to have longer limbs in relation to the rest of the body when compared to *M. mulatta* and were more similar to *M. fascicularis* in this regard even though they may have had longer limbs than the *M. fascicularis* parent (Bynum, 2002). While they had longer limbs in relation to their body, their inter-membral index was smaller than the Chinese rhesus macaque and the long-tailed macaque parental group but similar to the Indian rhesus macaque parental group and not significantly different (Bynum, 2002). PCA analysis indicated that the posited hybrid population was intermediate between the parental groups along PC1 which loaded positively for all morphometric measurements and accounted for 97% of the variance. PC2 only accounted for a small proportion of the variance but it indicated that hybrids had on average proportionally longer limbs when compared to the parental groups. Thus, here we see many things happening with different proportions being similar to different parental groups, the overall morphology of the hybrid is transgressive because it is different from what is seen in either parental group (Bynum, 2002).

Measurements were taken from developing gibbon siamang hybrids who were 18 and 5 months old (Wolkin and Myers, 1980). Siamangs are larger than gibbons because they grow for longer but they have similar initial masses; the 18 month old hybrid was the expected size for a *Hylobates,* while the 5 month old hybrid was smaller than the expected size (Myers and Shafer, 1979). The hybrid had the lower index characteristics of their siamang mother at 12 and 18 month old however these

variables could change with age. The relative lower limb length was also more similar to that of the siamang, regardless of the shorter lower limbs similar to the siamang, while the intermembral index of the hybrid was gibbon like, due to the fact that it also had shorter upper arms (Myers and Shafer, 1979; Wolkin and Myers, 1980).

Thus, in terms of morphometric traits there is a general trend for hybrids not to be transgressive in terms of absolute lengths of their limbs and instead they are intermediate in size but still more similar to one of the parents. However, we do see changes in the relationship between morphometric traits which might result in hybrids having an overall body shape which is transgressive even when the measurements might not be. Most of these data on post cranial and body measurements are from hybrids zones where there are very few studies with F1 hybrids, thus these trends might not reflect what you would see in F1 hybrids. F1 hybrids might be transgressive for size measurements while subsequent generations are not.

In summary primate hybridization is common, and has been shown to be a source of new variation. Primate hybrids like other hybrids can have intermediate phenotypes, or parental-like phenotypes as is the case with marmoset and macaque hybrids. They can have extreme phenotypes including being larger, or presenting with new coat colour patterns as is the case with the howler monkey hybrids. Many cryptic hybrids exist and in many multigenerational hybrid populations we see the dissociation of relationships between certain traits, with some hybrids grouping with one parental group in terms of coat colour or non-metric traits while being more similar to another parent in terms of morphometric measurements. Transgressive traits have also been identified in terms of dental morphology. However much of the research has been done on wild groups in which the pedigree of the parents is unknown. It will be important for more systematic research to be done on hybrids with known pedigree. It is also important to do more research on skeletal material which will be more informative for interpreting the fossil record.

Development and hybrid morphology

As described above transgressive traits displayed by hybrids are often thought to be the result of two co-adapted genomes coming together (Rieseberg, 1997; Chen, 2013). During development, the disconnect between the developmental systems which come together in the hybrid can result in the production of transgressive/heterotic phenotypic traits (Birchler *et al*., 2010; Chen, 2013). There has been a lot of research attempting to understand the developmental underpinnings of these transgressive traits. Research in the field is still in its initial stages but there is some theory regarding

how development systems change between two lineages over time and how this affects the phenotypic outcomes of hybridization.

Discordance between the genotype and phenotype: Developmental system drift and pheno-genetic drift

Developmental system drift (DSD) refers to the process whereby you see changes in the underlying molecular mechanisms which produce similar phenotypic traits in different organisms through differences in developmental processes (True and Haag, 2001; Johnson and Porter, 2007; Landry, Hartl and Ranz, 2007).. There can be uncoupling, over long evolutionary periods, of traits and developmental systems. In large networks it is possible that certain sections of the pathway might be lost during the developmental process (True and Haag, 2001; Johnson and Porter, 2007). Models are being produced to try and understand these processes (Johnson and Porter, 2007). One of the simplest models looked at how DSD could occur in a system where a few loci in a system control the development of specific traits. In this model one pleiotropic loci controlled the development of two traits, one of which is under stabilizing selection and another of which is under directional selection (Johnson and Porter, 2007). In this scenario, the trait under stabilizing selection doesn't change much over time even though the loci which control its development do; this is because the pleiotropic loci are responding to the directional selective pressure on the other trait, and therefore, compensatory mutants will be favoured in this system (Johnson and Porter, 2007). There can also be uncoupling of a phenotype and genotype which may or may not be tied to developmental process. This is known as pheno-genetic drift where the relationship between the phenotype and genotype drifts apart (Weiss and Fullerton, 2000). Even with a great deal of genetic conservation, the relationship between the genotype and a particular phenotype does not always hold (Weiss and Fullerton, 2000).

This research is still in its infancy, a lot work remains to be done especially as it relates to hybrids. When trying to understand DSD, most comparisons have been made between more distantly related groups. An example of a comparison would be the *Endo16* gene which is expressed at the same levels and performs the same function in the development of the endoderm of the sea urchin species S*trongylocentrotus purpuratus* and *Lytechinus variegatus*. But the components of the *Endo16* gene have diverged so much that the binding sites we find in *S. purpuratus* are not present in *L. variegatus* (Skaer and Simpson, 2000; Johnson and Porter, 2007).

Hybrids which are the result of crosses between *Drosophila melanogaster* flies and their sister species, *D. simulans,* provide an excellent example of DSD between parents producing dysgenic phenotypes in hybrid offspring. The parents share similar bristle patterns on their notums. The fly

hybrids are missing bristles and suffer from dysgenesis, with a reduction in the number of bristles present, even though the parents have an identical morphology (Skaer and Simpson, 2000).

Models have been designed to try and account for how developmental system drift could affect hybrid fitness; in models where there are linear pathways the prediction is that 25% of hybrids will be fitter or as fit as their parents while 75% of hybrids are predicted to be affected by the DSD which occurred in the parental groups (Johnson and Porter, 2007). This fits in with what we see from studies looking at variability in fitness and survival of hybrids in relation to their parents. The evolution of genes which have pleiotropic effects are important for understanding what happens during DSD (Johnson and Porter, 2007).

Many of the studies correlating DSD with phenotypic traits in hybrids focused on aberrant morphologies even though the parents have similar/identical morphologies such as the notum bristles in the flies (Skaer and Simpson, 2000). DSD can occur in a multitude of ways; it can be the result of genomic changes, changes in interaction between different parts of a developmental pathway, and due to differences in timing and patterning of gene expression in different organisms (Haag, 2007). Many traits are sensitive to variation in levels, timing and co-ordination of gene expression. These include the cranium (Kim *et al*., 1998; Liu *et al*., 1999; Zhou *et al*., 2000), the development of teeth and the development of the skeleton (Zakany *et al*., 1997; Litingtung *et al*., 2002; Yoshida *et al*., 2004). The same can be said for the development of plants (Galinha *et al*., 2007), and though there is work looking at the effect of gene expression and transgressive traits there has been less work trying to understand how DSD contributes to the production of heterotic and transgressive traits in hybrids. This will be important future research because DSD might play an important role in producing heterotic/transgressive phenotypes and not just dysgenic phenotypes.

DSD as a potential explanation for transgressive traits in hominin hybrids and baboons

Given the length of time during which Neanderthals and modern humans diverged before they came into secondary contact, there must have been DSD. One morphological trait where primate hybrids have shown a transgressive phenotype is that of the dentition (Ackermann, 2010). Thus understanding the underlying cause of transgressive dental traits will be important for understanding why the transgressive morphology has been produced and if we can expect it in the fossil record.

The limited work on DSD in animals has compared very distantly related model animals to each other and to humans. Comparisons have been made between mice and modern humans; these studies have been conducted because mice are used as model animals to understand human physiology and disease. Gene knock-in experiments (the addition of the homologous gene from organism A into organism B to determine how it interacts with the genes in organisms B and affects the development of organism B) have shown how human homologous are not always able to rescue mutants which have the mouse homologue inactivated, with only a subset of the target genes in the pathway activated in mice (Lynch, 2009). Knock-in experiments with human homologues also have different outcomes in different mouse strains. These show the extent of DSD overtime, and also show DSD between different mouse strains who respond differently in the knock-in experiments (Lynch, 2009).

Dental development

In developmental biology dentition provides an ideal system to study tissue development, especially in terms of temporal and spatial molecular patterns which occur during organogenesis (Peters and Balling, 1999). In palaeoanthropology, as a tissue group which preferentially survives in the fossil record, it has provided important insight into diet as well as development and life histories of fossil material (Anemone, Mooney and Siegel, 1996; Rougier, Crevecoeur and Wolpoff, 2006; T. M. Smith et al., 2010). Tooth morphology is also sensitive to changes in gene expression patterns, and thus transgressive traits in dentition might be a good indicator of developmental perturbation or breakdown (Jernvall, Keranen and Thesleff, 2000; Bei, 2009). Thus, understanding the role DSD might play in affecting dental morphology of primate and hominin hybrids is important. This is not only applicable to human, Neanderthal and Denisovan hybridization but also to other hominin species, as many hominins have lived concurrently on the landscape.

We know that there is interspecific variation in the timing of the development of the teeth, with many shifts in developmental processes throughout human evolution (Smith, 1991). Compared to chimps we have differences in timing and the pattern of tooth development (Smith, 1991; Anemone *et al*., 1996). Development of the dentition is also tightly correlated with traits such as brain size, age at first reproduction, life span and other life history traits (Smith, 1991). Because dentition survives in the fossil record, dental development in hominins is well studied and there is a lot of literature pertaining to development using dental material. Initial analysis of Neanderthal development using dental traits indicated that the development of the dentition in Neanderthals occurred at a faster rate than modern humans and that postnatal growth overall was faster (Macchiarelli *et al*. 2006; Smith *et al*. 2010). However more recent analysis indicates Neanderthals may have developed at a slower rate

than initially thought (Ponce de León, *et al*. 2016; Rosas *et al*. 2017). We also know that there are differences in cranial morphology between humans and Neanderthals. The characteristic shape of the Neanderthal and modern human cranium are present at birth and there is a large genetic component to this shape and they are not determined by environmental variation as development occurs post-natally (Ponce de León, and Zollikofer 2001). This means that there are differences early on in development in patterning of the crania at least between Neanderthals and modern humans (Ponce de León, and Zollikofer, 2001).

Within baboons, we see differences in timing of the eruption of the first deciduous teeth; for *P. hamadryas* they erupt at birth or soon thereafter, for *P. cynocephalus*, it occurs approximately between 2 to 4 weeks after birth (Smith, 1991). There are also differences in the pattern of eruption of dentition between different genera of callitrichids (Byrd, 1981). Gibbons and siamangs are also shown to have differences in the timing of the emergence of teeth including differences of the 1st and the 3rd molar (Dirks, 2007). Thus, divergence in the pattern of dental development is common in primates and is an indicator or slight divergence in these developmental pathways.

Transgressive dental traits in mammalian hybrids

It has been noted that in mammalian hybrids, there is an increase in non-metric cranial trait variation because of hybridization (Ackermann et al., 2006; Ackermann et al., 2010; Ackermann and Bishop, 2010). This has been recognized in several crosses including baboon, gorilla and wildebeest hybrids (Ackermann et al., 2006; Ackermann and Bishop, 2010; Ackermann et al., 2010), with hybrid offspring showing a common pattern of increased non-metric trait frequency and pattern of expression when compared to the parental species (Ackermann et al., 2006; Ackermann and Bishop, 2010). For the baboon hybrids, non-metric trait variation included an increased incidence of supernumerary teeth and atypical sutures for both males and females, with these differences significant between the hybrid males and the parents. The supernumerary teeth included distal molars at the end of the tooth row (this trait had the highest incidence with the fourth molars showing a range of variation), rotated premolars as well as supernumerary canines (Ackermann *et al*., 2006). These were present in the F1 hybrids, and in subsequent generations of hybrids which including backcrosses (Ackermann *et al*., 2014). In the wildebeest, non-metric sutural traits were found at a higher frequency compared to parents, while dental anomalies identified included the presence of a unilateral rotated premolar (Ackermann *et al*., 2010). In eastern lowland gorillas (*Gorilla beringei graueri*), which are hypothesized to have experienced hybridization, we also see an increased frequency of cranial non-metric traits including mandibular supernumerary teeth found at higher

proportions compared to mountain gorillas (*G. b. beringei*) and western gorillas (*G. gorilla*) (Ackermann and Bishop, 2010). Thus, such results pose the question to whether DSD in the molecular systems producing dental traits might play a key role in producing some of the common patterns we see in mammalian hybrid crania.

The development of the ectoderm and the connection between teeth and other soft tissue traits

Teeth and other appendages, including hair follicles, sweat glands, salivary glands and mammary glands are all derived from the ectoderm with many of the same genes controlling their development Figure 2.2 (Pispa and Thesleff, 2003) (Ackermann 2007). Many of the same genes and pathways are required for the coordinated development of these tissue groups during early development (Pispa and Thesleff, 2003). In particular, there is a relationship between pelage and teeth. Understanding DSD in this ectodermal developmental system might help us understand why transgressive non-metric trait variation as expressed in the dentition is so prevalent in mammalian hybrids (Ackermann *et al*., 2014). This line of investigation was proposed because, although hybrid baboons had an increase in non-metric trait variation in the cranium, heterosis was not as broadly expressed as expected in terms of cranial metric measurements (Ackermann *et al*., 2006; Ackermann, 2010), suggesting there was not a significant divergence in the underlying genetics for cranial traits (Ackermann, 2007). However, the increase in non-metric trait variation in the teeth might be explained by genetic divergence in the ectodermal developmental system for other traits such as pelage (Ackermann, 2007), with DSD occurring due to selection on different pelage traits. Compensatory changes in the developmental system could have occurred if trait such as teeth were under stabilizing selection while other traits such as the pelage, which is direct interaction with the environment as well as providing social cues, might be under more selective pressure to change very rapidly.

Thus, if we have a change in the morphology of a trait such as pelage colour or thickness there could be coevolution between different genes in these pathways in order to maintain other tissue morphology, in this case the morphology of the teeth formed by the same path way and under stabilizing selection. This could result in to two groups having similar morphology for a trait but the underlying genetics and developmental processes might be slightly different. Thus, when these co-adapted genomes come together during hybridization there is a break down in co-ordination during development resulting in the non-metric dental trait variation mentioned previously (Ackermann, 2007).

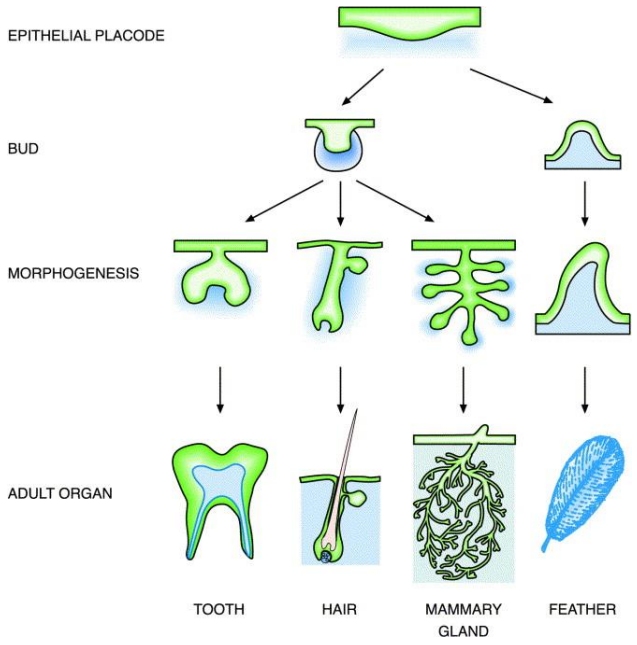

Fig. 2.2: This image displays how different tissue groups with very different functions develop from the same ectodermal germ layer. Taken from Pispa and Thesleff 2003.

There are indeed differences in pelage morphology and colour for all groups showing transgressive dental trait variation. Baboons have differences in coat colour, with Olive and Hamadryas baboons having differences in the texture and colour of their hair. Olive baboons have shorter, thicker, courser hair, which is darker in colour and consists of a brown-agouti banding pattern resulting from the alternative deposition of pheomelanin and eumelanin, while Hamadryas baboons have longer finer hairs which consists of alternating bands of tan/brown - very light almost unpigmented bands resulting in the lighter coat colour (Ito 2001). There are also differences in pelage between mountain gorillas and lowland gorillas. Mountain gorillas have thicker pelage and a larger amount of black hair. There is also a difference between Eastern Lowland Gorillas and Western Gorillas in the border between the silver region and the surrounding hair (Groves 2003). Blue and black wildebeest also have differences in their hair morphology. Blue wildebeest have longer black hair, which is concave in cross section with a thick cortex and small medium medulla. Black wildebeest have slightly shorter brown hair, circular shaped in cross section, with a thick cortex and no discernible medulla, they also have differences in scale patterns of their hair (Taru 2014).

Some of the genes involved in the development of these tissues derive from the ectoderm, and include *BMP*, *FGF*, *SHH*, *WNT*, *Eda* and *Edar*; these genes control the development of the different ectodermal tissues throughout their development (Pispa and Thesleff, 2003; Thesleff, 2006; Mikkola, 2007). With the timing and level of expression being tightly coordinated throughout development,

one could imagine that when two co-adapted genomes come together the patterning and the dynamics set up in hybrids will be different from that established in either parents resulting in the novel phenotypes such as the non-metric traits formed in hybrids. Thus, understanding the relationship between these traits which are intimately connected during early development, and determining whether these traits co-vary because of this is important. It could help us understand the non-metric traits we see, and possibly allow us make inferences about soft tissue based on non-metric trait variation.

This is not only applicable to dentition and pelage. Other traits also share developmental origins and are affected by pleiotropic genes. In mice, the development of dorsal ventral patterning is the result of differential expression of the agouti gene which determines which colour pigment is deposited in individual hairs (Millar *et al*., 1995). The differential pattern of expression in the dorsal and the ventral coat of mice forms part of the development of general body patterning, which includes skeletal development (Candille *et al*., 2004; Singh *et al*., 2005). Changes in genes important for the development of the limbs and the patterning of the skeleton also affect where the boundary between the dorsal and the ventral coat meets (Candille *et al*., 2004; Singh *et al*., 2005). In mice that have a loss of function mutation in the *Tbx15* gene, a reduced skeleton and cranium develops, resulting in the droopy eared phenotype (Candille *et al*., 2004; Singh *et al*., 2005). These mice also have a shift in the position of the dorsal ventral boundary (Candille *et al*., 2004). These are gross malformations and phenotypes produced by the loss of function of the *Tbx15* are very different from the wild type. We would expect more subtle morphologies in hybrids in terms of dorsal ventral patterning and skeletal patterning if differences in *Tbx15* gene and geneswith which it interacts were different between parental groups. This is but one gene which has pleiotropic effects and forms part of a series of genes activated during development of the mice and divergence anywhere along these pathways is likely to effect the development of the hybrid.

Developmental correlations and significant genes in human evolution

The Ectodysplasin (*EDAR*) gene has been identified as a possible candidate explaining the presence of supernumerary teeth in hybrids (Tucker *et al*., 2004; Ackermann, 2007). The expression level of this gene is associated with tooth morphology. Overexpression produces supernumerary teeth while under-expression produces reduced dentition (Tucker *et al*., 2004). It is suspected that in some hybrids the developmental breakdown results in the overexpression/underexpression of this gene and this results in the production of the supernumerary teeth or some of the reduced dentition noted (Ackermann *et al*., 2006; Ackermann, 2007). Variation in this gene is associated with head hair thickness in humans as well as dental morphology. The *EDAR1540C* variant produces thicker hair as

well as being associated with dental shovelling, a protostyloid cusp, and the absence of lower third molars; individuals with this variant also had larger molars in Asian and Native American populations, with the variant thought to have arisen 30 kya in China (Fujimoto *et al*., 2007; Kimura *et al*., 2009). There has been a selective sweep which resulted in this variant being found at high frequencies in East Asian populations. Experiments were done on mice in order to understand the evolutionary role of this variant and why it rose to such high frequencies (Kamberov *et al*., 2013). The wild type allele is the *EDART1540*/ *EDAR370V* allele and it is thought that the *EDAR370A* variant has better ability to bind to downstream products, in such a scenario overexpression of the wild type allele (*EDAR370V*) is expected to mimic the phenotype of *EDAR370A* (Kamberov *et al*., 2013).In protein dynamics overexpression of a variant of a protein which has a lower binding affinity to a substrate can simulate a protein with greater binding affinity. This is because there is more of the low affinity protein in the reaction and there is a better chance that the increased number of low affinity proteins can interact with the substrate even though it cannot bind as easily as the protein with the higher affinity, thus mimicking the phenotype produced by the high affinity protein(Kamberov *et al*., 2013). The overexpression of the wild type variant resulted in mice having thicker hair shafts similar to the phenotype displayed by humans with the *EDAR370A* allele; these mice also had enlarged mammary glands, a more intricate branching patterns as well as hyperplastic sebaceous glands, and meibomiane glands (glands in the eyelids) which "secrete hydrophobic films on the skin and the eye respectively as a barrier to water loss" (Kamberov *et al*., 2013). This led to the hypothesis that the *EDARV370A* variant was selected in response to cold and arid environmental conditions. In a follow up study in which the *EDARV370A* variant was knocked into mice, these mice had: thicker hair similar to the phenotype found in humans, but not significantly different meibomian glands from the wild type mouse, a more branched mammary gland and a larger mammary gland fat pad and an increased numbers of eccrine glands in the footpads (Kamberov *et al*., 2013). When looking at eccrine distribution in the hands of Han Chinese who had were homozygous for the *EDARV370A,* it was found that they had more active eccrine glands than their heterozygous counterparts (Kamberov *et al*., 2013). Thus, overexpression of this gene as well as genetic variation at this locus has resulted in changes across many traits which are under selective pressure and in direct interaction with the environment (Kamberov *et al*., 2013). It is thought that there was a selective sweep in Chinese populations favouring increased eccrine glands which would have assisted with dissipating heat in a humid Chinese climate (Kamberov *et al*., 2013). Other genes which have pleiotropic effects have also been introgressed in modern human populations. A good example of this are the Inuits who have an introgressed variant of the *TBX15* gene which is derived from a close relative to Denisovans (Racimo *et al*., 2017). This gene and the region on the genome which it encompasses is associated with cold

adaptation in Intuits (Racimo *et al*., 2017). This variant is also associated with variation in BMI and fat distribution as well as differential expression patterns in tissues such as fibroblasts and adipose, when compared to other non-introgressed variants. This is one example, but there were many pleiotropic genes which could have diverged and come together during hybridization between modern humans, Neanderthals and Denisovans. Understanding how these affected morphology and if we can see correlations is important. As previously discussed *Tbx15* is important for the development of the skeleton in mice. In modern humans a missense mutation in the *TBX15* gene results in a congenital disease known as Cousins Syndrome. Some of the defining traits of this syndrome include "complex cranial, cervical, auricular, and skeletal malformation syndrome with scapular and pelvic hypoplasia" (Lausch *et al*., 2008). These are similar to the features seen in the droopy phenotype presented by mice with the *Tbx15* deletion. Again these are gross malformations, but we know that there were differences in these developmental systems and that Denisovans and Neanderthals had body plans adapted to cold climates and, thus, we would expect these developmental differences to result in novel phenotypes in the hybrids. This is especially significant for the development of bones such as the scapula and pelvis, which don't develop properly if there isn't expression of the *TBX15* gene (Singh *et al*., 2005; Lausch *et al*., 2008). Thus, if were to hypothesise which traits might show transgressive phenotypes the scapula and the pelvis might be good candidates.Though there has been extensive research into development using mice because they are an easy to use model animal, and we have a great understanding of human developmental systems as they relate to medical issues, there has been relatively little research on these tissues in primates and thus there are large gaps in our knowledge.

Hybridization in the house mouse

This section focuses of morphology of the mouse as a model for considering morphological variation in other mammals, including hominins. Mice serve as a great model animal because they have short generation times, they reproduce in large numbers and there are many species of *Mus* and subspecies of *Mus musculus* (Guénet and Bonhomme, 2003). This provides an opportunity to produce a variety of crosses, with different histories of secondary contact in nature and different divergence times between the groups being crossed (Guénet and Bonhomme, 2003; Keane et al., 2011). Ancient DNA studies have shown that there has been very complex patterns of hybridization in the hominin past, and mice provide an opportunity for modelling a variety of possible scenarios that occurred in human evolution (Racimo *et al*., 2015).

For the model used here three subspecies of *M. musculus* were bred: *M. m. domesticus, M. m. castaneus* and *M. m. musculus.* These subspecies were initially identified due to their different defining mtDNA haplotype sequences (Boursot *et al*., 1996). *M. musculus* is thought to have originated somewhere on the Indian sub-continent; evidence for this is the high level of genetic diversity of the mice from this region relative to mice from peripheral populations (Figure 2.3) (Boursot et al., 1996; Bonhomme and Orth, 2013). This source population is thought to have settled in the region 900 kya and then dispersed and diverged in other regions a few hundred thousand years later (Figure 2.3) (Boursot *et al*., 1996; Din *et al*., 1996). The three lineages are thought to have diverged 300 kya to 500 kya ago (Boursot et al., 1996; Lundrigan, Jansa and Tucker, 2002; Milishnikov, Lavrenchenko and Lebedev, 2004; Geraldes et al., 2011; Staubach et al., 2012).

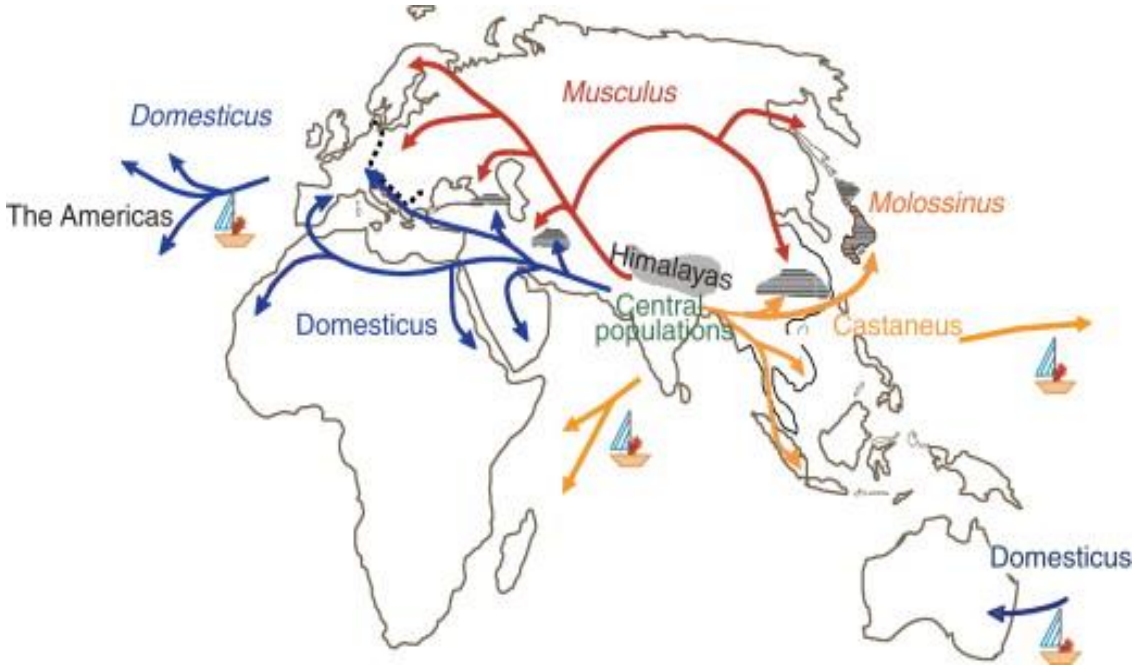

Figure 2.3: Hypothesized patterns of dispersal of *M. musculus* sub-species from the original population thought to be in Northern India. Figure taken from (Bonhomme and Orth 2013,)

They dispersed from the center of origin in Northern India to different regions of the world with defined geographic ranges (Figures 2.3 and 2.4). *M. m. musculus* has a geographic range from Eastern Europe to Japan; *M. m. domesticus* is found in Western Europe, Africa and the Near East; and the range of *M. m. castaneus* spans from Sri Lanka to South East Asia (Figure2.4) (Macholán, 2012). Although there is some discordance among various phylogenetic studies, a number of data sets support a closer phylogenetic relationship between *M. m. musculus* and *M. m. castaneus* with *M. m. domesticus* being more distantly related (Figure 2.5) (Boursot *et al*., 1996; Keane *et al*., 2011). *M. m.*

domesticus is thought to be older due to the fact that it has higher levels of mitochondrial and autosomal genetic diversity than the younger more closely related groups (Boursot *et al*., 1996), while *M. m. domesticus* and *M. m. musculus* are the least closely related (Figure 2.5)(Geraldes *et al*., 2011).

The three subspecies have ranges that overlap, resulting in secondary contact and the formation of two hybrid zones (Keane *et al*., 2011; Macholán, 2012). The *M. m. musculus - M. m. domesticus* hybrid zone in Europe is narrow and extends from Denmark to Bulgaria (Figure 2.5) (Macholán, 2012). The hybrid zone is thought to be a tension zone, where Bateson-Dobzansky–Muller incompatibilities result in hybrid infertility (Janoušek *et al*., 2012). The large X effect, which results in genes on the X-chromosome playinga key role in male hybrid infertility; however, epistatic interactions among autosomal genes have also been

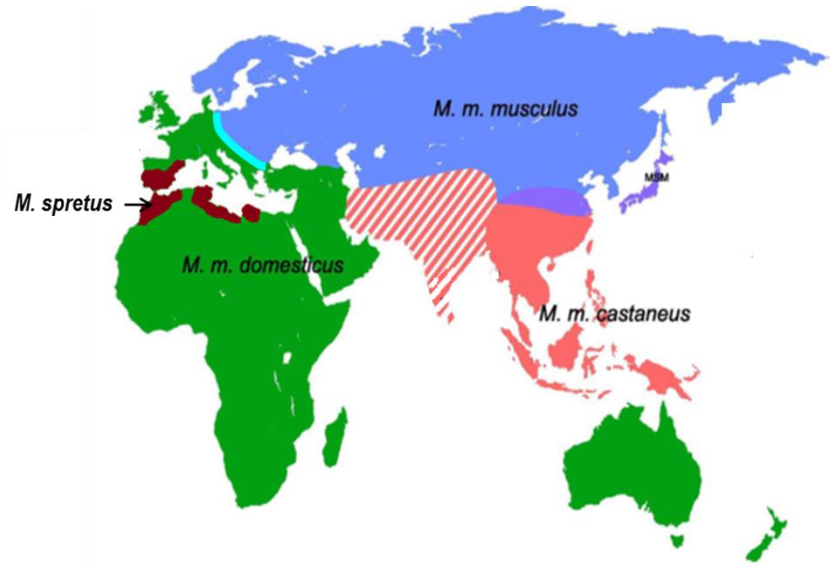

Figure 2.4: Adapted from Lilue et al 2013, this displays the range of the three subspecies of *M. musculus* with *M. m. domesticus* represented by green, *M. m. musculus* represented by blue, *M. m. casteneus* represented by pink. The *M. m. casteneus-M. m. musculus* hybrid zone is highlighted in purple and the *M. m. domesticus* – *M. m. musculus* hybrid zone highlighted in turquoise. The range of the species *M. spretus* is represented by maroon.

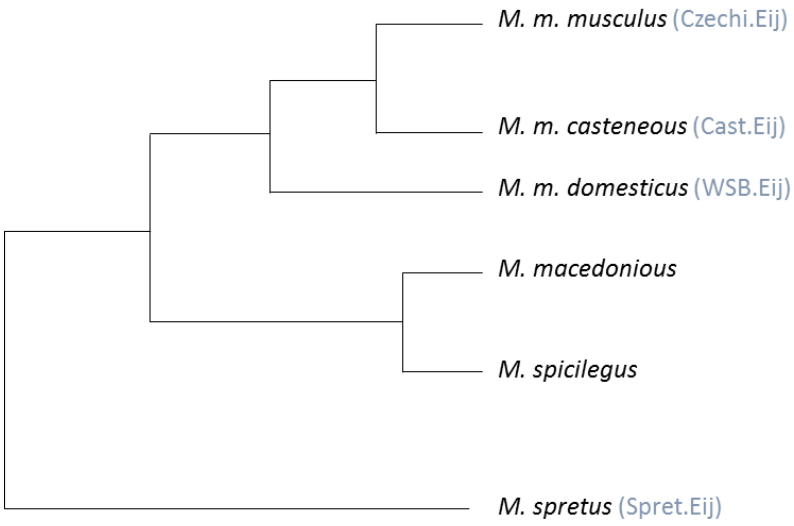

Figure 2.5: Reconstructed phylogeny displaying the phylogenetic relationships between the subspecies of *M. musculus which* diverged 300-500 kya and between *Musculus* species, with *M. spretus* diverging from *M. musculus* around 1.7mya. The strains representing these species used in this model are in brackets and highlighted in blue.

shown to play a role in the hybrid infertility (Janoušek *et al*., 2012). These two populations represent two lineages which are at a stage in the speciation process where hybridization can occur but there are post-zygotic barriers which produce offspring of variable fitness. This, however, has not stopped gene flow from occurring across this hybrid zone, and shaping the genomes of populations of *M. m. musculus* and *M. m. domesticus* close to the hybrid zone and further away from it (Staubach *et al*., 2012).The interplay between gene flow and selection resulted in many important genes with adaptive advantages spreading through the region as a result of hybridization (Staubach *et al*., 2012). Although the hybrid zone is thought to span 90km, populations outside of this hybrid zone are affected by hybridization, with the introgression of genes from the other taxon making up 9 to 25% of the genome of *M. m. musculus* from Czech Republic and Kazakhstan, and *M. m. domesticus* populations from France and Germany (Macholán 1997). An analysis of a hybrid zone in Denmark and Norway indicated that hybridization continues in the region and that beyond the hybrid zone there is evidence of introgression of *M. m. musculus* Y chromosomal alleles into populations considered to be *M. m. domesticus* (Prager *et al*., 1993). There is also evidence of *M. m. musculus* populations which carry *M. m. domesticus*-like mtDNA sequences in Germany; these are thought to be the result of a mouse hybrid founder event (Jones *et al*. 2010). Here we have an example of a hybrid population having a significant impact on descendent population even though there are biological barriers to hybridization. In these populations many cryptic hybrids exist with evidence of previous hybridization events only evident upon analysis of the genome (Milishnikov *et al*., 2004).

The *M. m. musculus - M. m. castaneus* hybrid zone is found in Asia, and is an extensive hybrid zone that resulted in the production of a hybrid species, *M. molossinus. M. molossinus* is endemic in Asia, with its range extending from the north-eastern part of China through to the Korean peninsula; it is also found on the island of Japan (Macholán, 2012). These Asian mice were originally thought to be a subspecies of *M. musculus*. Initial indications that *M. molossinus* could possibly be of hybrid origin were its affinity to *M. m. musculus* and the shorter genetic distance between these two groups (Minezawa, Moriwak and Kondo, 1981). Molecular analysis subsequently showed that *M. molossinus* mice had mosaic genomes composed of alleles derived from *M. m. musculus* and *M. m. castaneus*, and high levels of heterozygosity with very little geographical differentiation between the groups; it was determined that the species is the result of hybridization (Macholán, 2012). The Japanese populations of *M. m. molossinus* mice do not have their own defining mtDNA haplotype, and instead share haplotypes with *M. m. musculus* or *M. m. castenous* (Nunome *et al*., 2010). This hybrid zone has not been studied as well as the European hybrid zone even though it has resulted in the production of a hybrid species and is much more extensive.

To sum, three scenarios are represented in our model with the subspecies crosses. The first is hybridization between *M. m. musculus* and *M. m. domesticus,* a cross that in the wild manifests as a secondary contact with male hybrid fertility resulting in a narrow hybrid zone, but hybridization still shaping the genome of the descendent populations, as discussed above. The second is hybridization between *M. m. musculus* and *M. m. castenous,* with the wild situation consisting of secondary contact with an extensive hybrid zone, producing viable and fit offspring, and resulting in the production of a hybrid species, as above. The third cross is between *M. m. domesticus* and *M. m. castaneus* which do not come into secondary contact in the wild due to the geographic distance between them, and therefor do not hybridize naturally.

In addition to the subspecific crosses, crosses are also made between two species: *M. spretus* and *M. musculus* (*M. m. domesticus*). These two species are thought to have diverged around 1.7 Ma (Boursot *et al*., 1996). Previous data show that mouse hybrids between *M. musculus* and *M. spretus* suffer from male hybrid infertility (Bonhomme, 1996; Guénet and Bonhomme, 2003). Analysis of wild derived laboratory strains indicate that there is phylogenetic discordance when comparing *M. spretus* to *M. musculus* sub-species with 12% of the alleles not placing *M. spretus* as an out group (Keane *et al*., 2011). *M. spretus* and *M. m. domesticus* are sympatric in Africa and Europe and it is thought that natural hybridization occurs between the two and has occurred in the recent past as well as in deeper time (Orth *et al*. 2002, Liu 2015). Partial introgression has been detected using protein loci and DNA markers; the introgression detected was sporadic and occurred in a few individuals sampled (Orth *et*

al. 2002). Genome wide scans of *M. m. domesticus* mice for introgression of *M. spretus* genes were performed to determine whether other populations outside of the regions of sympatry also displayed indications of introgression or if the *M. spretus* introgression was confined to *M. m. domesticus* in sympatry. *M. m. domesticus* from regions of allopatry (*M. m. domesticus* from Europe) and sympatry (*M. m. domesticus* from Algeria) with *M. spretus* were analysed using genome wide sequences. Results indicate that *M. spretus* genes have introgressed into the genomes of *M. m. domestics,* but only on the autosomes, and that there was no introgression on the X chromosome, with a great deal of variation in terms of which autosomes showed indications of introgression. By analyzing the length distribution and sharing patterns of the introgressed regions researchers were able to determine that there were multiple hybridization events. There was a recent introgression event resulting in the group which had the introgressed *M. spretus Vkorc1* allele (Liu 2015). There are also short tracts of introgression shared across all three groups, which indicates an ancient hybridization event between *M. spretus* and that precedes the ancestor of all *M. m. domesticus* samples in the study; this took place before 2000 years ago before the colonization of Europe by *M. m. domesticus* (Liu 2015). The third hybridization event is a recent hybridization event which took place in Africa, with mice from *M. m. domesticus* in Morocco showing signatures of introgression (Liu 2015). The more recent introgression events took place within the last century, around 50 years ago, and this is as a result increased fitness due to warfarin resistance provided by the introgressed *Vkorc1* allele (Liu 2015). The allopatric mice from Europe have a larger proportion of their genome introgressed from *M. spretus* (Liu 2015). Introgressed tracks are shown to confer an adaptive advantage to the hybrids, regions shared between the groups thought to show selection for genes involved in olfaction which possibly provided an adaptive advantage and thus these introgressed regions remain present in both the sympatric and allopatric groups of *M. m. domesticus* (Liu 2015).

Genetic distance is thought to be a determining factor in hybrid phenotype and the addition of this cross will allow us to compared morphological outcomes between hybrids which are more closely related compared to hybrids which result from crosses which are more distantly related.

Chapter 3: Hybridization and Human Evolution

Recent African Origins (RAO) versus Multiregional (MR) models of human evolution

It is generally accepted that the first migration by hominins out Africa was the migration of *Homo erectus* into other regions of the world at around 2 million years ago. This initial dispersal resulted in the evolution of regional hominins inside and outside of Africa; what happened next was the question. Did modern humans evolve in one region (most likely Africa) and subsequently spread to other regions of the world, or did they evolve in the regions inhabited by *H. erectus* with gene flow between evolving groups resulting in modern humans? This debate over the emergence of modern humans has been raging for decades, with the model of total replacement generally favored, until recently.

The Recent African Origins (RAO)

The Recent African Origins (RAO) hypothesis posits that anatomically modern humans evolved in Africa and embarked on a (second) migration out of Africa, replacing all hominins found in regions outside of Africa, such as the *H. neanderthalensi* (C. B. Stringer and Andrews, 1988; C. Stringer, 2002). There has been a lot of support for this theory of modern human origins in part because the earliest fossils which are most similar to modern humans were found in Africa (C. B. Stringer and Andrews, 1988). Modern humans are described as those fossils which have derived traits found in modern populations, and are identified in the fossil record by the presence of a larger cranium with a round cranial vault and reduced facial size (Stringer and Andrews, 1988; . Bräuer, 2008). Early African fossils identified as modern humans include Omo I and II from Kibish (Bräuer, 2008). The Omo material from Kibish has been described as anatomically modern with primitive features, with Omo II being described as the more primitive specimen; they are dated to between 104 kya and 195 kya (Haldane, 1922; McDougall, Brown and Fleagle, 2005). Outside of Africa the modern humans fossil assemblages of Qafzeh and Skhul first appear in the Near East dated to around 100 kya (Grün *et al*., 2005). Thus, modern human traits first appear in the fossil record in Africa followed by the Near East and then they appear in the rest of Eurasia at around 40 kya with the appearance of Cro-Magnons in Eastern Europe and in the Iberian Peninsula (Anikovich *et al*., 2007).

Neanderthals also played a key role in the RAO narrative. Neanderthals are large brained hominins that evolved in Eurasia and inhabited Europe and Western Asia from the period of around 200kya to 30kya (Tattersall and Schwartz, 1999). They inhabited large geographic regions with regional variation in traits (Smith, 1991). Neanderthals are morphologically different from modern humans based on many cranial and post cranial traits, but they have many shared derived traits with hominins which evolved in Eurasia during the last 500kya (Smith, 1991). It was initially questioned whether they were *H. sapiens,* however, it soon became clear that they are a different lineage with their own defining traits (Smith, Janković and Karavanić, 2005). Though they were successful in the region for a long time upon the arrival of modern humans in the region at around 40kya they started to disappear and we don't see them in the fossil record after 27kya, with the youngest Neanderthal Mousterian culture site found in the Iberian Peninsula at around 27kya (Finlayson *et al*., 2006). The fact that Neanderthals were so morphologically different from AMH emerging in Africa was used as support for the ROA theory, as it provided evidence for a lack of continuity or interbreeding between modern humans and Neanderthals (Stringer and Andrews, 1988; Smith, 1991; Stringer and Gamble, 1993). Many researchers interpreted their disappearance from the fossil record, coincident with the appearance of modern humans in the regions, as evidence that modern humans replaced Neanderthals (Stringer and Andrews, 1988; Stringer and Bräuer, 1994). This replacement scenario is one of the defining features of the ROA theory, postulated as the result of Neanderthals being outcompeted (Stringer and Andrews, 1988; Stringer and Gamble, 1993; Stringer and Bräuer, 1994).

Early genetic evidence also provided support for the ROA hypothesis, with mitochondrial DNA (mtDNA) from modern populations indicating that the most diversity is found in Africa, pointing to a recent African origin for all living people (Ingman *et al*., 2000). The first sequencing of ancient Neanderthal mtDNA also indicated that modern humans from different geographic regions were more closely related to each other than to Neanderthals (Caramelli *et al*., 2003). Neanderthals were four times more different from modern humans than modern humans are from each other (Caramelli *et al*., 2003). The RAO theory was also supported by Y chromosomal data and autosomal data. Non-recombining regions of the Y chromosome have a young molecular age and geographic structure allows one to determine the effects of genetic drift and natural selection on male populations (Underhill *et al*., 2001). This region of the Y chromosome is also sensitive to change in population structure (Underhill *et al*., 2001). Analysis if this region showed that Y chromosomal haplotypes from Africa are more diverse than haplotypes form other regions (Underhill *et al*., 2001). This data was also used to create a phylogeography to determine the relationships between global populations (Underhill *et al*., 2001). These data indicate an early dispersal and diversification took place within Africa (Underhill *et al*., 2001). Analysis of the relationship between Fst (a measure of population

differentiation due to genetic structure) and geographic distance also indicates that there is a relationship between genetic distance and geographic distance from Africa (Ramachandran *et al*., 2005). The results show that global genetic variation is the result of a serial founder effect as modern humans moved outwards from a central point which was most likely Africa (Ramachandran *et al*., 2005). With each migration resulting in a subsampling of the population left behind as they moved further away from the center. Heterozygosity also decreases with increasing distance from Africa which is what is expected if modern humans first evolved in Africa and then subsequently spread to other regions of the world (Prugnolle, Manica and Balloux, 2005). The order of genetic diversity for different continents matches the order in which different regions were colonized There is decreased variation with increased distance and the largest level of variation occurs within Africa (Ramachandran *et al*., 2005). Genetic data from East Asian populations also supported the ROA theory, showing that all East Asians had a shared common ancestry and that the tree is rooted by African populations (Jin and Su, 2000). However, the statistical power of these tests were questioned and it was questioned whether the samples used for worldwide analysis were sufficient to refute the MR theory (Jin and Su, 2000). Y chromosomal data also did not support the trellis model of gene flow proposed by the MR theory (Jin and Su, 2000). It clearly showed that Y chromosomal haplotypes originated in Africa and diversified after migration out of Africa. There are no ancient local contributions to the Asian populations, to indicate that there was regional evolution. The haplotypes are also relatively young if there was gene-flow between different groups you would expect to find older haplotypes in all regions and not just in Africa (Jin and Su, 2000). In sum there was evidence from the fossil record as well as molecular data which supported the ROA theory. These multiple lines of evidence made this the accepted theory for modern human origins.

Multiregional (MR) hypothesis

In contrast, the MR hypothesis posits that *H. erectus* migrated out of Africa and modern human traits subsequently evolved regionally with gene flow between regional populations (Thorne and Wolpoff, 1992). This is based mainly on evidence for continuity of traits between ancient and modern regional populations, especially between *H. erectus* and modern populations (Thorne and Wolpoff, 1992). Proponents of this hypothesis maintained that fossil evidence was a better source of for understanding human origins than mtDNA. Continuity of traits between ancient fossils from Java and modern Australian aboriginal populations were used as evidence for MR evolution (Thorne and Wolpoff, 1981; Hawks et al., 2000). Fossils from mainland Asia were also thought to show some continuity with modern people, however the traits which are thought to be continuous are different from the traits recognized for the Australian and Javanese fossils (Thorne and Wolpoff, 1992). The third region supporting this hypothesis was Europe, where it was argued that more recent

Neanderthal fossils share traits with modern Europeans, and some Neanderthal derived traits are found in modern human populations outside of Africa (Thorne and Wolpoff, 1992; Wolpoff et al., 2004). Thus, there were three regions that were thought to independently support MR evolution, or to show continuity of traits.

Additional evidence supporting MR evolution and refuting the RAO hypothesis included the rapid rate at which AMH colonized new environments. It was hypothesized that modern humans could not adapt as rapidly to new environments as would be required by the ROA hypothesis. There must have been gene flow allowing for beneficial genes to be shared between independently evolving populations (Thorne and Wolpoff, 1992; Wolpoff, Hawks and Caspari, 2000). Archaeological evidence was also used to support this hypothesis with proponents claiming that there was no discontinuity in stone tool cultures in Eurasia that could indicate replacements of populations occupying this region (Thorne and Wolpoff, 1992). There is very little evidence for invasion in these regions as well as no African material culture leaving the continent; instead they surmised that there was a shared material culture between modern humans and Neanderthals, in the Middle East where occupation is thought to overlap for a significant period of time (Thorne and Wolpoff, 1992). Modern human remains have also been found in conjunction with Mousterian stone tool culture (usually associated with Neanderthals) in Israel (Mayer, Vandermeersch and Bar-Yosef, 2009).

Criticisms of RAO and MR

Though the ROA model has had the most support and it is widely accepted that modern humans evolved in Africa and subsequently migrated to other regions of the world. What happens subsequent to human migration out of Africa is still questioned. Both proposed models were extreme and were modified as new data became available. Below are some of the criticisms of both theories.

One of the main criticisms of the MR evolution models is that some of the derived traits which are supposed to represent continuity are not unique to the Asian fossil record neither those attributed to Asian *H. erectus*, nor those attributed to modern Asian populations (Bräuer and Mbua, 1992; Lieberman, 1995; Bräuer, 2008). Studies of traits said to be continuous did not show patterns of continuity, some traits were too variable to be of used, and others did not show any significant regional distribution (Lieberman, 1995; Lieberman, 1995; Bräuer, 2008). With regards to the Australian fossils the defining traits were thought to be plesiomorphies found in archaic humans from the Middle East and Africa (Bräuer and Mbua, 1992; Lieberman, 1995). Another criticisms of the morphological data used for the MR theory included the fact that there was very little consensus on how traits are defined, resulting in different researchers interpreting the same specimens very

differently (Bräuer and Mbua, 1992; Lieberman, 1995;Bräuer, 2008). There is also a large gap in the East Asian fossil record with archaic hominins found at around 100kya with a 60 kya gap before modern humans appear in the fossil record at around 40 kya. This does not indicate continuity instead it indicates that the 100 kya archaic population disappeared and that the region was repopulated by modern humans at a later stage (Jin and Su, 2000).

The ROA model needed more flexibility, because of evidence for some continuity. In spite of the mtDNA evidence which indicated than Neanderthals and modern humans where two separate groups, it was still possible that gene flow between the two groups occurred without any Neanderthal mtDNA surviving in modern populations (Bräuer, Collard and Stringer, 2004). Modern humans and Neanderthals being classified as separate paleospecies also didn't mean that hybridization couldn't occur, even if they had diverged significantly over time (Stringer and Bräuer, 1994). As noted previously hybridization between primates is common even though they are considered different species, highlighting the possibility of hybridization occurring even though groups might still maintain species differences (Bräuer *et al*., 2004). Hybridization was proposed because there is some evidence for continuity in the fossil record and the ROA model needed to be modified to accommodate this (Bräuer *et al*., 2004). A lot of support for the ROA theory also came from molecular data, specifically Y chromosomal data and mtDNA. However when using X chromosomal and autosomal data to determine demographic events in the past there were some indications that admixture might have occurred. Using divergence times for different genomic regions there were indications that some regions in modern human genomes had divergence times as far back as 535 kya to 186 kya ago predating the hypothesized time when modern humans migrated out of Africa (Jin and Su, 2000). Other regions of the autosome and the X chromosome supported a more recent divergence time (Jin and Su, 2000). Sequencing of the β-globin gene indicated some Asian ancestry for this region of the genome was more than 200 kya and thus did not support the ROA hypothesis and supported the MR hypothesis (Harding *et al*., 1997). X chromosomal data from a non-coding region which is not under selection and that has low recombination rates indicated that the highest level of diversity was found in Africa, however it also indicated that a deep divergence time occurred in Asia. This data supported the ROA hypothesis because of the higher diversity in Africa. However, the earlier divergence time for Asia did not align with the ROA model (Kaessmann *et al*., 1999). Because of the slower mutational rates of this region of the X chromosome when compared to the mtDNA, older demographic events can be determined (Kaessmann *et al*., 1999). This data indicated that the most recent common ancestor existed at 535 kya, with two aboriginal Australians and an individual from Georgia having ancient genotypes along with African individuals (Kaessmann *et al*., 1999). The African haplotypes are more widely distributed than the other two sequences reflecting a higher genetic diversity

(Kaessmann *et al*., 1999). The age of divergence was too young to be the result of similarity due to shared ancestry because the last common ancestor would have been *H. erectus* and thus the required divergence time would have had to be older than 1 mya (Kaessmann *et al*., 1999). The authors of this article concluded that the data supported the ROA theory but did not reconcile it with the possibility of hybridization or recent admixture events, even though this might have been a plausible explanation for the results (Kaessmann *et al*., 1999). Other genetic data from the X chromosome also showed discordance with the mtDNA data showing that there was a divergence time of ~200 kya for the X linked *PDHA1* locus (Harris and Hey, 1999). This discordance between the mtDNA and Y chromosomal data and the autosomal and X chromosomal data indicated that there was second dispersal from Africa. However, certain regions of the genome also showed deeper divergence times which indicated gene flow and introgression of genes present in a population in Eurasia predating the second dispersal. This molecular data was not accommodated for in the ROA model. There was also evidence in the fossil record for continuity or gene flow between modern humans and Neanderthals as the migrated out of Africa. Many of the modern humans from Eurasia had traits associated with Neanderthals, and many later Neanderthals had traits associated with modern humans (Bräuer, 1981; Stringer and Bräuer, 1994; Smith et al., 2005; Bräuer, 2008) .

Both theories were extreme, there needed to be a way to merge the molecular and morpholigcal data, which clearly indicated the modern humans evolved in Africa. However there was also a need to accommodate some of the evidence of continuity between archaic fossils from different regions of Eurasia and modern humans, molecular data showing haplotypes with deep divergence times in modern populations as well as the archaeological data which showed interaction between modern humans and Neanderthals. Theories were needed to reconcile the fact that modern humans did not emerge in Europe, but that Neanderthals might have had a major influence on the evolution of modern humans once they left Africa (Smith, 1991).

Alternative models for modern human origins

As mentioned above alternative models were needed. One of the alternative models proposed was the assimilation model which was largely based on the on the fossil record prior to aDNA becoming available (Smith *et al*., 2005). This theory was proposed to explain the rapid expansion of modern humans into regions with very different climates. It posseted that modern humans needed to interact with other groups already established and adapted to those regions such as the Neanderhtals in the colder northern climates (Smith, 1991; Smith *et al*., 2005). The assimilation model posited that

hybridization and the introgression of beneficial genes was necessary to move into the colder climates of Europe at a later stage. Neanderthals were thought to be a good candidate for interbreeding and providing modern humans with beneficial genes (Smith, 1991; Smith *et al*., 2005). It was much easier to move into Western Asia because of the milder climate compared to the rest of Europe (Smith *et al*., 2005). Under the assimilation model this interaction did not lead to the elimination of Neanderthals but simply and exchange of genes, with Neanderthals being absorbed into modern human populations and not outcompeted (Smith, 1991). Under the assimilation model continuity was seen in certain traits and particular anatomical features and not in the overall morphology (Smith *et al*., 2005). It was also thought that Australian aborigines showed indications of continuity. This is due less to the presence of particular traits, but the presence of a combination of traits seen in the Javan skulls and in modern aboriginal Australians (Smith, 1991; Smith *et al*., 2005). Other fossil evidence supporting possible continuity included the fact that more recent Neanderthals had modern human derived traits (Smith *et al*., 2005). Cro-magnons were also thought to have Neanderthal derived traits, including the presence of occipital bunning and suprainaic fossa (Smith, 1991; Smith *et al*., 2005). Both traits were not found in archaic African fossils, and thus must have evolved independently in modern humans in Europe or as is more likely are the result of interaction with Neanderthals. The Modern humans might have a slightly different morphology when compared to Neanderthals, but you would not expect off spring resulting from admixture between two groups who have diverged for relatively long period of time to have the exact same morphology as one of the parents (Smith, 1991; Smith *et al*., 2005). This theory is also supported by archeological evidence which indicated an overlap in human and Neanderthal material culture and transfer between these cultures, with the Le Chaperion culture which includes the use of bone as a raw material by Neanderthals thought to be a result of their interaction with modern humans who by the time had been using the Aurignacian stone tool culture. There are also modern human fossils in the Middle East found in association with the Mousterian Stone tool culture. Before the sequencing of the Neanderthal genome there were also indications from modern genomes as mentioned above that admixture occurred, this in conjunction with the fossil evidence was seen as clear indications for interactions between Neanderthals and humans and assimilation of Neanderthals through interbreeding (Smith, Janković, and Karavanić 2005, 7-19).

It was recognized that the ROA model needed to be modified. Studies showed that hybridization between mammals occurred with some frequency and there was a possibility that Neanderthals and modern humans were capable of hybridizing (Stringer and Bräuer, 1994). Thus the hybrid theory of modern human origin was proposed (Bräuer, 1981; Stringer and Bräuer, 1994; Bräuer, 2008). Even though the mtDNA didn't show any shared ancestry, it was acknowledged that limited hybridization could occur without out Neanderthal derived mtDNA being present in modern human populations

and it was recognized that brief interactions between different groups was a possibility (Stringer and Bräuer, 1994). It was recognized that there could have been gene flow recently after modern humans migrated out of Africa, however, it was not long term gene flow since the time *H. erectus* occupied different regions of the world (Stringer and Bräuer, 1994). In this model Africa provided the prime evolutionary source for modern human morphology (Bräuer, 1981; Stringer and Bräuer, 1994; P. Bräuer, 2013). These hybridization events were not necessarily the same as the extended gene flow patterns which were proposed by the MR model, and the hybridization events might not necessarily have resulted in a continuation between Neanderthals modern populations however there was evidence that it occurred (Bräuer, 1981; Stringer and Bräuer, 1994; Bräuer, 2013). Thus the ROA model with hybridization proposed that modern humans and Neanderthals were the same species and that they differentiated at the sub-species level (Stringer and Bräuer, 1994). Thus they were capable of interacting and reproducing (Stringer and Bräuer, 1994). This model hypothesized that hybridization occurred but there was still replacement and not assimilation off other groups. It was also recognized that gene flow was possible and that these are paleo species and their might were most likely limited real barriers to gene flow between these different groups, but that they would still be able to reproduce as many mammalian hybrids do.

Ancient DNA studies post-2010

The sequencing of the Neanderthal genome profoundly affected our understanding of the emergence of our species, and especially the question of whether humans emerging form Africa truly did replace other hominins such as Neanderthals. Sequencing the autosomal genome of the first Neanderthal and comparing it to the genomes of modern humans made it clear that admixture occurred between humans leaving Africa and the hominins they encountered (Green *et al*., 2010). The first study indicated that 2-4% of the genome of non-African modern humans are derived from the Neanderthals (Green *et al*., 2010). Subsequent sequences of additional Neanderthal genomes along with full genome sequences of modern populations showed that Asians have a larger proportion of their genome derived from Neanderthals compared to Europeans (Wall *et al*., 2013). Considering the fact Neanderthals are thought of as inhabiting mostly Europe and the Middle East this has led to many questions about where and how often modern humans and Neanderthals interacted.

Some argued that these shared derived genes were the result of population substructure within Africa before the first migration took place and these alleles predated the split between modern humans and Neanderthals (Eriksson and Manica, 2012). However, studies of the shared regions

indicate that these genomic regions are most likely the result of introgression because the divergence time between the introgressed regions in modern human genomes and the Neanderthal sequences are around the time period when humans and Neanderthals would have made secondary contact and long after the split between modern humans and Neanderthals occurred (Sankararaman *et al*., 2012). If they were shared derived genomic sequences then we would expect larger divergence times between the regions of the genome more similar to the Neanderthal genome than to the same regions in modern African populations. The expected divergence time would have been would have been 400 kya if the shared region was due to population substructure within Africa before the first migration (Sankararaman *et al*., 2012).

The sequencing of aDNA from the Oase 1 mandible, a modern human dated to 37kya to 47kya ago and thought on the basis of morphology to be a product of admixture, confirmed that Neanderthal derived sequences present in modern humans are the result of admixture (Fu *et al*., 2015). This fossil had Neanderthal ancestors as recently as 6 to 8 generations prior, with 6-9 % of its genome derived from its Neanderthal ancestor (Fu *et al*., 2015). Oase1 also had longer stretches of Neanderthal derived genes when compared to modern populations, which indicated that the genes had been recently introgressed into this individual resulting in longer stretches of Neanderthal derived DNA that had not been broken down by recombination; other modern human remains from Siberia showed a similar pattern (Fu *et al*., 2014; Fu *et al*., 2015).

Hybridization did not only occur between Neanderthals and modern humans but also between a group known as the Denisovans (Prüfer *et al*., 2014). They are a sister group to Neanderthals and more distantly related to modern humans; they were identified as a separate lineage based solely on aDNA evidence taken from a phalynx and three teeth providing us with genomic data for four individuals (Krause *et al*., 2010; Slon *et al*., 2017). Denisovans were initially identified as a distinct group based on analysis of mtDNA sequences indicating that they had haplotypes outside the range of variation of modern humans and Neanderthals. Additional sequences from 3 other Denisovans show that they group together to the exclusion of Neanderthals and modern humans (Krause 2010). Denisovans and Neanderthals are thought to have diverged between 190 and 470 kya. Denisovans contributed substantially to the genomes of South Asian and Oceanic populations with 3-4% of their genome derived from the Denisovans (Reich *et al*., 2011; Sankararaman *et al*., 2016). Not much is known about the morphology of the Denisovans because other than the phalange and dental remains no other cranial and skeletal remains are available (Krause *et al*., 2010; Meyer *et al*., 2012; Slon *et al*., 2017). Ancient DNA for Denisovans are from three different time periods; the oldest is Denisova 2 thought to be 54.2 to 99.4 kya older than Denisova 3 and 20.6 to 37.7 kya older than Denisovan 8 (Slon *et al*.,

2017). Denisova 2 which is the oldest remain, shared fewer derived alleles with Denisovan 3 which indicates that it is more distantly related to Denisova 3, 4 and 8 (Slon *et al*., 2017). Analysis of the Denisovan genome gives some phenotypic information and it is likely that Denisovans had dark skin, hair and eyes like some modern human populations (Meyer *et al*. 2012). Denisovan sequences also indicate that Denisovans experienced admixture in the past with a lineage which predates the split between humans, Neanderthals and Denisovans (Meyer et al., 2012; Vernot and Akey, 2014). Gene flow didn't only occur in one direction; East Asian Neanderthals from the Altai Mountains also have genes which were introgressed from modern humans (Prüfer *et al*., 2014; Kuhlwilm *et al*., 2016). These genes are derived from a population of modern humans that diverged early from modern African populations and contributed to the Neanderthals from the Altai Mountains at around 100kya prior with contact most likely taking place in the Near East (Prüfer *et al*., 2014; Kuhlwilm *et al*., 2016).

Ancient DNA along with full genomic sequences has allowed us to gain some insight into the frequency of hybridization and when it occurred. When looking at the patterns of introgression in modern humans we don't see the same patterns of introgression in European, Asian and Oceanic populations (Sankararaman *et al*., 2016). We also know that modern populations from Southern Asia have a large proportion of their genomes derived from Denisovans with a smaller contribution from Neanderthals (Sankararaman *et al*., 2016). Using this genomic data from modern populations it is thought that admixture with Neanderthals took place around 50 to 60 kya while admixture with the Denisovans took place at around 44 to 55 kya (Sankararaman *et al*., 2016). The Ancient gene flow from modern humans into Neanderthals also indicates that there was an admixture event at around 100 kya (Kuhlwilm *et al*., 2016). This admixture event took place after the Neanderthals split from Denisovans (Kuhlwilm *et al*., 2016). There is also some evidence that hybridization may have occurred as far back as 270 kya resulting in mtDNA from an African population being introgressed into the Neanderthal populations (Posth *et al*., 2017). We know from analysis of modern genomes that hybridization occurred multiple times, with additional pulses of admixture between Neanderthals and modern humans showing up in the genomes of European, East Asian and South Asian populations compared to Melanesians (Sankararaman *et al*., 2016).

Ancient DNA has also allowed us to make certain inferences about the processes of hybridization. It is suspected that there might have been male hybrid infertility which could have been a barrier to gene flow between the populations (Currat and Excoffier, 2011). This is because there is a large reduction in introgression on the X chromosome compared to the rest of the genome, as well as areas around genes which are expressed in the testes more than other tissue groups (Currat and Excoffier, 2011; Sankararaman et al., 2016).

Population demographics of Neanderthals and Denisovans based on aDNA

In addition to contributing to our knowledge of hybridization and introgression, aDNA has allowed us to learn a lot about population structure. Analyses have indicated that Neanderthals had smaller population sizes and lower levels of genomic variation when compared to modern human populations (Prüfer *et al*., 2014). Smaller population sizes are inferred from the fact that Neanderthals have more deleterious mutations which could not be weeded out by selection, as well as the fact that they had much longer stretches of homozygosity when compared to modern humans (Prüfer *et al*., 2014; Kuhlwilm *et al*., 2016). It is also thought that there were many consanguineous relations between Neanderthals (Prüfer *et al*., 2014). Early studies of Denisovans indicate that they also have low levels of heterozygosity, but because they do not have long tracts of homozygousity the low levels of heterozygosity is most likely a result of small population size and not consanguineous relations/inbreeding (Slon *et al*., 2017). Genomic data also indicated that when modern humans started spreading we see a decline in the population of Denisovans. However, the recent sequencing of the fourth Denisovan individual, also from the Denisova cave, shows that they had similar sequence diversity to the Neanderthals (Slon *et al*., 2017). Both groups are at the lower range of modern human genomic diversity (Slon *et al*., 2017). This is surprising since all four Denisovan sample come from the cave while Neanderthal genomic samples come from a large geographic range but still had less variation (Slon *et al*., 2017).

The limitations of aDNA and evidence for extensive gene flow from modern African genomes

Ancient DNA gives us a window into past interactions and confirms that hybridization occurred multiple times. There are however still many questions as to where it occurred and how often. We know that there are many other hominin populations which overlapped in time and space. Genomic analysis of human, chimp and gorilla DNA indicates that there has been admixture and secondary contact between these groups after the initial divergence (Patterson *et al*., 2006). This is indicated by the fact that there are stretches of the genome which show smaller levels of divergence when compared to the rest of the genome, indicating that some genes were introgressed after the initial split, possibly as a result of secondary contact (Patterson *et al*., 2006). Analysis of genomes from modern African populations also indicates that there was extensive admixture in Africa. Regions of the genome which are the result of introgression in modern populations are expected to have larger divergence times between haplotypes when compared to the rest of the genome (Hammer *et al*., 2011). This method was used to identify whether introgression and admixture occurred even though

aDNA samples are not available for African samples (Hammer *et al*., 2011). This research indicates that introgression occurred around 35 kya (Hammer *et al*., 2011). The two groups which are thought to have hybridized diverged at least 700 kya (Hammer *et al*., 2011; Hsieh *et al*., 2016). There was also a stretch of chromosome 4 which is thought to have been introgressed more recently from an extinct population that may have lived in Central Africa. Ancient admixture has also been identified by looking at shared short regions of African populuations, Neanderthals and Denisovans. These short regions which are shared and thought to be identical by descent and predate when secondary contact took place were used to infer admixture in deeper time (Hammer *et al*., 2011). Analysis of modern genomes has also showed that hybridization is thought to have occurred 1.2 – 1.3 mya in African populations using genomic sequences from Tanzanian hunter gatherer and farming populations (Lachance *et al*., 2012). These data combined with aDNA data suggests that at many points along our evolutionary trajectory, between various hominin species, hybridization could have occurred. However, we are not able to get aDNA from older material. Thus it will be important to understand how morphology is affected by hybridization in order to identify hybrids from deep time, identify possible hybrid zone and the dynamics of those hybrid zones.

Identifying hybrids in the fossil record

Thus far a number of hybrids have been proposed in the fossil record based on morphological data. These are mostly fossils found in the transitional zones between Africa and Eurasia with fossil and archaeological evidence indicating that there might have been hybridization between modern humans moving out of Africa and Neanderthals (Wolpoff *et al*., 2004; Smith *et al*., 2005; Ackermann, 2010). An example would be in the Middle East where there was an overlap in occupation. First modern humans occupied the region at around 100 kya, subsequently the region was occupied by Neanderthals at around 52kya (Smith *et al*., 2005). Many of the first modern human fossils found in Europe also indicate that admixture has occurred due to the presence of mosaic phenotypes and traits that are considered Neanderthal derived traits (Smith *et al*., 2005; Ackermann, 2010). More recent Neanderthals are also thought to exhibit modern human traits such as the presence of an incipient chin becoming more frequent in younger Neanderthal samples (Smith *et al*., 2005; Condemi *et al*., 2013). Some fossils also show indications of developmental instability especially in terms of dental development which will be discussed further below. Other signs of developmental instability include by facial asymmetry as is the case of Skhul V (Ackermann, 2010). Developmental instability is thought to be one of the consequences of hybridization due to new genomes coming together in the hybrid.

Fossils also exhibit other outcomes of hybridization such as heterosis in certain dental traits as will be discussed below.

Oase 2 displays what might be considered heterosis in their facial size being described as having a "large facial skeleton and dentition, which gets progressively larger distally" (Rougier *et al*., 2007). This fossil also has other traits which have been identified in mammalian hybrids such as the presence of an ossicle found between entomion and asterion (Rougier *et al*., 2007). Ossicles were found at higher frequencies in baboon and mouse hybrids (Ackermann *et al*., 2006). Oase 2 also has large molars outside of the range of modern humans despite being classified as modern humans overall (Rougier *et al*., 2007). The patterning of the molar progression is also strange for a modern human with the size progression being similar to Neanderthals (Rougier *et al*., 2007). The third molar also has an interesting occlusal morphology which is very distinct (Rougier *et al*., 2007). Oase 2 also has what can be described a hemibun along with the modern human features of the crania. This hemi-bun is a Neanderthal derived trait seen in many modern humans in Europe at the time (Rougier *et al*., 2007). This is a good example of a hybrid identified based on morphological traits, it has indications of heterosis as well as a mosaic morphology.

Other possible hybrids have been identified as far back as 130 kya, the fossils from Krapina Croatia show indications of hybridization (Schwartz 1995). This is a collection composed of 24 adults and juveniles classified as Neanderthal with many Neanderthal derived traits (Rougier *et al*., 2006). They also have other interesting dental traits such large teeth and higher proportions of rotated P3 molars compared to both modern human populations and other comparative Neanderthal populations. This high frequency is thought of as a unique feature of this group and is similar to the pattern identified in primate and wildebeest hybrids (Rougier *et al*., 2006; Ackermann *et al*., 2006; Ackermann, 2010).

In the Middle East where AMH and Neanderthals are present there are not majour archaeological differences even though there are two morphologically different groups. Material culture cannot be used to distinguish the two groups significantly. The oldest fossils in the region identified as hybrids are Qafzeh and Skhul fossils found in cave sites in Mount Carmel (McDermott *et al*., 1993). Both groups of fossils have cranial and post cranial traits indicating that they are anatomically modern humans, they are however found in association with the Mousterian stone tool culture which has been associated with Neanderthals in Europe (Mayer *et al*., 2009). The Skhul collection is dated to 100kya to 135kya and is made up of ten individuals, that were intentionally buried (Grün *et al*., 2005). Though classified as modern humans these remains have traits that are associated with Neanderthals including a retro-molar space and the lack of a chin. This mosaic phenotype could point to possible hybridization. Other traits associated with hybridization have been identified such as rotated teeth

found in Skhul IV, and cranio-facial asymmetry in Skul V (Ackermann, 2010). Skhul IV and IX are intermediate for some characteristics between humans and Neanderthals lending more support to the theory that this group represents a population descended from hybrids. The Qafzeh collection is younger and dated to 95 kya and is composed of 12 individuals. The Qafzeh sample shows high levels of variation (Smith, 1991; Ackermann, 2010). One Qafzeh individual (Qafzeh 11) has rotated molars, and others (Qafzeh 6,8,9) exhibit dental crowding which could represent transgressive traits or developmental instability during dental development as a result of hybridization (Ackermann, 2010). Also from the Middle East are Near Eastern Neanderthals from Tabun, Amud, Kebara and Dederiyah (Trinkaus, 1995). These remains have been identified as possible hybrids. Near East Neanderthals are seen as distinct from Western European Neanderthals because of differentiating traits which include a higher cranial vault in the range of variation seen in modern humans, with other features being slightly different from what we see in classic Neanderthals (Trinkaus, 1995). Amud I is from Israel and dated to 50 kya, it has a very large cranium outside of the range of variation seen in other hominins from the same time period (Ackermann *et al*., 2006; Ackermann, 2010). This fossil has a reduced 3rd molar which might represent dysgenesis in dental development which would be an outcome of hybridization (Ackermann, 2010). The Tabun cave has the remains of Tabun I which is composed of a cranium and skeleton, and has been classified as a Neanderthal. Tabun II is composed of a mandible and might come from a layer below Tabun I which has been difficult to classify. The difficulty with the classification of Tabun II is due to the fact that is has human traits such as a chin, found in conjunction with other Neanderhtal mandibular traits. Morphometric analysis has grouped Tabun II with early modern humans (Quam, 1995). However, more recent research shows that Tabun II does not group with Neanderthals or Modern Humans and may predate both groups (Harvati and Lopez, 2017). Tabun II also has a retromolar space which some have interpreted as a Neanderthal trait, however it has been argued that the morphology for this is different when comparing modern humans to Neanderthals from the Middle East (Trinkaus, 1995). With the retromolar space in modern humans being a result of a deeper alveolar notch while in Neanderthals it is the positioning of the M3 which results in the retromolar space (Trinkaus, 1995). If the retromolar space in archaic humans in the Middle East are the result of hybridization we would not expect the phenotype to be exactly the same as the parental group. The retromolar spacet is only introduced into modern populations at a much later period after hybridization with Neanderthals was a possibility.

We also find possible hybrids in Europe which modern humans colonize at a much later stage. These include the Oase 1 and 2 fossils (Ackermann, 2010). Oase 1's status as a descendent of a hybrid has been confirmed by aDNA analysis, here we have confirmation of the hybrid nature of a specimen which was first identified mainly on morphology (Rougier *et al*., 2007; Ackermann, 2010). A frontal

bone from Hamburg dated to 36 kya which shows both Neanderthals and modern human traits is also thought to be a hybrid (Bräuer, 1981). This was identified as a hybrid due to the fact that PCA grouped it with Neanderthals while it had some AMH traits (Bräuer, 1981). The most recent fossil identified as a hybrid is a juvenile found in Portugal dated to around 20 to 30kya. It is thought to be a modern human but has some traits which are associated with Neanderthals, traits that show intermediate morphology, and other traits that are thought to be extreme, such as the chin which is thought to be well developed for estimated age of the fossils (Duarte *et al*., 1999). This specimen also has post cranial traits which are Neanderthal like including body proportion more similar to Neanderthals (Duarte *et al*., 1999). The combination of a mosaic phenotype, with traits having intermediate measurements as well as the presence of extreme traits all point to the possible hybrid nature of the fossil (Duarte *et al*., 1999). Other specimens identified as descendants of hybrids include the Mladěc assemblage from the Czech republic identified as a group of modern humans associated in association with the Aurignacian stone tool culture dated to 31kya (Wolpoff *et al*., 2001; Wild *et al*., 2005) . Mladěc 5 has many Neanderthal characteristics such as "suprainiac fossa of elliptical form, extensive lambdoidal flattening, and a short posterior face on the occiput" (Wolpoff *et al*., 2001). The Mladěc fossils are also thought to be hybrids because they display sagittal traits and dimensions similar to Neanderthals, other interesting traits include large maxillary canines (Wolpoff *et al*., 2001). The Skhul/ Qafzeh modern humans and Neanderthal fossils are thought to be good candidates as ancestors for these specimens. Cioclovina has also been identified as a hybrid and is dated to the 28 kya (Soficaru *et al*., 2007). This modern human has Neanderthal derived traits such as the superior nuchal morphology producing a mosaic phenotypic pattern which indicates hybridization (Soficaru *et al*., 2007). The post cranial body proportions of the skeleton also indicate that it is the result of admixture (Soficaru *et al*., 2007). We also see changes in the morphology of later Neanderthals, indicating that this population as a whole was affected by hybridization. Later Neanderthals are thought to have a more modern chin region with an incipient mental trigone, which has a different morphology from earlier Neanderthals (Condemi *et al*., 2013). A fossil which displays this trait is the Mezzena Neanderthal from Italy (Smith, 1991; Condemi *et al*., 2013). mtDNA from the Mezzena fossil indicates that it is Neanderthal, as well as other features of the mandible including the height at the mental foramina and at the M2 being within the range of variation recognized for Neanderthals along with some non-metric traits (Condemi *et al*., 2013). However, geomorphometric analysis groups the Mezzena fossil with modern humans (Condemi *et al*., 2013). Other younger Neanderthals such as Spy, La Ferrassie 1, Saint-Césaire, Vindija and Las Palomas are thought to show the same pattern (Condemi *et al*., 2013).

Many of the modern human remains with indications of hybridization are found during a period when Neanderthals no longer inhabited Europe. This brings up the important question of whether we

expect to see traits which are the result of hybridization in the fossil record in later generations of backcrossed hybrids which these fossil remains would represent. The Oase 1 mandible which was identified as a hybrid and confirmed by sequencing still displayed hybrid traits even though the hybridization event occurred 6 to 8 generations back (Fu *et al*., 2015). A recent article looking at modern humans, showed that there was a correlation between overall morphology being more similar to Neanderthals in modern human populations and the proportion of the genome derived from Neanderthals (Gregory *et al*., 2017).Thus it is possible that these phenotypes would still be present in skeletal material long after hybridization has occurred and there had been backcrossing back into modern populations. However more work needs to be done to determine the frequency of the traits and whether they change across generations of hybrids.

In sum many of these hypothesized hybrids show patterns identified in mammalian hybrids such as the presence of rotated teeth, transgressive dental size, dysgenesis with reduced dentition and the presence of ossicles. It is thus important gather more data which helps us understand how hybridization affects skeletal phenotype, in order to make it easier to identify hybrids in the fossil record. It is also important to understand how these phenotypes might have developed. Understanding how the genomes of Neanderthals, Denisovans and modern humans might have been different is important in this regard.

Post cranial remains and human evolution

Post cranial remain have not been used a phylogenetic resource as extensively as cranial remains when looking at evolutionary change in humans even though they are thought of as just as informative (Pearson, 2000). They are thought to show the same patterns during human evolution as cranial material (Pearson, 2000). Some hypothesized hybrid fossils such as Skhul V and the Lagar Velho 1 juvenile for which post cranial material is available have Neanderthal derived traits (McCown and Keith, 1939; Roberts et al., 1994; Duarte et al., 1999; Pearson, 2000). Neanderthals and modern humans had differences in limb proportions with Neanderthals being shorter, having proportionally shorter distal limbs, short limbs in relation to body mass and a broad pelvis, with bodies which are especially adapted to arctic climates (Pearson, 2000). Modern humans have elongated distal limbs, long limbs in relation to their trunks, along with a narrow pelvis and low estimated body mass (Pearson, 2000). Both groups were adapted to their particular environments following Bergman's and Allen's rules which state that animals closer to the poles and inhabiting colder climates will be larger in size with shorter extremities (limbs), while those in more equatorial climates will be smaller in size and have longer extremities allowing for more exposed surface area making it easier to dissipate heat

(Holliday, 1997; Pearson, 2000). Thus both modern humans and Neanderthals had big differences in body shape and the relationships between different regions of the body. It is therefore important to understand how these traits are affected by hybridization. Neanderthals were also more robust than modern humans, with thicker long bone diaphysis relative to the length of the long bone. These are thought to be adaptations to colder climates (Pearson, 2000). Though there is a great deal of selective pressure determining body shape and size there is a large genetic component to body size (Pearson, 2000). Differences in body proportions appear early in ontogeny, and genes associated with height in some Africa populations are thought to be the result of introgression resulting in shorter statue in some modern African populations (Hammer *et al*., 2011). Thought much of the variation in body size is thought to be due to climatic variation it has also been shown that population structure and history might play a role in variation in body size and shape in modern populations (Roseman and Auerbach, 2015). Regardless of this there were clear differences in body shape and size between Neanderthals and modern humans and it will be important to investigate how post cranial elements are affected by hybridization in order to identify hybrids in the fossil record.

The importance of introgression for adaptation in modern human evolution

Understanding hybridizations role in human evolution is important humans moved into new regions with new climates, sources of food and pathogens. Hybridization may have proven an important way of introducing new beneficial genes through introgression (Ding et al., 2013; Huerta-Sanchez and Casey, 2015; Dannemann et al., 2016; Racimo et al., 2017). There is a lot of evidence indicating that humans leaving Africa gained advantages genes from Neanderthals and Denisovans (Ding et al., 2013; Huerta-Sanchez and Casey, 2015; Dannemann et al., 2016; Racimo et al., 2017). These include genes related to immunity, metabolism and variation in skin and hair traits (Ding et al., 2014; Huerta-Sanchez and Casey, 2015; Racimo et al., 2015; Sankararaman et al., 2016). These will be explored further below.

Adaptation to climatic variation

For adaptation, to new climates genes were introgressed which are associated with dermatological traits and responses to varying levels of UV exposure in Northern climates (Ding *et al*., 2014). Some of the Neanderthal derived genes found in modern human populations include a Neanderthal derived haplotype that includes a loss of function mutation in the *MC1R* gene associated with many skin colour related, and other demonological traits associated with response to UV radiation (Ding *et al*., 2014). This haplotype is found at low frequencies in Europe (~5%) while

occurring at higher frequencies in East Asian populations (30-60%) (Ding *et al* 2014). Genes expressed in keratinocytes are also thought to be introgressed in both European and Asian populations including a variant of the *BNC2* gene which is also associated with variation in pigmentation. The introgressed haplotype is found at high frequencies in Europeans (~70%) while being absent form East Asian populations (Vernot and Akey, 2014). A variant of the *POU2F3* gene is also thought to be the introgression, and is associated with the control of keratinocyte proliferation and differentiation, this is found at a high frequency in East Asian populations ~ 60% while being found at a lower frequency in European populations (Vernot and Akey, 2014). It is also believed that SNPs affecting the functionality of genes such as *HYAL2* which is important for tissue repair after exposure to UV-B irradiation might, have disappeared from populations which migrated out of Africa as they were not functionally important (Ding *et al*., 2013). However, they may have been reintroduced through introgression from Neanderthals as populations moved into new regions with climates that required adaptation to increased UV exposure (Ding *et al*., 2013). This functional variant of the *HYAL2* gene is found at high frequencies in East-Asians populations and contains the same SNP as found in the functional variant found in African populations with the non-functional variant found in other non-African populations (Ding *et al*., 2013). Thus there is a great deal adaptation to new levels of UV exposure as modern humans moved across different habitats.

Other genes that were beneficial to adapting to new climate include the *TBX15* gene which was under strong positive selection and is found at high frequencies in Inuit populations, this gene is on a haplotype thought to be introgressed from a group closely related to the Denisovans (Racimo *et al*., 2017). This introgressed haplotype includes the *TBX15* and *WARS2* genes. The introgressed haplotype has a different expression pattern from the modern human haplotype (Racimo *et al*., 2017). Variation in *TBX15* is associated with variation in fat distribution in modern humans (Racimo *et al*., 2017). Denisovan derived genes are also thought to be associated with adaptation to high altitudes by Tibetans (Huerta-Sanchez and Casey, 2015).

Metabolic adaptations were also important as modern humans moved into new regions such as Europe. Genes associated with lipid catabolism were introgressed into European populations from Neanderthals (Khrameeva *et al*., 2014). These genes are also thought to have gone through a positive selective sweep in modern European populations. Lipid metabolism genes are enriched for in modern European populations with these regions containing three times more introgressed genes when compared to other populations that also experienced introgression (Khrameeva *et al*., 2014). Europeans have differential expression of genes expressed in lipid catabolism pathways along with an excess of lipid concentration when compared to East Asian, African populations and Chimps

(Khrameeva *et al*., 2014). The genes which showed the most divergent expression are genes which are have the most sequence similarity to Neanderthal genes (Khrameeva *et al*., 2014). A lot of work still needs to be done in order to really understand the significance of the differences gene expression levels. However some of the genes identified have been shown to be associated with glucose dependent insulin expression, obesity, hypertriglyceridemia a condition in which individuals have higher levels of triglycerides and heart disease (Khrameeva *et al*., 2014). It is thought that this introgression is the result of regionally specific benefits resulting in its concentration in Europeans and not East Asians. The genes might assist with adaptation to the colder climate (Khrameeva *et al*., 2014). Introgressed genes have also been associated with type 2 diabetes in Mexican populations and these are also genes associated with lipid catabolism (SIGMA Type 2 Diabetes Consortium *et al*., 2014).

How can aDNA help us understand the morphological outcomes of hybridization? Looking beyond the the use of aDNA to determine introgression for adaptation

Many of the genes which have been introgressed are genes that primarily effect soft tissue traits and metabolic functions. Many genes such at *TBX15* have pleiotropic effects as they are expressed early on in development (Singh *et al*., 2005; Lausch *et al*., 2008). Ancient DNA has assisted with understanding which genes were introgressed into human populations and aided our ability to spread into other regions of the world. However, we could also use aDNA to understand what happened when hybridization occurred and the biological consequences thereof. We already know that there was male hybrid sterility and that there are deserts in modern human genomes which don't have any introgressed Neanderthal and Denisovan derived genes (Sankararaman *et al*., 2014). These are due to the fact that there were many differences between AMH, Neanderthals and Denisovans and it is just as important to understand what these differences are and how they affected the outcomes of hybridization. Understanding these genetic differences could help us understand how hybridization affected the phenotype of hybrid offspring and the possible causes of developmental instability in hybrids

There hasn't been research which looks at genes that were functionally different between modern humans and Neanderthals. However, we known that there was enrichment for genes associated with metabolism, the cardiovascular system and hair distribution among other things in the line leading to Neanderthals and Denisovans after they split from modern humans (Meyer *et al*., 2012). In the human

lineage, we see enrichment in genes associated with behavior, skin pigmentation and eye development (Meyer *et al*., 2012). Most of the diverged genes are expressed early on in development and have pleiotropic effects. Another method that has been used to identify divergent genes was by determining which genes may have been purged from the human genome after hybridization because they were most likely deleterious (Sankararaman *et al*., 2014). This analysis showed that the Neanderthal and Denisovan depleted region overlap more than expected by chance and some of the regions with very little to no introgression contain genes that are differentially expressed in different tissues particular in the developing cortex (Sankararaman *et al*., 2014; Kuhlwilm *et al*., 2016). We also know that some of the other genomic differences included genes which are associated with skeletal differences (Green *et al*., 2010). One of the major ways in which Neanderthal derived genes function in modern humans is through differential gene expression or gene regulation (Dannemann, Prüfer and Kelso, 2017). Archaic genes which are found at high frequencies in modern human populations contribute to differences in gene expression (Dannemann *et al*., 2017). We know that in hybrids (mammalian and plant hybrids) there is overall genome wide changes in gene expression these would have affected the phenotypic outcomes of hybridization.

Identifying these differentiating genes will be useful because DSD between hybridizing hominin groups might have affected phenotypic outcomes of hybridization. We know that the tissues in direct interaction with the environment are under a great deal of selective pressure but that they also share developmental pathways with other tissue groups including craniam and skeletal materials. Thus adaptation to different environments might have driven DSD in hominin groups which occupied very different environments and had specific adaptations prior to their secondary contact. Thus getting a better understanding of the genomic differences combined with what we know from developmental biology could help us make informed predictions of the outcomes of hybridization. It could also help explain some of the traits we see in hybrid fossil material.

Chapter 4: Methods and Materials

Mouse sample

The mice used for this experiment are wild derived inbred mice collected in different regions and at different times. Wild derived lab mice are sib mated in order to produce inbred homozygous stocks of different species and subspecies of *Mus.* All mice were purchased from Jackson laboratories (Sacramento, California) and the strains used include: Cast.Eij, representing *M. m. casteneaus* with the original parental population trapped in Thailand; WSB.Eij, representing *M. m. domesticus* with the parental population trapped in in Eastern Shore Maryland; Czechi.Eij, which represents *M. m. musculus* with the original population captured in Morovia, Czechoslovakia; and Spret.Eij, which represents *M. spretus* with the original population trapped in Cadiz, Spain (Eisen, 2005). The short generation times of mice result in the phylogenetic relationship between the three subspecies being similar to the phylogenetic relationship between humans and chimpanzees in terms of genetic distance. Phylogenetic analysis shows that the *M. musculus* subspecies form a clade to the exclusion of the other species in the genus *Mus*, with *M*. *macedonias* and *M. spicilegus* forming a sister group, and *M. spretus* forms an out group basal to all the other species for most phylogenetic trees constructed (Lundrigan *et al*., 2002).

The mouse model consists of multigenerational hybrid groups. There are three sub-specific crosses which will be used in this model, as well as one specific cross. The first subspecies/species cross will result in the production of F1 hybrids (Figure 4.1). When these F1 hybrids mate and reproduce they produce F2 hybrids (Figure 4.1). F1 hybrids can also reproduce with mice from the parental population and this produces B1 hybrids (Figure 4.1). The B1 backcrossed hybrids can again backcross into the parental population and these produce B2 backcrossed hybrids (Figure 4.1 and Table 4.1).

Data collection

Metric variation in coat colour

Photographic set up

In order to measure variation in colour, digital photos were taken of the mice from above using a Canon EOS Rebel T3 camera (Ohta-ku, Tokyo 146-8501, Japan) placed on a Voyager Tripod T200 at a

height of 58 cm (Tiffen, Hauppauge, New York). Mice were placed inside a 24 inch black and white lighting tent, with translucent sides that diffuse light from multiple sides and allowing for shadow-less lighting against the black back ground, with lights positioned above the lighting tent and lab lights serving as the main source of light (overhead florescent beam lights). The mice were placed on the black material background along with a ruler which served as a scale and the Calibr8 ColourChart® SG colour standard (Munchen, Germany), which will be referred to as the colour chart. Three photos were taken per mouse: one of the dorsal aspect, one of the ventral aspect and one of lateral aspect. Photos were saved as JPEG images for further analysis via digital photography software (Figure 4.2).

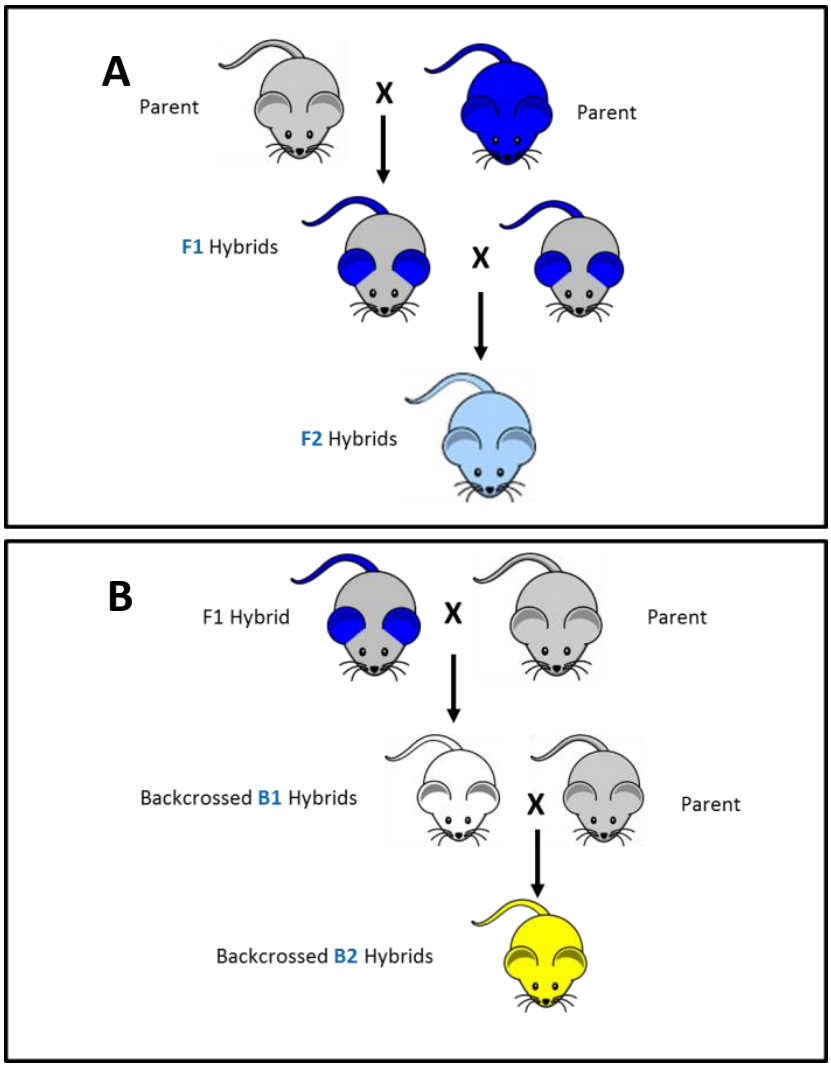

Figure 4.1: Schematic of the breading plan used to produced multigenerational hybrid populations, consisting of (A) F1, F2, (B) B1 and B2 hybrids.

Generation	Cross	Name
Parents	*M. m. casteneaus* x *M. m. casteneaus*	*M. m. casteneaus*
	M. m. domesticus x *M. m. domesticus*	*M. m. domesticus*
	M. m. musculus x *M. m. musculus*	*M. m. musculus*
	M. spretus x *M. spretus*	*M. spretus*
F1 Hybrids	*M. m. casteneaus* x *M. m. domesticus*	*M. m. casteneaus* x *M. m. domesticus* F1 hybrids
	M. m. casteneaus x *M. m. musculus*	*M. m. casteneaus* x *M. m. musculus* F1 Hybrids
	M. m. musculus x *M. m. domesticus*	*M. m. musculus* x *M. m. domesticus* F1 Hybrids
	M. spretus x *M. m. domesticus*	*M. spretus* x *M. m. domesticus* F1 Hybrids
F2 Hybrids	(*M. m. casteneaus* x *M. m. domesticus* F1 hybrids) x (*M. m. casteneaus* x *M. m. domesticus* F1 hybrids)	*M. m. casteneaus* x *M. m. domesticus* F2 hybrids
	(*M. m. casteneaus* x *M. m. musculus* F1 hybrids) x (*M. m. casteneaus* x *M. m. musculus* F1 hybrids)	*M. m. casteneaus* x *M. m. musculus* F2 Hybrids
B1 Hybrids	(*M. m. casteneaus* x *M. m. domesticus* F1 hybrids) x *M. m. domesticus*	*M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids
	(*M. m. casteneaus* x *M. m. domesticus* F1 hybrids) x *M. m. casteneaus*	*M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids
	(*M. m. castaneus* x *M. m. musculus* F1 hybrids) x_*M. m. castaneus*	*M. m. castaneus x M. m. musculus_M. m. castaneus* B1 hybrids
	(*M. m. castaneus* x *M. m. musculus* F1 hybrids) x *M. m. musculus*	*M. m. castaneus x M. m. musculus_M. m. musculus* B1 hybrids
	(*M. m. musculus x M. m. domesticus* F1 hybrids) x *M. m. musculus*	*M. m. musculus x M. m. domesticus_M. m. musculus* B1 hybrids
	(*M. m. musculus x M. m. domesticus* F1 hybrids) x *M. m. domesticus*	*M. m. musculus x M. m. domesticus_ M. m. domesticus* B1 hybrids

Table 4.1: Multigenerational hybrids and parents used for the Hybrid Mouse Project.
***Cross indicates crosses between different hybrids and parental groups to produce hybrid**
***Name indicates the name of particular hybrid used throughout the thesis**

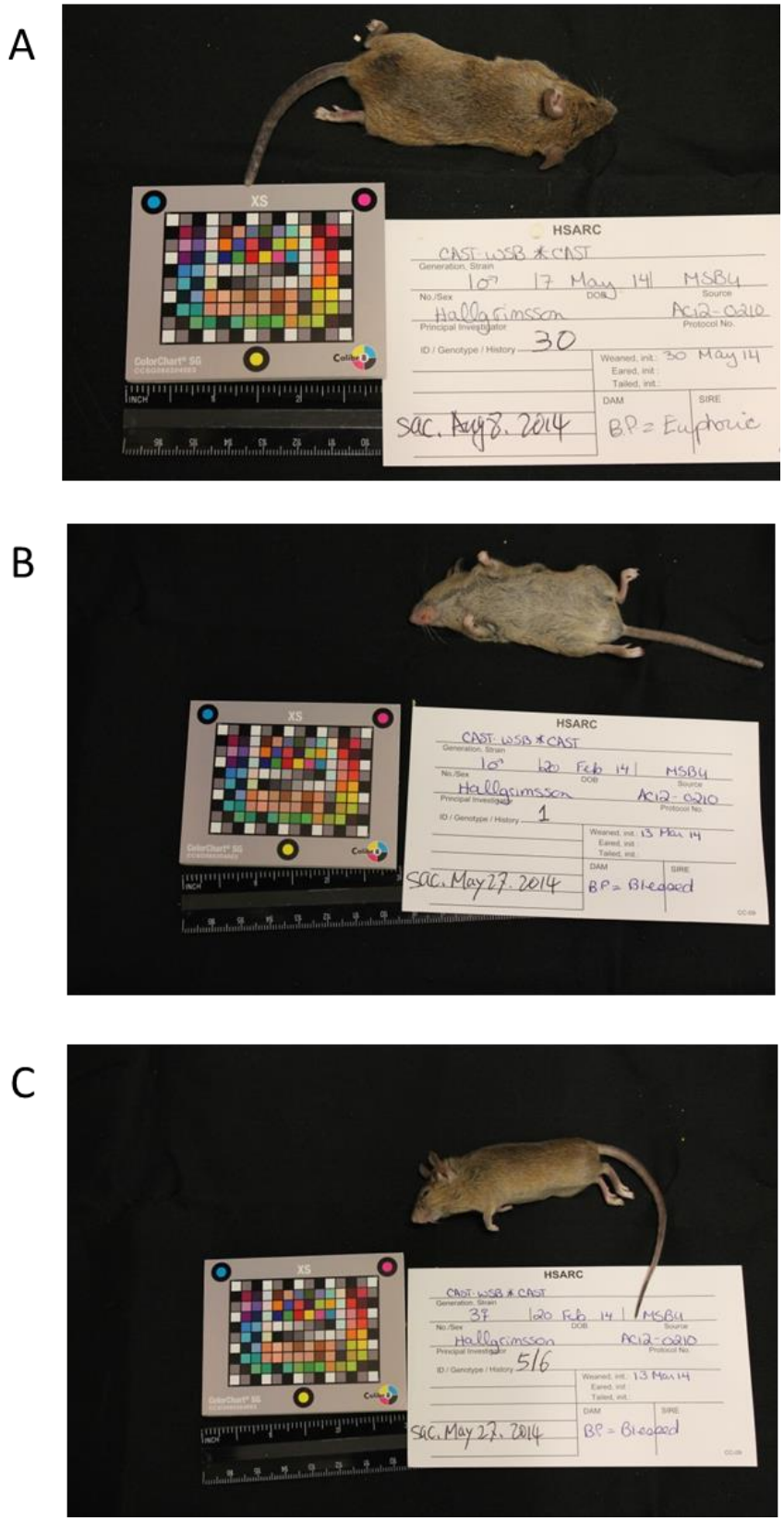

Figure 4.2: Examples of the photos taken of the (A) dorsal, (B) ventral and (C) lateral aspect of mice used for the Hybrid Mouse Project.

Collection of metric coat colour data using digital photographs

Colour spaces are ideal for measuring differences in colours among individuals, especially if those differences are subtle. Different colour spaces and values have been used to determine differences in hair, skin and eye colour between human populations (Norton *et al*., 2016), as well as differences in coat colour between different regions of the body, and differences in colour between different groups (Hamada et al., 2006; Bergman and Beehner, 2008). Because the mice have subtle differences in coat colour and are various shades of brown, to properly quantify variation in coat colour quantitative methods are required. When determining variation in colour between different groups, certain protocols have to be followed in order to standardize photos. Protocols need to be followed because variation in lighting conditions can affect measurement of colour. RGB values are a measure of Red (R), Green (G) and Blue (B) detectors activated in the digital camera with R sensors having peak sensitivity at 580 nanometers (nm), G sensors having peak sensitivity at 540nm, and B sensors having peak sensitivity at 450nm. The colour that is produced is determined by which combination of sensors are excited and how much they are excited. Light at any wavelength between 400 to 700 nm, exciting one or a combination of these sensors, produces a colour within the RGB colour space. There are different methods for obtaining these values which include the use of objects such as tristimulus colorimeters, which can determine colour by measuring wavelengths being emitted from the object directly. However these instruments only provide measurements of small regions of the object and thus many measurements need to be taken in order to determine the average colour of the whole object. They also require special equipment and for biologists who might not focus on variation in coat colour this might not seem cost effective. Digital images are an easy and cost effective way of obtaining colour information and this could allow for comparison across different data sets.

Standardization of digital photographs

Before collecting RGB information from the digital images, they were standardized using a colour card included in every photograph. Jpeg photographic files were imported into the Nip2 image processing package programme for standardization of digital photographs (Martinez and Cupitt, 2005). Standardization of photographs is required because subtle differences in lighting might result in variation in colour measurements for the same object. Colour is standardized by the inclusion of the colour chart composed of a 140 squared checkered array of colours with multiple six step grey scales as well as an array of natural colours (Figure 4.3). The six-step grey scale is made up a set of neutral colours ranging from absolute black, which has 25% reflectance, through to absolute white each which has 95% reflectance. Once standardized, there should be a linear increase in RGB measurements from the black to the white squares, and the R, G and B values should have nearly the

same value for each square (Bergman and Beehner, 2008). Nip2 is designed to standardize photos using known measurements of the colour chart, and uses that to calibrate the rest of the photo to correct for variation in lighting. All the colour squares in the colour chart were not used; in this case only 24 of the 140 colour squares were used for standardization. These correspond to the 24 squares of the Gregtag Mcbeth colour chart, a colour chart frequently used in the photographic industry and for which most programmes are designed. Instead of using the pre-set collaboration values for the Gregtag Mcbeth colour the XYZ (another standardized colour space) values for the corresponding colour squares in the Calibr8 ColourChart® SG colour chart were imported into Nip2. These were measurements taken under D65 lighting (which is the lighting of indoor lighting such as florescent lights). When colour correction is successful this results in a more vivid photograph with colours correctly presented in the photograph, as can be seen from the colour card in Figure 4.3 before and after correction.

Linearization and Equalization

If standardization is successful this resulted in a linear increase in R, G and B values as we move from the black colour standard, which would have the lowest reflectance of 25% as well as the smallest RGB values, to the white colour standard which would have the highest reflectance of 95% and the largest RGB measurements (Bergman and Beehner, 2008). The RGB values would also equalize thus they would have similar or equal values RGB values for the six grey scale squares on the chart (Bergman and Beehner, 2008).

To determine if linearization and equalization of the photographs occurred, mean RGB measurements of the six step grey scale patches were taken. This was done for a subset of photographs from the parental and F1 hybrid groups. The grey scale data were collected and analyzed, by importing calibrated Jpeg images into image J (Schneider, Rasband and Eliceiri, 2012), the square marque tool was used to select regions within the each of the six grey scales squares. The histogram tool was used to determine the mean RGB values for each square. These data were then imported into Excel, and scatter plots were created to calculate R^2 values for the RGB measurements, to determine if there was a linear relationship between the measurements for the six grey squares. Mean R^2 values were calculated to determine if there was linearization of images. Data were linearized with R^2 values having a mean±SD for RGB measurements of 0.99±0.0011, 0.99±0.0013 and 0.99±0.0015, respectively; an example of the RGB data before calibration and after calibration can be found in Figure 4.3.

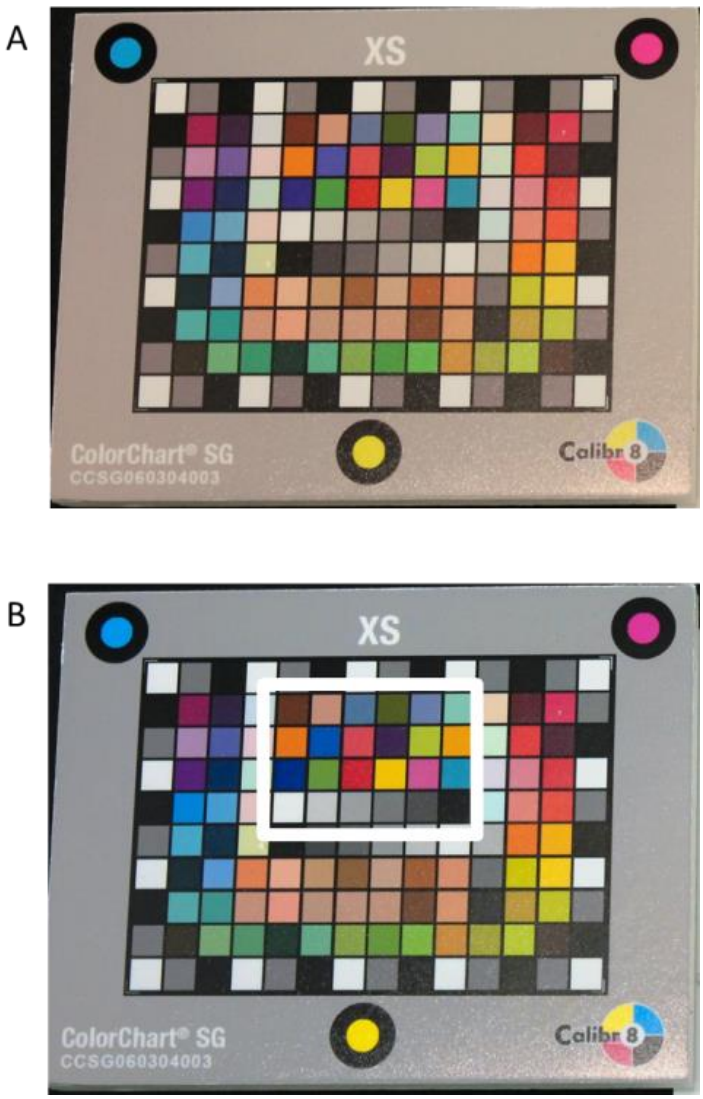

Figure 4.3: Calibr8 ColorChart SG (A) before collaboration with Nip 2 and (B) after colour collaboration using Nip2 with colours becoming more vivid after collaboration and reflecting the actual colour. The white block highlights the regions used in collaboration process for this project.

In order to determine if there was equalization of the images the differences between the R, G and B values were determined. These data were analyzed to determine the minimum and maximum difference between these three colour measurements as well as the mean difference. In terms of equalization the range of difference between R and G, R and B and G and B are 0.04 to 10.55, 0.06 to 10.34 and 0 to 10.23 respectively. The mean±SD in difference between R and G, R and B and G and B are 0.059±2.03, 3.89±2.3 and 3.71±1.39 respectively, with 97.4% of the R and G values, 95.36% of the R and B and 99.26% of the G and B measurements with a smaller than 5% difference between the two measurements.

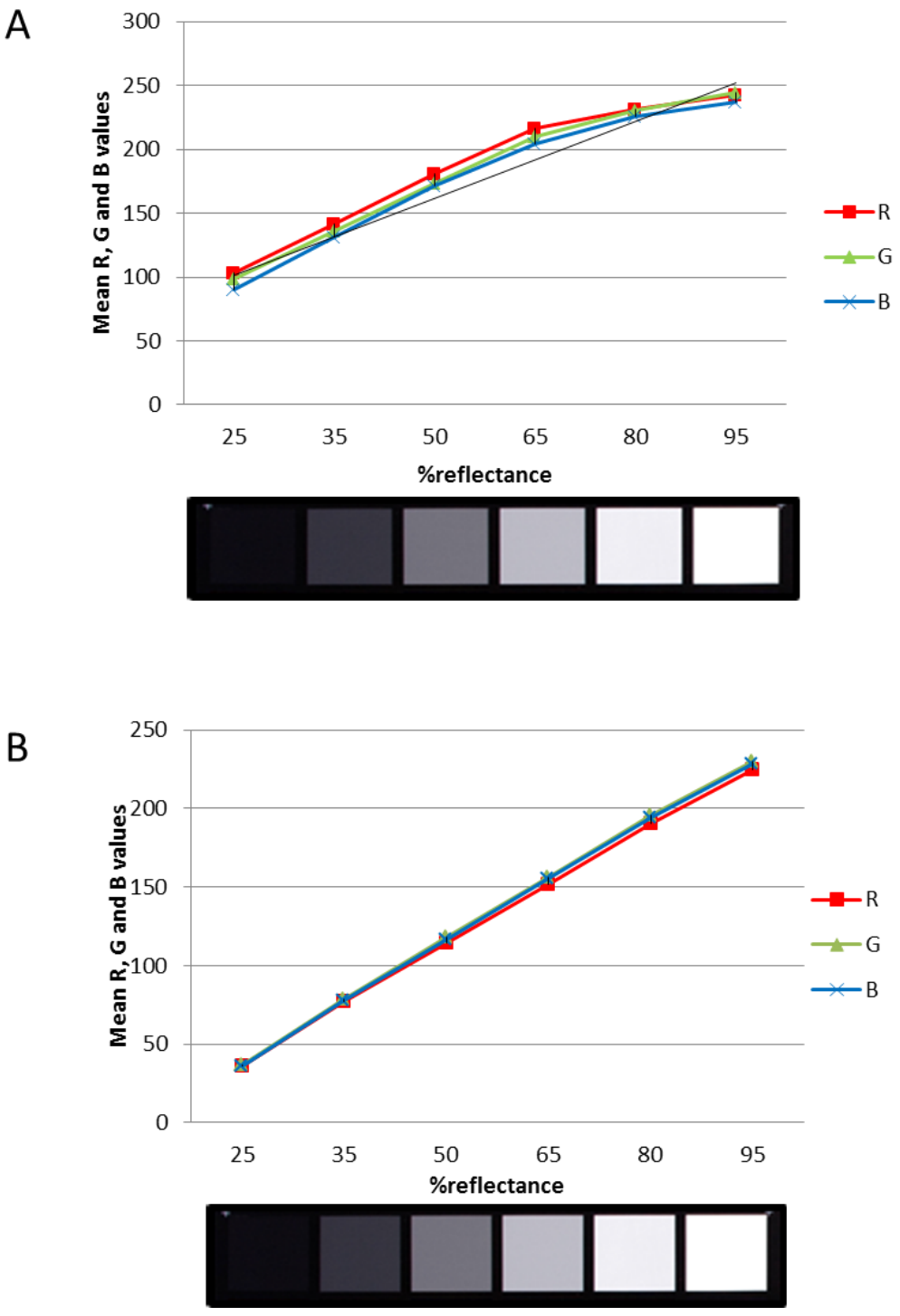

Figure 4.4: Graph of mean R,G and B values vs reflectance value of Cast.Eij 30 dorsal coat photo (A) plot of data collected from a photo which has not been calibrated in Nip2 ($R^2_{(R)}$= 0.954 , $R^2_{(G)}$ = 0.971 , $R^2_{(B)}$ = 0.962) and (B) a plot of data collected from a photo after calibration in Nip2 ($R^2_{(R)}$= 0.9994, $R^2_{(G)}$ = 0.999 , $R^2_{(B)}$ = 0.999). This results in equalization and linearization of photos.

Collection of coat colour data for the dorsal and ventral coat

To analyse the variation in dorsal and ventral coat colour, first photos were selected in which the dorsal and the ventral coat were clearly visible and where these regions were flattened out to some extent and would allow for RGB data to be collected. Lateral aspect photos were not used because they were inconsistently taken. Data collection was not possible for all 50 specimens for each cross due to variation in the positioning of the mice during the photographic process. Once photos were selected they were imported into Nip2 for standardization.. In image J, the circular marque tool was used to collect data from different regions of the ventral and dorsal coat. The histogram tool was used in order to collect the mean RGB values for the selected regions. For the dorsal coat three regions were selected: just above the base of the tail, in the abdominal region, and the thoracic region (interscapular region) of the dorsal coat (Figure 4.5.A). For the ventral coat two regions were selected: the abdominal region and, if it was exposed in the digital photograph, the pelvic region just above the leg (Figure 4.5.B). For the dorsal coat the three measurements were combined in order to provide the average dorsal coat measurement, i.e. average dorsal R (ADR), average dorsal B (ADB) and the average dorsal G (ADG) (the number of regions collected -n- varied due to differences in positioning of mice and whether the coat was smooth enough to collect colour data). The average ventral values are calculated from two measurements taken from the ventral coat, resulting in average ventral red (AVR), average ventral green (AVG) and average ventral blue (AVB) values (the number of regions once again varied due to differences in positioning of mice and whether the coat was smooth enough to collect colour data). These values were combined and the average values for the dorsal coat and for the ventral coat were used to determine variation in dorsal coat colour and variation in ventral coat colour.

Measuring dorsal ventral contrast

To determine the level of contrast between the dorsal and the ventral coat, the mean RGB values for the dorsal coat represented by the values ADR, ADG and ADB were subtracted from the mean RGB values of the ventral coat represented by AVR, AVG and AVB, producing differences in dorsal ventral contrast R (DVR), G (DVG) and B (DVB). This gave an indication of contrast in colour between the dorsal coat and the ventral coat. A larger dorsal ventral contrast is the result of a lighter ventral coat and a darker dorsal coat. This is one of the clear patterns seen in the parental groups with one strain, Czechi.Eij (*M. m. musculus*), having a very light yellow/agouti ventral coat and a brown dorsal coat, while the other two strains (Cast.Eij, *M. m. castenous* and WSB.Eij, *M. m. domesticus*) have brown

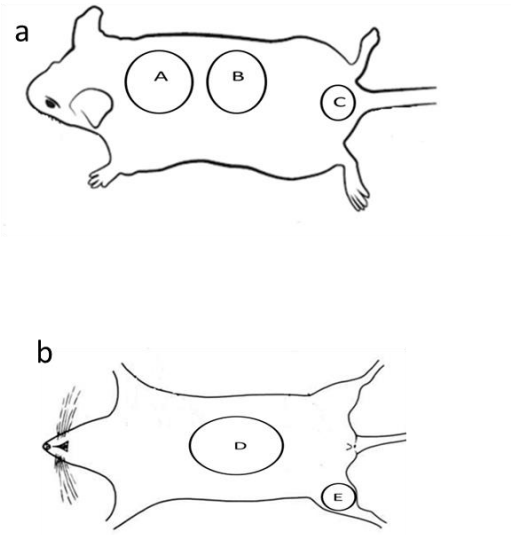

Figure 4.5: (a) Regions on the dorsal coat from which colour measurements were collected: the abdominal region (A), the thoracic region (B) and the region above the tail (C) of the dorsal coat. (b)Regions on the ventral coat from which colour measurements were collected: the abdominal region (D) and the pelvic region (E).

dorsal coats with ventral coats which are a slightly lighter shade of brown and having a much smaller contrast (Figure 4.6.A and B). The Spretus.Eij (*M.* spretus) mice have a brown dorsal coat and a grey ventral coat. This method has been previously used to look at contrast in colour between the upper and lower part of the body of Rhesus macaques having a bipartite colouring pattern with the upper body being lighter than the lower body (Hamada *et al*., 2006). The subtraction of the smaller (darker) dorsal values from the larger (lighter) ventral values gives a numerical indication of the contrast and also allows us to see how this changes in the hybrids. These represent differences in dorsal ventral contrast (DVC).

Determining levels of technical variation

Due to the fact that there was very little stratification of data, with batches of the same strain of mice often euthanised and photographed on the same day, and slight variation in photo location, a colour chart was included to standardize the photos. Although this allowed for standardization of the photos, the colour chart itself has certain problems because the white paper it is printed on fluoresces slightly, the greys are not spectrally neutral which is required for the card to appear neutral under all illuminants, and the grey squares also has an increased red reflectance. Because the photos were taken under slightly different conditions and the grey card squares on the colour chart are not

spectrally neutral, analysis was performed to determine how much of the variation in coat colour was due to technical variation and how much was biological variation in coat colour.

Analyses were performed using ADR, ADG and ADB values for the dorsal coat and the AVR, AVG and AVB values for the ventral coat. To determine how much of the variation in the colour measurements was the result of variation in when/where/how the photo was taken, and how much was due to actual differences in colour between the strains, a linear model was used. Analyses were done separately for the dorsal values and the ventral values because not all mice were used for both data sets (Table 4.2). For the linear model, the dependent variables are ADR, ADG, ADB for the dorsal coat, and AVR, AVG and AVB for the ventral coat. The independent variables were the date on which the photo was taken and group affiliation (the different strains of mice which were bred). Different linear models were used for each independent variable. First linear models were created with the whole data set for each F1 hybrid and its respective parent (Table 4.2). Then a smaller stratified dataset (Tables 4.3, 4.4, 4.5, 4.6,4.7, 4.8) was created in which different groups were photographed on the same day, and linear models were created with these data sets to determine if the variation was due mainly to biological differences versus technical variation. When analyzing the three complete ventral data sets the date taken accounts for a lot of the variability, often accounting for more variability than group affiliation when we look at the adjusted R^2 value (Table 8). However this could due to the fact that there was little stratification of the data (Table 4.9).

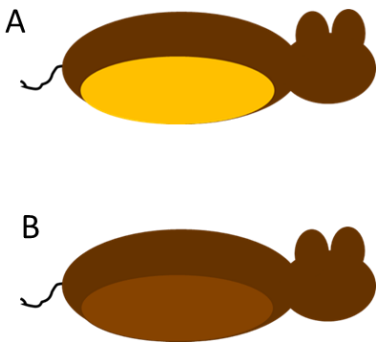

Figure 4.6: Illistrationrepresenting differences in dorsal ventral contrast. Parental populations vary in dorsal ventral contrast with some strains having a larger dorsal ventral contrast as in (A) examples of this would be Czechi. Eij and smaller dorsal ventral contrast as in (B) an example of this would be Cast.Eij and WSB.Eij .

When looking at the results from the stratified analysis for three groups what we see is that for the *M. m. casteneaus* x *M. m. musculus* F1 hybrids and the parents most of the variation is significantly correlated with variation in group affiliation, with 94.15%, 93.47%, and 75.03% of variation in AVR, AVG and AVB, respectively, being accounted for by variation in group affiliation; this is significant in all cases ($P<0.001$; Table 4.9).

When looking at the *M. m. musculus* x *M. m. domesticus* F1 hybrids and their parents in (Table 4.9) in the stratified sample the date taken accounts for 28.19% , 29.18%, and 20.16% of variation in AVR, AVG and AVB, respectively, and this is significant. With group affiliation as an independent variable, 71.6%, 53.09% and 36.31% of variation for AVR, AVG or AVB is accounted for, and this is significant ($p<0.001$). Though it is significant for AVB, this is not much larger than the R^2 for the linear model where date taken was an independent variable. Though some of the variation in AVR, AVG and AVB is due to variation in the date taken it was difficult to achieve a stratified sample where all three groups are represented equally. *M. m. musculus* x *M. m. domesticus* F1 hybrids also have an extreme ventral coat colour. In two of the days used for the stratified sample (19 December 2013 and 27 June 2014) *M. m. musculus* x *M. m. domesticus* F1 hybrids are found in higher numbers along with *M. m. musculus* which also has a much lighter ventral coat in comparison to the dark dorsal coat of *M. m. domesticus*. This observation could contribute to the larger proportion of variation being determined by the date taken.

For the *M. m. casteneaus* x *M. m. domesticus* F1 group in table 4.9 we see the same pattern as what we see in the *M. m. casteneaus* x *M. m. musculus* F1 group, with the stratified sample showing very little association between the date taken and variability in AVR, AVG and AVB values; this is not significant ($p>0.05$). With "Group" as an independent variable 41.41%, 43.38% and 47.86% of variation in AVR, AVG and AVB is accounted for and this is significant ($p<0.001$). This accounts for much less of the variation than is seen in the other two crosses and this could be because *M. m. casteneaus*, *M. m. domesticus* and *M. m. casteneaus* x *M. m. domesticus* F1 hybrid mice do not differ that much in terms of ventral coat colour with all three having dark brown ventral coats.

Thus, overall the stratified samples indicate that most of the variation we see in AVR, AVG and AVB values are due to differences between the mice and not due to technical variation, however we see slightly different results for the *M. m. musculus* x *M. m. domesticus* mice, largely due to the fact that it was not possible to get a good stratified sample for this group. Because most of the variation in average ventral colour was due to differences between groups we used these data to determine mean differences in these values between the various groups.

Parents and F1 hybrids	Number of mice for ventral coat sample	Number used of mice for dorsal coat analysis
M. m. casteneaus	29	32
M. m. musculus	42	32
M. m. domesticus	32	21
M. m. casteneaus x *M. m. musculus* F1 hybrids	18	20
M. m. musculus x *M. m. domesticus* F1 hybrids	18	32
M. m. casteneaus x *M. m. domesticus* F1 hybrids	24	25
Total	163	162

Table 4.2: Sample size for data collected from the ventral and dorsal regions for F1 hybrids and parents

***M. m. casteneaus* x *M. m. musculus* F1 hybrid stratified sample**				
Parents and F1 Hybrid	Date photographed			n
	19-Dec-13	27-Aug-15	17-Sep-15	
M. m. Casteneaus	4	4	4	12
M. m. musculus	10	0	0	10
M. m. casteneaus* x *M. m. musculus	2	8	3	13
	16	12	7	35

Table 4.3: Sample size of parents and F1 hybrids for *M. m. casteneaus* x *M. m. musculus* cross stratified ventral data set

***M. m. musculus* x *M. m. domesticus* F1 hybrid stratified sample**						
Parents and F1 Hybrid	Date photographed					n
	19-Dec-13	13-Mar-14	24-Apr-14	27-Jun-14	20-Nov-14	
M. m. domesticus	2	3	7	1	1	14
M. m. musculus	2	0	5	5	4	16
***M. m. musculus* x *M. m. domesticus* F1 hybrids**	4	2	0	2	0	8

Table 4.4: Sample size of parents and F1 hybrids for *M. m. musculus* x *M. m. domesticus* F1 hybrids cross stratified ventral data set

***M. m. casteneaus* x *M. m. domesticus* F1 hybrid stratified sample**				
Parents and F1 Hybrid	Date photographed			n
	8-Nov-13	19-Dec-14	11-Mar-14	
M. m. domesticus	4	2	2	8
M. m. casteneaus	8	4	7	19
M. m. casteneaus* x *M. m. domesticus	7	1	15	23
Total	12	6	9	27

Table 4.5: Sample size of parents and F1 hybrids for *M. m. casteneaus* x *M. m. domesticus* F1 hybrid cross stratified ventral data set

M. m. casteneaus x *M. m. musculus* F1 stratified sample				
Parents and F1 Hybrid	Date photographed			n
	19-Dec-13	27-Aug-15	17-Sep-15	
M. m. casteneaus	4	4	2	10
M. m. musculus	1	8	4	13
***M. m. casteneaus* x *M. m. musculus* F1 hybrids**	11	0	0	11
Total	16	12	6	34

Table 4.6: Sample sizes of parents and F1 hybrids for *M. m. casteneaus* x *M. m. musculus* stratified dorsal data set

M. m. musculus x *M. m. domesticus*						
Parents and F1 Hybrid	Date photographed					n
	11 Augutst 2013	13-Mar-14	24-Apr-14	25-Apr-14	27-Jun-14	
M. m. domesticus	3	5	5	5	6	18
M. m. musculus	1	0	3	0	1	4
***M. m. musculus* x *M. m. domesticus* F1 hybrids**	4	7	0	3	3	14
Total	8	12	8	8	10	36

Table 4.7: Sample sizes of parents and F1 hybrids for *M. m. musculus* x *M. m. domesticus* F1 hybridsstratified dorsal data set

M. m. casteneaus x *M. m. domesticus*					
Parents and F1 Hybrid	Date photographed				n
	8-Nov-13	19-Dec-14	11-Mar-14	13-Mar-14	
M. m. domesticus	4	4	1	7	9
M. m. casteneaus	9	3	8	4	20
***M. m. casteneaus* x *M. m. domesticus* F1 hybrids**	9	1	10	0	20
Total	22	8	19	11	49

Table 4.8: Sample sizes of parents and F1 hybrids for *M. m. casteneaus* x *M. m. domesticus* F1 hybrids stratified dorsal data set

	M. m. casteneaus x *M. m. musculus*			*M. m. musculus* x *M. m. domesticus*			*M. m. casteneaus* x *M. m. domesticus*		
	AVR	AVG	AVB	AVR	AVG	AVB	AVR	AVG	AVB
Independent variable Group									
Multiple R-squared	0.81	0.77	0.60	0.56	0.33	0.26	0.39	0.41	0.43
Adjusted R-sqaured	0.81	0.76	0.59	0.55	0.31	0.24	0.37	0.39	0.44
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Independet variable Date Taken									
Multiple R-squared	0.60	0.64	0.66	0.64	0.60	0.57	0.48	0.45	0.44
Adjusted R-sqaured	0.48	0.52	0.55	0.47	0.43	0.38	0.35	0.32	0.30
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Stratified analysis (different groups photographed on the same day) independent variable Date Taken									
Multiple R-squared	0.09	0.06	0.12	0.38	0.39	0.31	0.1374	0.07661	0.04
Adjusted R-sqaured	0.03	-0.00	0.06	0.28	0.29	0.2	0.09	0.03	-0.00
P value	0.23	0.39	0.13	p<0.05	p<0.05	p<0.05	0.05	0.20	0.41
Stratified analysis (different groups photographed on the same day) independent variable Group									
Multiple R-squared	0.95	0.94	0.76	0.73	0.56	0.41	0.44	0.46	0.5
Adjusted R-sqaured	0.94	0.93	0.75	0.72	0.53	0.36	0.41	0.43	0.48
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Table 4.9: Results from different linear models used to determine how much variation in AVR, AVG and AVB values are determined by differences in lighting conditions vs actual differences in average ventral coat colour (biological variation).

When looking at the dorsal data for the *M. m. casteneaus* x *M. m. musculus* F1 hybrids and their parents what we see is that when the data are not stratified date taken accounts for more of the variability than the group affiliation (Table 4.10). However, when we look at the stratified data what we see is that with group affiliation as an independent variable accounts for 65.7% of the variation in ADR ($p<0.001$), 55.2% of the variation in ADG ($p<0.001$) and 38% of the variation in ADB ($p<0.05$). This is significant in all cases. The date taken only accounts for 20% of the variation in ADR ($p<0.05$), while only accounting for approximately 10% of the variation in the ADG and ADB values, and this is not significant (Table 4.10).

When we look at the *M. m. musculus* x *M. m. domesticus* F1 hybrids F1 hybrid stratified data set in Table 4.10 the group affiliation as an independent variable accounts for more of the variation in ADR and ADG accounting for 55% and 35% of the variation respectively and this is significant($p<0.001$). Group affiliation only accounts for ADB 2% ($p=0.34$) of the variation and date taken accounts for and 7% ($p=0.10$) and in both cases it is not significant.

For the *M. m. casteneaus* x *M. m. domesticus* F1 hybrids stratified data set results in Table 4.10 what we is that group affiliation as an independent variable accounts for 46.31% of variation in ADB values ($p<0.001$), in the stratified data set while date taken only accounts for 3% ($p=0.24$) of the variation and this is not significant. Group affiliation and date taken separately each account for around 10% of the variation in and the ADG values in the stratified data set in both cases this is significant ($p<0.05$). Group affiliation and date taken are separately associated with less than 1% and around 1% of the variation in ADR and this is not significant.

Taken together, these data indicate that most of the variation in the data is accounted for by biological differences in coat colour between the groups. In the stratified data sets, in most cases, group affiliation as an independent variable accounted for most of the variation. In cases where group did not account for most of the variation, the amount of variation determined by date taken is minimal and or similar to what we see when group affiliation is used as the independent variable, which indicates that there most likely is very little variability between the groups for those measurements.

	M. m. casteneaus* x *M. m. musculus			*M. m. musculus* x *M. m. domesticus*F1			*M. m. casteneaus* x *M. m. domesticus* F1		
	ADR	ADG	ADB	ADR	ADG	ADB	ADR	ADG	ADB
Independent variable group affiliation									
Multiple R-squared	0.42	0.31	0.13	0.16	0.06	0.04	0.14	0.19	0.28
Adjusted R-sqaured	0.40	0.29	0.11	0.14	0.04	0.016	0.12	0.17	0.26
P value	p<0.001	p<0.001	p<0.001	p<0.001	0.066	0.19	p<0.05	p<0.001	p<0.001
Independent variable date taken									
Multiple R-squared	0.68	0.69	0.68	0.59	0.58	0.7	0.33	0.33	0.38
Adjusted R-sqaured	0.58	0.60	0.59	0.45	0.43	0.58	0.19	0.19	0.25
P value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.05	p<0.05	p<0.05
Stratified analysis (photos taken on same day different groups) date taken									
Multiple R-squared	0.19	0.16	0.16	0.28	0.16	0.18	0.07	0.16	0.092
Adjusted R-sqaured	0.13	0.10	0.10	0.19	0.06	0.03	0.02	0.11	0.03
P value	p<0.05	0.08	0.08	p<0.05	0.21	0.34	0.28	0.04	0.24
Stratified analysis (photos taken on same day different groups) group affiliation									
Multiple R-squared	0.81	0.58	0.52	0.62	0.39	0.13	0.05	0.14	0.49
Adjusted R-sqaured	0.79	0.55	0.48	0.59	0.35	0.08	0.00	0.11	0.46
P value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	0.10	0.36	0.02	p<0.001

Table 4.10: Results from different linear models used to determine how much variation in ADR, ADG and ADB values are determined by differences in lighting conditions vs actual differences in average ventral coat colour (biological variation).

Statistical methods

Comparisons of average dorsal and ventral coat colours and DVC

MANOVA to determine if there are significant difference in coat colour between various hybrids and parental groups

Shapiro wilk tests were used to determine if the data were normally distributed. To determine if there were significant differences in dorsal coat colour multivariate analysis of variance (MANOVA) was performed in R statistical programme (Team, 2013) using the ADR, ADG and ADB values as the dependent variables and the different strains as the independent variables. Parents were compared to each other, and to their F1 hybrid offspring. For each cross parents were compared to subsequent generations of hybrids including F2 hybrids and B1 hybrids. In order to determine if there were differences in colour in the ventral coat AVR, AVG and AVB measurements served as dependent variables with strain being the independent variable; this was used to determine if there were significant differences in ventral coat colour. To determine if there were significant differences in dorsal ventral contrast MANOVA was used with DVR, DVG and DVB serving as dependent variables and groups/strains serving as dependent variables. An α-level of 0.05 was used to determine if the differences were signicant. However, because of multiple testing which results in a higher likelihood of Type II errors a more conservative α-level is also reported. The Bonferroni corrected p-value is 0.016 for comparisons between parents and F1 hybrids. For comparisons beween F2 hybrids and F1 hybrids and F2 hybrids and their original parental groups as well as between B1 hybrids and its respective parental groups an α-level of 0.012 was used.

Pairwise comparisons of average dorsal and ventral coat colour measurements

To determine whether there were significant differences in ADR, ADG and ADB measurements, and which measurement were driving variation in coat colour, pairwise t test were performed of ADR, ADG and ADB values. Comparisons were done between the parents, parents and F1 hybrids. F2 hybrids were compared to the F1 parents as well as to their original parental groups. B1 hybrids were compared to their F1 parents as well as to the two original parental groups. The same was done for

ventral coat colour using pairwise comparisons of the AVR, AVG and AVB values as well as the dorsal ventral contrast using the DVR, DVG and DVB values.

Principal Components Analysis (PCA)

PCA was performed in R statistical programme using the average dorsal measurements and average (ADR, ADG and ADB) and average ventral measurements (AVR, AVG and AVB). PCA determines the spread of the data, and where the most variance in the data lies. PCA allows you to determine which measurements account for most of the variability in your data set and thus which measurements account for most of the variability and differences you see between the different groups you are comparing. PCA was done to determine which traits accounted for most of the variation in overall mouse coat colour. This method allowed us to determine how the hybrid mice varied in coat colour in relation to the parental groups. PCA was performed with parental groups and F1 hybrids for both species and sub species crosses; F2 hybrids were compared to their F1 hybrid parents as well as the original parental strains; and B1 hybrids were compared to the F1 parents as well as to their original parental strains. PCA also reduces the dimensionality of the data set and makes it easier to analyse a data set and represent differences between groups.

Morphometric measurements of long bones:

Maximum limb length measurements

All measurements of the long bones were taken using Avizo Fire 8.1.1. (Thermo Fisher Scientific, Waltham,Massachusetts, USA) Measurements were taken of long bones of both the left and the right hind limbs.

Forelimb long bone measurements

Maximum limb lengths for the fore limbs were collected. Maximum length of the humerus was measured from the most proximal point on the humeral head to distal end of trochlea (Sargis, 2002). Maximum length for the ulna was measured from the proximal edge of olecranon process to distal edge of styloid process (Sargis, 2002). Measurements were taken for both the left and right long bones of the forelimb. The total length of the forelimb was determined by adding the maximum length measurement for the humerus to the maximum length for the hybrids. The relationship between the humerus and the ulna was measured by determining the ratio between the humerus and the ulna.

This ratio was determined by dividing the maximum length of the humerus by the maximum length of the ulna.

Hind-limb long bone measurements

Maximum length measurements were also collected for the hind limbs. Maximum length for the femur was measured from the proximal edge of the greater trochanter to the distal end of the lateral condyle. Maximum length of the fibula was measured from the distal end of the head to the distal end of the lateral malleolus. The total length of the hind limb was determined by adding the maximum measurements of the femur and the fibula. The relationship between the long bones of the hind limb was determined by calculating the ratio between the femur and the fibula.

The inter-membral index(IM) was also calculated for the samples measured, the calculation for the inter-membral index can be found below.

$$\frac{(Humerus + Radius)}{(Femur + Tibia)} x100 = IM$$

Statistical analysis forelimb and hind limb measurements

Statistical analysis was performed using statistical programme R Version 3.1.2014-07-03. Data were tested for normality using the Shapiro-Wilks test. Subsequent to establishing that the data were normally distributed, t-tests (two tailed) were performed to determine if there was a significant difference between the mean values for the left and right limb lengths. Subsequent to establishing that there was no significant differences between left and right limbs, t- tests (two tailed) were performed to determine if there if there were significant differences between parents, parents and their F1 hybrids and between F1 hybrids for the left long limb measurements as follows: length of humerus and ulna, total length of the forelimb, humerus:ulna ratios, femur length, fibula length, total hindlimb length and the femur:fibula ratio. An α-level of 0.05 was used to determine if the differences were signicant. However, because of multiple testing which results in a higher likelihood of Type II errors a more conservative α-level is also reported. The Bonferroni corrected p-value is 0.016 for comparisons between parents and F1 hybrids.

Chapter 5: Results

Results for differences in coat colour data in sub specific crosses

Results for F1 hybrids and parents

Variation in pelage colour parents and F1 hybrids

Multivariate analysis of variance (MANOVA) was used to determine if there were significant differences in colour between the parents and between the parents and the F1 hybrids. The dependent variables were ADR, ADG and ADB for the dorsal coat analysis and AVR, AVG and AVB for the ventral analysis; the independent variable is the group/strain.

Comparisons between parents

M. m. musculus has the largest mean ADR and ADG values while *M. m. domesticus* has the largest average ADB values (Table 5.1). All the parents are significantly different from each other in terms of dorsal coat colour (MANOVA; $p<0.001$; Table 5.2). *M. m. musculus* has the overall lightest dorsal coat. *M. m. domesticus* has intermediate ADR and ADG values and *M. m. castaneus* has the smallest mean values and the darkest coat (Table 5.1). The parents are also significantly different from each other in terms of average ventral coat colour (MANOVA; $p<0.001$; Table 5.2). *M. m. musculus* has the lightest ventral coat colour with the largest mean AVR, AVG and AVB values, with *M. m. domesticus* having an intermediate coat colour, and *M. m. castaneus* has the darkest ventral coat with the smallest mean AVR, AVG and AVB values (Table 5.11). all the differences were significant based on the Bonferonni corrected p-value of 0.012.

M. m. castaneus x *M. m. domesticus* F1 hybrids compared to parents

For dorsal coat colour the *M. m. castaneus x M. m. domesticus* F1 hybrids have smaller mean ADR, ADG and ADB values than *M. m. domesticus* (Table 5.1) thus having a darker dorsal coat; this difference is significant according the the Bonferonni corrected p-value ($p<0.001$; Table 5.2). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus* F1 hybrids and *M. m. domesticus* for ADR, ADG and ADB values using t-tests indicate that they are significantly different but not at the Bonferonni corrected p-value of 0.0125 ($p<0.05$; Table 5.3). When compared to *M. m. castaneus*, the *M. m. casteneaus* x *M. m. domesticus* F1 hybrids have a lighter dorsal coat colour with larger mean ADR, ADG and ADB values (Table 5.1). This difference however is not significant ($p= 0.46$; Table 5.2).

Pairwise comparisons of ADR, ADG and ADB values between the *M. m. casteneaus* x *M. m. domesticus* F1 hybrids and *M. m. castaneus* indicate that they are not significantly different (Table 5.3). The average dorsal coat colour for *M. m. casteneaus* x *M. m. domesticus* F1 hybrids are close to the expected mid-parental value (MPV) (Table 5.1). The MPV was determined by taking the means for the two parental groups and deviding by two.

The *M. m. casteneaus* x *M. m. domesticus* F1 hybrids were significantly different from both parents for average ventral coat colour at the Bonferonni corrected p-value ($p<0.001$; Table 5.2). Hybrid mean AVR, AVG and AVB values are intermediate between the mean AVR, AVG and AVB values of the parents, and close to the expected MPV for *M. m. castaneus* x *M. m. domesticus* F1 hybrids (Table 5.1). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus* F1 hybrids and both parental groups indicate that there are significant differences in AVR, AVG and AVB means at the Bonferonni corrected p-value ($p<0.001$; Table 5.3).

The results for the PCA analysis of *the M. m. castaneus* x *M. m. domesticus* F1 hybrids and their parental groups are displayed in Figure 5.1A and Table 5.4. PC1 is represented on the x-axis of Figure 5.1A and accounts for 81% of the variation in coat colour. PC1 represents variation in overall lightness/darkness of the coat colour with ventral coat measurements accounting for most of this variation. Large negative loadings are associated with ventral colour (AVR (-0.61), AVG(-0.55), AVB (-0.47)) and small negative loading are associated with dorsal coat colour (ADR (-0.20), ADG (-0.21) and ADB (-0.18)). *M. m. domesticus* and *M. m. castaneus* separate along this axis. *M. m. domesticus,* which has the lighter overall coat colour (a brown dorsal coat, and a lighter grey brown ventral coat), has negative PC1 scores, and *M. m. castaneus,* the parent with the darker overall coat (a dark brown dorsal coat and a slightly lighter brown ventral coat) has positive scores. The hybrids overlap with both parents along this axis (Figure 5.1A). PC2 (y-axis) accounts for 16% of the variation, dorsal coat measurements have large negative loadings (ADR(-0.57), ADG (-0.57), ADB (-0.48)) and ventral measurements have smaller positive loadings (AVR(0.19), AVG(0.19), AVB(0.21)). Variation in dorsal coat colour makes up most of PC2 with the *M. m. castaneus* x *M. m. domesticus* F1 hybrids and parents overlapping along this axis because all three groups have dark brown dorsal coats. Overall the *M. m. castaneus* x *M. m. domesticus* F1 hybrids look like one or the other parental group in terms of variation in dorsal and ventral coat colour.

M. m. castaneus x *M. m. musculus* F1 hybrids compared to parents

The *M. m. castaneus* x *M. m. musculus* F1 hybrids have smaller mean ADR, ADG and ADB values than *M. m. musculus* and larger values than *M. m. castaneus*, thus having an intermediate dorsal coat

colour (Table 5.1). These differences in dorsal coat colour between the *M. m. castaneus* x *M. m. musculus* F1 hybrids and their parental groups are significant at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.2). Their mean ADR, ADG and ADB values are similar to the expected MPV (Table 5.1). Pairwise comparisons of the dorsal coat measurements between *M. m. castaneus* x *M. m. musculus* F1 hybrids and *M. m. musculus* show that there are significant differences in mean ADR, ADG are significant at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.3) and ADB values is not at the the Bonferonni corrected p-valueof 0.016 ($p<0.05$; Table 5.3). Pairwise comparisons between *M. m. castaneus* x *M. m. musculus* F1 hybrids and *M. m. castaneus* show that there are no significant differences in mean ADR, ADG and ADB values when using t-tests (Table 5.3).

The *M. m. castaneus* x *M. m. musculus* F1 hybrids have a darker ventral coat with smaller mean AVR, AVG and AVB values than *M. m. musculus* (Table 5.1). This difference in ventral coat colour is significant at the Bonferonni corrected p-value of 0.016 ($p<0.01$; Table 5.2). The hybrids have larger mean AVR, AVG and AVB values than *M. m. castaneus* (Table 5.1) thus having a significantly lighter ventral coat at the Bonferonni corrected p-value of 0.016 ($p<0.01$; Table 5.1). The *M. m. castaneus* x *M. m. musculus* F1 hybrids thus have an intermediate ventral coat colour when compared to the parental groups with the AVR, AVG and AVB values being slightly smaller than the expected MPV (Table 5.1). Pairwise comparisons of average AVR, AVG and AVB values between *M. m. castaneus* x *M. m. musculus* F1 hybrids and *M. m. musculus* using t-tests indicate that these values are significantly different as well as for comparisons between *M. m. castaneus* x *M. m. musculus* F1 hybrids and *M. m. castaneus.* These differences are significant at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.3).

The PCA results *M. m. castaneus* x *M. m. musculus* F1 hybrids and their parents are presented in Table 5.5 and Figure 5.1B. PC1 is on the x-axis of Figure 5.1B and accounts for 90% of the variation in coat colour. Overall lightness or darkness of the coat is again represented by PC1. The ventral coat makes up most of the variation with large negative loadings (AVR(-0.69), AVG(-0.53), AVB(-0.37)), because there is a big difference between the parental groups for ventral coat colour. *M. m. musculus* has a yellow/agouti ventral coat resulting in larger AVR, AVG and AVB values, *M. m. castaneus* has a brown ventral resulting in smaller AVR, AVG and AVB values. The dorsal coat measurements have small negative loadings (ADR (-0.24), ADG (-0.20) and ADB (-0.12)), again the *M. m. musculus* parent has a slightly lighter dorsal coat with larger ADR, ADG and ADB values than the *M. m. castaneus* parent. The two parental groups separate along the x-axis, *M. m. musculus* has negative PC1 scores because it has a ligher ventral coat which is agouti coloured, *M. m. castaneus* has positive PC1 scores because it has a darker brown ventral coat. The hybrids occupy an intermediate space along the x-axis and

some individuals overlap slightly with *M. m. musculus*, having a dark brown dorsal coat with a ligher yellow brown ventral coat. PC2 accounts for 7% of the variation and this is represented on the y-axis of Figure 5.1B. Most of the variation in PC2 is the result of variation in dorsal coat colour coat, with large negative loadings for dorsal coat colour values (ADR (-0.52), ADG(-0.57), ADB(-0.51)), the parents mostly overlapping along this axis and the hybrids have positive PC2 values,overall occupying the space represented by lighter dorsal coat colour but still in the range of dorsal colour of the parental groups.

M. m. musculus x M. m. domesticus F1 Hybrids compared to parens

The *M. m. musculus* x *M. m. domesticus* F1 hybrids have smaller mean ADR, ADG and ADB values than *M. m. musculus* and thus on average have darker dorsal coats than *M. m. musculus* (Table 5.1). This difference is significant at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.2). Pairwise comparisons of dorsal coat values between *M. m. musculus* and *M. m. musculus* x *M. m. domesticus* F1 hybrids indicate that they are significantly different for mean ADR and ADG measurements at the Bonferonni corrected p-value of 0.016 ($p<0.01$) but not for mean ADB measurements ($p= 0.44$; Table 5.3). The *M. m. musculus* x *M. m. domesticus* F1 hybrids have larger mean ADR values than *M. m. domesticus*, but slightly smaller ADG and ADB values (Table 5.1). This difference in dorsal coat colour is significant at the Bonferonni corrected p-value of 0.016 ($p<0.01$; Table 5.2). Pairwise comparisons of dorsal coat measurements between *M. m. musculus* x *M. m. domesticus* F1 hybrids and *M. m. domesticus* indicate that there are significant differences in mean ADB ($p<0.05$) but not at the Bonferonni corrected p-value of 0.016. Mean ADR ($p=0.43$) and ADG ($p=0.72$; Table 5.3) are not significantly different. Thus the *M. m. musculus* x *M. m. domesticus* F1 hybrid has a darker dorsal coat than both its parental groups and the mean ADR, ADG and ADB values are smaller than the expected MPV (Table 5.1).

For the ventral coat the *M. m. musculus* x *M. m. domesticus* F1 hybrids have smaller mean AVR and AVG values than *M. m. musculus* while having larger mean AVB values (Table 5.1); the difference in ventral coat colour is significant at the Bonferonni corrected p-value of 0.016 ($p<0.01$; Table 5.2). The *M. m. musculus* x *M. m. domesticus* F1 hybrid mice have a coat colour which is different from that seen in either parental group, with the *M. m. musculus* parent having a yellow/agouti coat colour, while the hybrid has a very light cream ventral coat colour. Pairwise comparisons of ventral coat measurements between *M. m. musculus* x *M. m. domesticus* F1 hybrids and *M. m. musculus* indicate that there are significant differences in AVR ($p<0.001$) and AVB ($p<0.01$) at the Bonferonni corrected

p-value of 0.016 but not AVG mean values (p=0.52; Table 5.3). The *M. m. musculus* x *M. m. domesticus* F1 hybrid has larger mean AVR, AVG and AVB values than *M. m. domesticus* (Table 5.1) and thus has a lighter ventral coat colour with this difference in colour being significant at the Bonferonni corrected p-value of 0.016 (p<0.001; Table 5.2). Pairwise comparisons of ventral coat measurements between *M. m. musculus* x *M. m. domesticus* F1 hybrids and *M. m. domesticus* indicates that there are significant differences in AVR, AVB and AVG values at the Bonferonni corrected p-value of 0.016 (p<0.001; Table 5.3). *M. m. musculus* x *M. m. domesticus* F1 hybrid mean AVR values are similar to that of the expected MPV but the AVG and the AVB values are larger than the expected MPV (Table 5.1).

The results for the PCA for *M. m. musculus* x *M. m. domesticus* F1 hybrids and their parental strains are displayed in Figure 5.1C (x-axis) and Table 5.6. PC1 accounts for 76% of the variation and represents overall variation in lightness or darkness of the coat colour, with variation in ventral coat colour accounting for most of the variation (with large negative loadings (AVR (-0.64), AVG (-0.50) ,AVB (-0.36))) and the dorsal coat somewhat less (smaller negative loadings (ADR(-0.306), ADG(-0.265), ADB(-0.164))). Thus, differences in ventral coat colour accounts for most of the variation once again. *M. m. musculus* with the overall lighter yellow/agouti ventral coat separates from *M. m. domesticus* with the light brown grey ventral coat along the x-axis, while the *M. m. musculus* x *M. m. domesticus* F1 hybrids overlap with both parental groups along PC1. The dorsal coat accounts for less of the variation along this axis with the parents both having brown dorsal coats; *M. m. musculus* has a lighter brown dorsal coat than the *M. m. domesticus* while the *M. m. musculus* x *M. m. domesticus* F1 hybrids have dark brown dorsal coats. PC2 accounts for 13% of the variation in colour with dorsal coat colour measurements having large negative loadings (ADR (-0.42), ADG (-0.40), ADB(-0.32)) and ventral AVR having a smaller negative loading of -0.131. AVG has a positive loading of 0.24 and AVB has a large positive loading of 0.69. For PC2 most of the variation is in dorsal coat colour as well as variation in ventral coat colour. Those with lighter ventral coat (*M. m. musculus* x *M. m. domesticus* F1 hybrids - cream ventral coat) have large positive scores, while *M. m. musculus* (slightly darker tan yellow coat) and *M. m. domesticus* (brown coat) have negative scores. The difference in AVB as well as the dark brown dorsal coat separates the F1 hybrid from the two parental groups along this axis. The hybrid with the positive scores along PC2 has a cream/light yellow ventral coat, which is different from the yellow and brown ventral coat colours seen in the parenal groups which are represented by negative PC2 scores. The F1 hybrids also have a dark brown dorsal coat with a cream/lighter yellow ventral coat which is what we see along PC2 – a combination not present in either parental group. The *M. m. musculus* parent has a dark brown dorsal coat while having a yellow/augoti ventral coat and the *M. m. domesticus* has a dark brown dorsal coat with a slightly ligher grey brown ventral coat. Thus,

along PC2 we see a difference in dorsal ventral contrast (DVC) with negative PC2 scores associated with a smaller difference between the dorsal and ventral coat colour while positive scores are associated with a larger difference in dorsal and ventral coat colour. The *M. m. musculus* x *M. m. domesticus* F1 hybrids thus have a different phenotype from the parental groups on average with a dark brown dorsal coat and a lighter ventral coat which is cream/light yellow in colour.

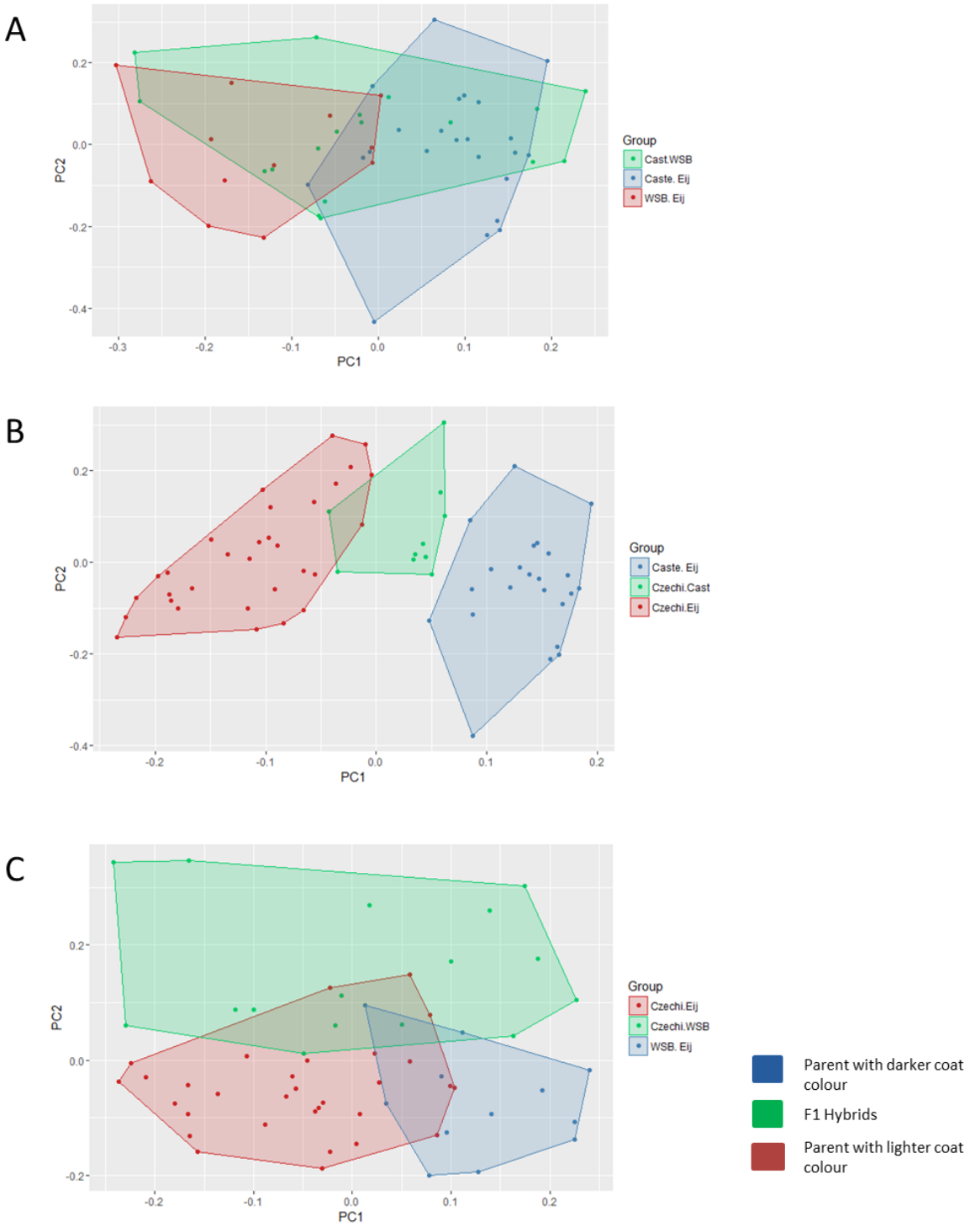

Figure 5.1: PCA based on average dorsal and ventral colour measurements of parents, F1 (A) Distribution of Czechi.eij and Cast.Eij parents and their F1 hybrids and parents according to the first two principal components. (B) Distribution of WSB.Eij and Cast.Eij parents and their F1 hybrids and parents according to the first two principal components and (C) Distribution of Czechi.Eij and Cast.Eij parents and their F1 hybrids. *M. m. casteneaus* = Cast.Eij, *M. m. domesticus* = WSB.Eij, *M. m. musculus* = Czechi.Eij.

Dorsal and ventral R,G,B values	*M. m. musculus*			*M. m. domesticus*			*M. m. castaneus*			*M. m. musculus* x *m. castaneus* F1		*M.*	*M. m. musculus x m. domesticus* F1		*M.*	*M. m. castaneus* x *m. domesticus* F1		Expected MPV		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	*M. m. musculus* x *M. m. castaneus* F1	*M. m. musculus* x *M. m. domesticus* F1	*M. m. castaneus* x *M. m. domesticus* F1
ADR	32	105.2	14.42	21	90.44	12.66	32	76.18	15.12	20	84.90	15.56	31	93.54	15.34	25	80.86	15.48	90.69	97.82	83.31
ADG	32	91.78	14.39	21	84.98	11.99	32	68.80	14.48	20	75.61	16.52	31	83.64	15.11	25	73.36	14.82	80.29	88.38	76.89
ADB	32	59.47	13.15	21	63.22	10.13	32	47.13	11.48	20	51.32	13.92	31	57.05	14.82	25	49.92	11.22	53.3	61.345	55.17
AVR	42	212.8	16.95	32	159.50	17.08	29	126.9	11.58	18	166	23.18	18	184.7	28.69	24	145.5	21.22	169.9	186.15	143.2
AVG	42	186	16.64	32	155	15.48	29	124.8	10.16	18	150.1	19.058	18	181.9	24.85	24	140.3	18.64	155.4	170.5	139.9
AVB	42	137.1	18.34	32	126.4	15.57	29	96.66	8.38	18	107.2	17.113	18	157.9	22.38	24	109.61	16.3	116.9	131.75	111.5

Table 5.1: Mean AVR, AVG and AVB for ventral coat colour and ADR, ADG and ADB values for dorsal coat colour, n (indicates sample size), SD (standard deviation) and the expected MPV

Comparison	Dorsal coat colour	Ventral coat colour
	p-value	p-value
M. m. castaneus v *M. m. musculus*	p<0.001	<0.001
M. m. castaneus v *M. m. domesticus*	p<0.001	<0.001
M. m. musculus v *M. m. domesticus*	p<0.001	<0.001
M. m. castaneus v *M. m. domesticus* x *M. m. castaneus* F1	p<0.001	<0.001
M. m. castaneus v *M. m. castaneus* x *M. m. musculus* F1	0.46	<0.001
M. m. musculus v *M. m. musculus* x *M. m. castaneus* F1	p<0.001	<0.001
M. m. musculus v *M. m. musculus* x *M. m. domesticus* F1	p<0.001	<0.001
M. m. domesticus v *M. m. castaneus* x *M. m. domesticus* F1	p<0.001	<0.001
M. m. domesticus v *M. m. musculus* x *M. m. domesticus* F1	p<0.001	<0.001

Table 5.2: Results for MANOVA used to determining if there is a significant difference in average dorsal coat colour and average ventral coat colour between F1 hybrids and their respective parents

Average dorsal and Ventral values	*M. m. castaneus* v *M. m. musculus*	*M. m. castaneus* v *M. m. domesticus*	*M. m. musculus* v *M. m. domesticus*	*M. m. castaneus* v *M. m. musculus* x *M. m. castaneus* F1	*M. m. musculus* v *M. m. musculus* x *M. m. castaneus* F1	*M. m. castaneus* v *M. m. castaneus* X *M. m. domesticus* F1	*M. m. domesticus* v *M. m. castaneus* x *M. m. domesticus* F1	*M. m. musculus* v *M. m. musculus* x *M. m. domesticus* F1	*M. m. domesticus* v *M. m. musculus* x *M. m. domesticus* F1
ADR	p<0.001	p<0.001	p<0.001	0.054	p<0.001	0.25	p<0.05	p<0.001	0.43
ADG	p<0.001	p<0.001	0.069	0.14	p<0.001	0.25	P<0.05	p<0.05	0.72
ADB	p<0.001	p<0.001	0.25	0.27	p<0.05	0.36	p<0.001	0.44	p<0.05
AVR	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.05	p<0.001	p<0.001
AVG	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.05	0.53	p<0.001
AVB	p<0.001	p<0.001	p<0.05	p<0.05	p<0.001	p<0.001	p<0.05	p<0.05	p<0.001

Table5.3: Pair wise comparisons of AVR, AVG and AVB for the ventral coat and ADR, ADG and ADB for the dorsal coat between parents, parents and F1 hybrids to determine if there were significant differences in the three colour measurements.

M. m. castaneus x *M. m. domesticus* F1 hybrids						
	PC1	PC2	PC3	PC4	PC5	PC6
SD	34.85	15.67	5.82	3.12	1.67	0.89
Proportion of Variance	0.81	0.16	0.02	0.01	0.00	0.00
Cumulative Proportion	0.81	0.97	0.99	1.00	1.00	1.00
	Loadings					
ADR	-0.20	-0.57	0.00	0.58	0.00	-0.54
ADG	-0.21	-0.57	0.00	0.11	0.00	0.78
ADB	-0.18	-0.48	-0.11	-0.80	0.00	-0.29
AVR	-0.61	0.19	0.59	0.00	-0.48	0.00
AVG	-0.55	0.19	0.00	0.00	0.81	0.00
AVB	-0.47	0.21	-0.79	0.12	-0.32	0.00

Table 5.4: PCA results for *M. m. castaneus* x *M. m. domesticus* F1 hybrids and their *M. m. castaneus* and *M. m. domesticus* parents.

*M. m. castaneus*x *M. m. musculus* F1 hybrids						
	PC1	PC2	PC3	PC4	PC5	PC6
SD	60.16	16.79	10.06	4.53	3.68	1.31
Proportion of Variance	0.90	0.07	0.03	0.01	0.00	0.00
Cumulative Proportion	0.90	0.97	0.99	1.00	1.00	1.00
Loadings						
ADR	-0.24	-0.52	-0.26	0.40	0.42	0.53
ADG	-0.20	-0.57	0.00	0.00	0.11	-0.79
ADB	-0.12	-0.51	0.00	-0.62	-0.50	0.31
AVR	-0.69	0.38	-0.48	-0.35	0.17	0.00
AVG	-0.53	0.00	0.20	0.55	-0.62	0.00
AVB	-0.37	0.00	0.81	-0.20	0.40	0.00

Table 5.5: PCA results for *M. m. castaneus* x *M. m. musculus* F1 hybrids and their *M. m. musculus* and *M. m. castaneus* parents.

. m. musculus x *M. m. domesticus* F1 hybrids						
	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	43.90	18.05	15.88	4.30	1.87	1.40
Proportion of Variance	0.76	0.13	0.10	0.01	0.00	0.00
Cumulative Proportion	0.76	0.89	0.99	1.00	1.00	1.00
Loadings						
ADR	-0.31	-0.42	0.19	0.63	0.00	-0.54
ADG	-0.26	-0.40	0.37	0.14	0.20	0.75
ADB	-0.16	-0.33	0.49	-0.71	-0.14	-0.31
AVR	-0.65	-0.13	-0.6	-0.26	0.36	0.00
AVG	-0.51	0.24	0.00	0.00	-0.80	0.17
AVB	-0.36	0.69	0.47	0.00	0.40	-0.11

Table 5.6: PCA results for *M. m. musculus* x *M. m. domesticus* F1 hybrids and their *M. m. musculus*and *M. m. domesticus* parents.

Dorsal ventral patterning

DVC is a measure of the difference between colour of the ventral and the dorsal coat. It is determined by subtracting the dorsal ADR, ADG and ADB values from the ventral AVR, AVG and AVB values this provides a value which indicates DVC (The dorsal ventral R (DVR), dorsal ventral G (DVG) and the dorsal ventral (DVB)). Large DVR, DVG and DVB values indicate that there are large differences in colour between the dorsal and ventral coat while small values indicate very little difference between the dorsal and ventral coat colour. MANOVA was used to determine if there were significant differences between parents and parents and F1 hybrids dorsal ventral patterning using the DVR, DVG and DVB as dependent variables.

Differences in dorsal ventral patterning between parents

Parents were all significantly different from each other at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.7), *M. m. castaneus* had the lowest contrast *M. m. domesticus* was intermediate and *M. m. musculus* had the largest contrast (Table 8). Parents were all significantly different from each for pairwise comparisons DVR, DVG and DVB using t-tests at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.9). The two with the smallest contrast (*M. m. castaneus* and *M. m. domesticus)* had very subtle differences in colour between the dorsal and the ventral coat.

Comparisons between F1 hybrid and parents

Using MANOVA, the F1 hybrids are significantly different from both their parents ($p<0.001$), except for *M. m. domesticus* not being significantly different from *M. m. castaneus* x *M. m. domesticus* F1 hybrids using MANOVA ($p=0.07$; Table 5.7). *M. m. castaneus*x *M. m. musculus* F1 hybrids have intermediate DVR, DVG and DVB values when compared to the two parental groups (Table 5.8). The *M. m. castaneus* x *M. m. domesticus* F1 hybrids also have intermediate values when compared to parental groups but is not significantly different from the parent with the largest contrast (*M. m. domesticus*) ($p=0.07$; Table 5.7 and 5.8). The *M. m. musculus* x *M. m. domesticus* F1 hybrids have larger DVG and DVB values than both parents and are extreme in this regard with a lighter cream coloured ventral coat and a darker brown dorsal coat (Table 5.8). The *M. m. musculus* x *M. m. domesticus* F1 hybrids, DVR is smaller than that of *M. m. musculus* which could be because *the M. m. musculus* has a tan yellow almost orange in colour ventral coat resulting in larger AVR values, it is still larger than the expected MPV (Table 5.7). The *M. m. musculus* x *M. m. domesticus* F1 hybrid mean DVR is larger than the *M. m. domesticus* DVR and this difference is statistically significant at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.8 and 5.9).

In terms of pairwise comparisons of the DVR, DVG and DVB values, *M. m. castaneus* x *M. m. musculus* F1 hybrids are significantly different from both parents in t-test pairwise comparisons of DVR, DVG and DVB values at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.9). *M. m. musculus* x *M. m. domesticus* F1 hybrids are significantly different from the parent with the smallest contrast *M. m. domesticus* in pair wise t-test comparisons of DVR, DVG and DVB at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.9).It is also significantly different from *M. m. musculus* for DVR, in terms of pair wise t-test comparisons at the Bonferonni corrected p-value of 0.016 ($p<0.001$; Table 5.9), it is not significantly different at the Bonferonni corrected p-value for pairwise comparisons of mean DVB ($P<0.05$; Table 5.9). It is not significantly different from *M. m. musculus* for DVG ($p=0.12$; Table 5.9). The *M. m.*

castaneus x *M. m. domesticus* F1 hybrid had a significantly different mean DVR, DVG and DVB from *M. m. castaneus* at the p value of 0.05 (the parent with the smallest DVC) for pairwise comparisons using t-tests but not at the Bonferonni corrected p-value of 0.016 ($p<0.05$; Table 5.9). It was not significantly different from the other parent *M. m. domesticus* for DVR, DVG and DVB (Table 5.9).

Comparison	p value
M. m. castaneus v *M. m. musculus*	p<0.001
M. m. castaneus v *M. m. domesticus*	p<0.001
M. m. musculus v *M. m. domesticus*	p<0.001
M. m. castaneus v *M. m. castaneus* x *M. m. musculus* F1	p<0.001
M. m. castaneus v *M. m. castaneus* x *M. m. domesticus* F1	p<0.05
M. m. musculus v *M. m. castaneus* x *M. m. musculus*	p<0.001
M. m. musculus v *M. m. musculus* x *M. m. domesticus* F1	p<0.001
M. m. domesticus v *M. m. castaneus* x *M. m. domesticus* F1	0.07
M. m. domesticus v *M. m. musculus* x *M. m. domesticus* F1	p<0.001

Table 5.7: P values for MANOVAs used to determine if there significant differences in DVC

F2 Hybrids

Pelage results for F2 hybrids

M. m. castaneus x *M. m. musculus* F2 hybrids

The *M. m. castaneus* x *M. m. musculus* F2 hybrids have observably lighter brown dorsal coats and larger ADR, ADG and ADB values than the *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 5.10), but a MANOVA with ADR, ADG and ADB measurements as independent variables indicates that this difference is not significant ($p=0.23$; Table 5.11). Pairwise comparisons of ADR, ADG and ADB mean values between *M. m. castaneus* x *M. m. musculus* F2 and *M. m. castaneus* x *M. m. musculus* F1 hybrids indicate that all three measurements are not significantly different (Table 5.12). *M. m. castaneus* x *M. m. musculus* F2 hybrids have smaller mean ADR, ADG and a slightly larger ADB mean value than *M. m. musculus* and are significantly different in average dorsal coat colour at the Bonferroni corrected p-value of 0.0125($p<0.01$;

Difference between Dorsal and Ventral RGB values	*M. m. musculus*			*M. m. castaneus*			*M. m. domesticus*			*M. m. castaneus* x *M. m. musculus* F1			*M. m. musculus* x *M. m. domesticus* F1			*M. m. castaneus* x *M. m. domesticus* F1			Expected mid parental values		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	*M. m. castaneus* x *M. m. musculus* F1	*M. m. musculus* x *M. m. domesticus* F1	*M. m. castaneus* x *M. m. domesticus* F1
DVR	31	108.94	11.8	23	46.92	15.20	12	70.04	14.9	11	76.5	16.5	16	96.6	19	18	60.11	19.7	77.9	89.49	58.48
DVG	31	96.47	9.53	23	52.25	14.70	12	70.48	13.9	11	78.1	8.98	16	103.8	17.9	18	63.63	17.7	74.36	83.48	61.36
DVB	31	80.7	12.4	23	46.61	13.56	12	62.58	13.9	11	63.7	9.06	16	106	16.7	18	58.25	16.2	63.67	71.64	54.6

Table 5.8: Mean and SD for DVR, DVG and DVB values, and n for each parent and F1 hybrid

Difference between Dorsal and Ventral RGB values	*M. m. castaneus* v *M. m. musculus*	*M. m. musculus* v *M. m. domesticus*	*M. m. castaneus* v *M. m. domesticus*	*M. m. castaneus* v *M. m. castaneus* x *M. m. domesticus*	*M. m. castaneus* v *M. m. castaneus* x *M. m. musculus*	*M. m. domesticus* v *M. m. castaneus* x *M. m. domesticus*	*M. m. domesticus* v *M. m. musculus* x *M. m. domesticus*	*M. m. musculus* v *M. m. musculus* x *M. m. domesticus*	*M. m. musculus* v *M. m. castaneus* x *M. m. musculus*
DVR	p<0.001	p<0.001	p<0.001	p<0.05	p<0.001	0.13	p<0.001	p<0.05	p<0.001
DVB	p<0.001	p<0.001	p<0.001	p<0.05	p<0.001	0.44	p<0.001	p<0.001	p<0.001
DVG	p<0.001	p<0.001	p<0.001	p<0.05	p<0.001	0.25	p<0.001	0.12	p<0.001

Table5.9: Results from pairwise comparisons of DVR, DVG and DVB values which were compared between parents and parents and F1 hybrids to determine if there were significant differences in mean DVC

Table 5.10 and 5.11). The F2 hybrids thus have a darker brown dorsal coat colour than *M. m. musculus* wich has a dark chestnut brown dorsal coat colour. Pairwise comparisons between *M. m. castaneus* x *M. m. musculus* F2 hybrids and *M. m. musculus* indicate that there is a significant difference in mean ADR, but this is not significant at the Bonferroni corrected p-value of 0.012 ($p< 0.05$) values but not mean ADG and ADB values (Table 5.12). The *M. m. castaneus* x *M. m. musculus* F2 hybrids had larger mean ADR, ADG and ADB values indicating that they have lighter dorsal coats than *M. m. castaneus* and this difference in colour is significant ($p<0.01$; Table 5.10 and 5.11). Pairwise comparison between *M. m. castaneus* x *M. m. musculus* F2 hybrids and *M. m. castaneus* parents indicate that there are significant differences in mean ADR, ADG and ADB values this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.12)

For the ventral coat, the *M. m. castaneus* x *M. m. musculus* F2 hybrids have larger AVR, AVG and AVB values than the *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 5.10) and there is a significant difference in mean ventral coat colour this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.11). Pairwise comparisons between *M. m. castaneus* x *M. m. musculus* F2 hybrids and *M. m. castaneus*x *M. m. musculus* F1 hybrids indicate that are significant differences in AVR, AVG and AVB values ($p<0.01$; Table 5.12). The *M. m. castaneus* x *M. m. musculus* F2 hybrids have smaller mean AVR and AVG values than *M. m. musculus* population and slightly larger AVB values indicating that on average they have a darker ventral coat than *M. m. musculus* (Table 5.10). This difference is significant this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.11). The *M. m. musculus* have yellow/agouti ventral coats, which results in larger AVR measurements while the F2 hybrids have larger mean AVB values which could be a result of having a more light mustard brown ventral coat which is still very light relative to the dorsal coat but not as yellow as *M. m. musculus*. Pairwise comparisons between the *M. m. castaneus* x *M. m. musculus* F2 hybrids and *M. m. musculus* show that there are significant differences in mean AVR and AVB ($p<0.05$) but this is not significant at the Bonferroni corrected p-value of 0.012 values but not mean AVG values (Table 5.12). The *M. m. castaneus* x *M. m. musculus* F2 hybrids have larger AVR, AVG and AVB values than *M. m. castaneus* and this is significant this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.10 and 5.11). This indicates that the *M. m. castaneus* x *M. m. musculus* F2 hybrids have a lighter ventral coat than *M. m. castaneus*. Pairwise comparisons between *M. m. castaneus* x *M. m. musculus* F2 hybrids and *M. m. castaneus* indicates that there are significant differences at the Bonferroni corrected p-value of 0.012 in mean AVG, AVB and AVR values ($p<0.01$;Table 5.12).

PCA was used to look at trends in coat colour variation with the ADR, ADG, ADB, AVR, AVG and AVB values. PCA was performed with the data for the *M. m. castaneus* x *M. m. musculus* F2 hybrids, the *M. m. castaneus* x *M. m. musculus* F1 hybrids, *M. m. castaneus* and *M. m. musculus* (sample sizes listed in Table 5.13). The PCA results are presented in Figure 5.2A and Table 5.14. PC1 accounts for 86% of the variation; ventral colour measurements have large negative loadings (AVR(-0.66), AVG(-0.53), AVB(-0.42)) and the dorsal measurements have smaller negative loadings (ADR(-0.24), ADG(-0.2) ,ADB (-0.14)). PC1 represents the overall lightness/darkness of the coat colour with the parent with the lightest coat (*M. m. musculus*) with a chestnut brown dorsal coat and a yellow/agouti ventral coat having negative scores and the parent with the darkest coat (*M. m. casteneaus*) which is a deep brown dorsal coat and a slightly lighter brown ventral coat having positive scores for PC1. Most of the variation in the first component is due to variation in the ventral coat colour. PC1 is represented on the x-axis in Figure 5.2A. The F2 hybrids overlap with *M. m. musculus* with the lightest overall coat colour while not overlapping with *M. m. castaneus* and overlapping slightly with the *M. m. castaneus* x *M. m. musculus* F1 hybrids along the x-axis. PC2 accounts for 8.6% of the variation; the dorsal coat colour measurements has large negative loadings (ADR(-0.58), ADG(-0.57), ADB(-0.45)) while ventral coat values has small positive loadings (AVR(0.33), AVG(0.136))(Table 5). PC2 is represented on the y-axis and most of the variation along this axis is due to variation in dorsal coat colour (Figure 5.2A). The F2 hybrids overlap with both *M. m. casteneaus*, *M. m. musculus* and *M. m. castaneus* x *M. m. musculus* F1 hybrids along PC2. Because the F2 hybrids have a lighter ventral coat more similar to *M. m. musculus*, they overlap with them on the x-axis. They also have a lighter dorsal coat and overlap with *M. m. musculus* parent the y-axis, and separates from *M. m. castaneus* x *M. m. musculus* F1 hybrids which has the slightly darker ventral coat, and is intermediate between the original parental populations (Fig 5.2A).

Average R,G,B values	Parents									F1 Hybrids						F2 Hybrids					
	M. m. musculus			*M. m. domesticus*			*M. m. casteneaus*			*M. m. castaneus* x *M. m. domesticus*			*M. m. castaneus* x *M. m. musculus*			*M. m. castaneus* x *M. m. domesticus*			*M. m. castaneus* x *M. m. musculus*		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
ADR	32	105.19	14.42	21	90.44	12.7	32	76.2	15.1	25	80.9	20.01	20	84.9	15.56	26	97.23	17.66	25	95.6	18.32
ADG	32	91.78	14.39	21	84.98	12	32	68.8	14.5	25	73.4	18.47	20	75.6	16.52	26	88.38	15.47	25	86.1	15.58
ADB	32	59.47	13.15	21	63.22	10.1	32	47.1	11.5	25	49.9	14.17	20	51.3	13.92	26	62.92	12.8	25	59.9	11.08
AVR	42	212.8	16.95	32	159.5	17.1	29	127	11.6	24	146	18.56	18	166	23.18	33	163.2	14.52	27	196.4	18.43
AVG	42	186	16.64	32	155	15.5	29	125	10.2	24	140	16.37	18	150	19.06	33	156.8	12.83	27	181.8	16.31
AVB	42	137.1	18.34	32	126.4	15.6	29	96.7	8.38	24	110	18.48	18	107	17.11	33	130.2	15.95	27	145.9	20.75

Table 5.10: Means, sample sizes and SD for *M. m. castaneus*, *M. m. musculus* and *M. m. domesticus* parents and their F1 and F2 hybrids.

Comparison	Dorsal Coat	Ventral Coat
	p value	p value
M. m. castaneus x *M. m. musculus* F1 v *M. m. castaneus* x *M. m. musculus* F2	0.23	p<0.01
M. m. musculus v *M. m. castaneus* x *M. m. musculus* F2	p<0.01	p<0.01
*M. m. castaneus*v *M. m. castaneus* x *M. m. musculus* F2	p<0.01	p<0.01
*M. m. castaneus*x *M. m. domesticus* F1 v *M. m. castaneus* x *M. m. domesticus* F2	p<0.01	p<0.01
M. m. castaneus v *M. m. castaneus* x *M. m. domesticus* F2	p<0.01	p<0.01
M. m. domesticus v *M. m. castaneus* x *M. m. domesticus* F2	p<0.01	p<0.01

Table 5.11: Manova results of the dorsal and the ventral coat to determine if there are significant differences in coat colour between the F2 hybrids and F1 hybrids and original parental populations.

Average dorsal and ventral values	***M. m. castaneus* x *M. m. musculus* F1 v *M. m. castaneus* x *M. m. musculus* F2**	***M. m. castaneus* v *M. m. castaneus* x *M. m. musculus* F2**	***M. m. musculus* v *M. m. castaneus* x *M. m. musculus* F2**	***M. m. casteneaus* x *M. m. domesticus* F1 v *M. m. castaneus* x *M. m. domesticus* F2**	***M. m. castaneus* v *M. m. castaneus* x *M. m. domesticus* F2**	***M. m. domesticus* v *M. m. castaneus* x *M. m. domesticus* F2**
	p value	p value	p value	p value	p value	p value
ADR	0.11	p<0.001	p<0.05	p<0.05	p<0.05	0.173
ADG	0.086	p<0.001	0.156	p<0.05	p<0.001	0.0.46
ADB	0.058	p<0.001	0.9	p<0.05	p<0.001	0.93
AVR	p<0.001	p<0.001	p<0.001	0.06372	p<0.001	0.49
AVG	p<0.001	p<0.001	0.8897	0.05059	p<0.001	0.71
AVB	p<0.001	p<0.001	p<0.05	p<0.05	p<0.001	0.45

Table 5.12: Pairwise comparison results for F2 hybrids compared to F1 parents and to parents

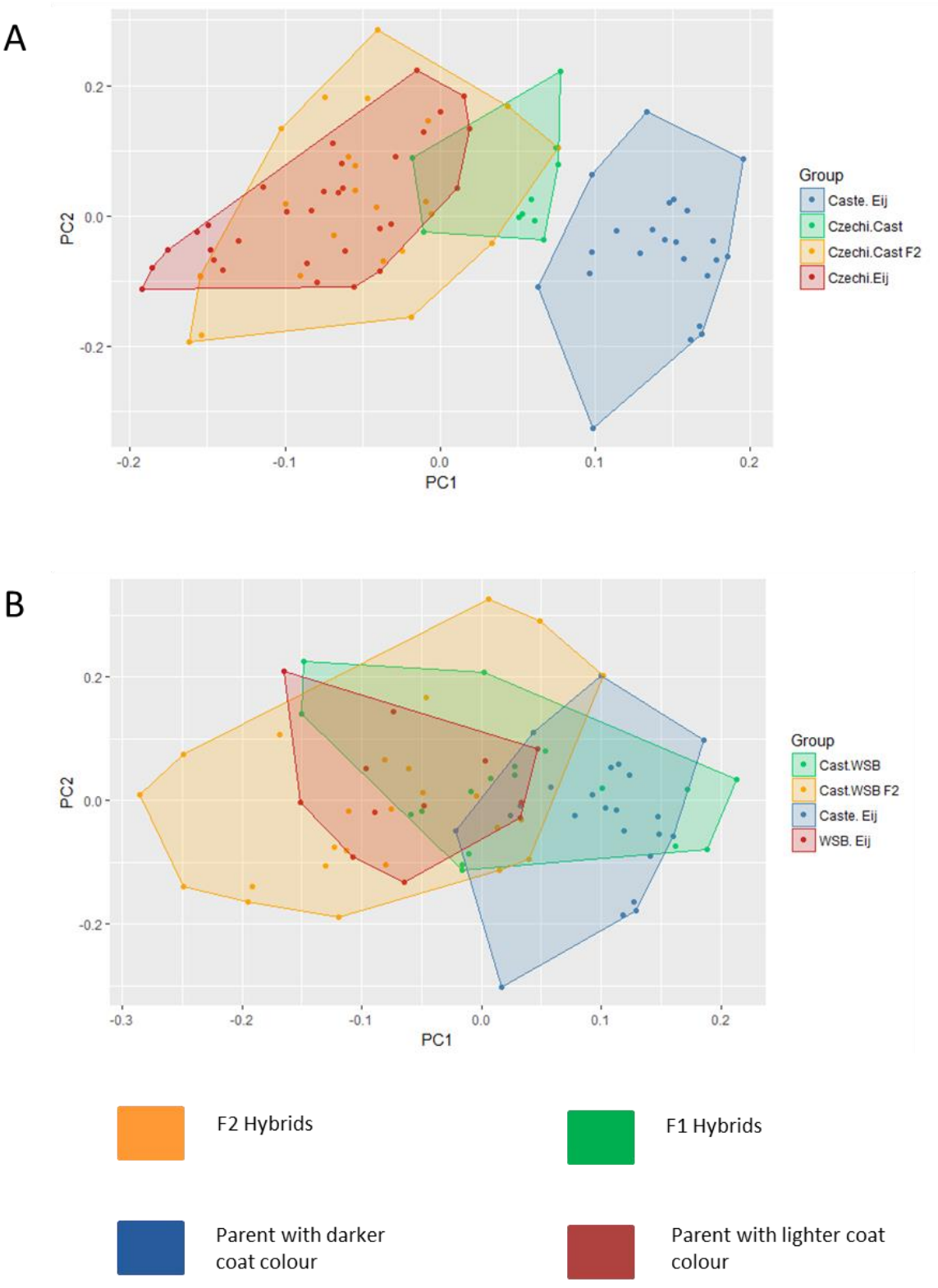

Figure 5.2: PCA based on average dorsal and ventral colour measurements of parents, F1 and F2 hybrids. (A) Distribution of Czechi.eij and Cast.Eij parents and their F1 and F2 hybrids and parents according to the first two principal components. (B) Distribution of WSB.Eij and Cast.Eij parents and their F1 and F2 hybrids and parents according to the first two principal components. *M. m. casteneaus* = Cast.Eij, *M. m. domesticus* = WSB.Eij, *M. m. musculus* = Czechi.Eij

Generation	Strain	n
Parents	*M. m. domesticus*	12
	M. m. casteneaus	23
	M. m. musculus	31
F1 Hybrids	*M. m. castaneus* x *M. m. musculus*	10
	M. m. castaneus x *M. m. domesticus*	18
F2 Hybrids	*M. m. castaneus* x *M. m. domesticus* F2	25
	M. m. castaneus x M. m. musculus F2	25

Table 5.13: Sample sizes for the PCA for F1, F2 hybrids and parents

	***M. m. castaneus* x *M. m. musculus* F2 hybrids**						***M. m. castaneus* x *M. m. domesticus* F2 hybrids**					
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
SD	56.91	18.03	12.45	4.22	3.77	1.24	40.38	18.05	6.473	3.68	1.68	0.99
Proportion of Variance	0.86	0.09	0.04	0.01	0	0	0.81	0.16	0.02	0.01	0.001	0
Cumulative Proportion	0.86	0.95	0.99	1	1	1	0.81	0.97	0.99	0.99	0.99	1
Loadings												
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
ADR	-0.24	-0.58	-0.23	0.52	0.18	-0.5	-0.3	-0.55	-0.1	0.58	0	-0.50
ADG	-0.2	-0.57	0	0	0	0.79	-0.28	-0.54	0	0	0.22	0.76
ADB	-0.14	-0.45	0.12	-0.79	-0.2	-0.33	-0.22	-0.43	0	-0.81	-0.15	-0.31
AVR	-0.66	0.33	-0.53	-0.24	0.35	0	-0.55	0.32	-0.6	0	-0.46	0.15
AVG	-0.53	0.136	0.12	0.2	-0.8	0	-0.49	0.27	0	0	0.79	-0.21
AVB	-0.42	0	0.80	0	0.42	0	-0.48	0.22	0.79	0.11	-0.29	0

Table 5.14: PCA results for parents, F1 hybrids and F2 hybrids.

M. m. castaneus x *M. m. domesticus* F2 Hybrids

The *M. m. castaneus* x *M. m. domesticus* F2 hybrids have larger mean ADR, ADG and ADB values for the dorsal coat than the *M. m. castaneus* x *M. m. domesticus* F1 hybrids indicating that they have a lighter brown dorsal coat overall (Table 5.10). This difference is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.11). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus* F2 and *M. m. castaneus* x *M. m. domesticus* F1 hybrids using t-tests indicate that there are significant differences in mean ADR, ADG and ADB values but this is not significant at the Bonferroni corrected p-value of 0.012 ($p<0.05$; Table 5.12). When compared to the original parental populations, the *M. m. castaneus* x *M. m. domesticus* F2 hybrids have larger ADR and ADG values than *M. m. domesticus* and slightly smaller ADB values (Table 5.10); they are significantly different for dorsal coat colour at the Bonferroni corrected p-

value of 0.012 ($p<0.01$; Table 5.11). Pairwise comparisons using t-tests indicate that there are no significant differences in mean ADR, ADG and ADB values between *M. m. castaneus* x *M. m. domesticus* F2 hybrids and *M. m. domesticus* (Table 5.12). The *M. m. castaneus* x *M. m. domesticus* F2 hybrids also have larger mean ADR, ADG and ADB values than *M. m. castaneus* (Table 5.10) indicating that the F2 hybrids have a lighter dorsal coat than *M. m. castaneus*, this difference is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.11). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus* F2 hybrids and *M. m. castaneus* indicate that there are significant differences in ADR, ADG and ADB values ($p<0.01$; Table 5.12). Thus the *M. m. castaneus* x *M. m. domesticus* F2 hybrids have lighter brown dorsal coats than both the original parental populations and their F1 hybrid parents.

The *M. m. castaneus* x *M. m. domesticus* F2 hybrids have significantly larger mean AVR, AVG and AVB values than the *M. m. castaneus* x *M. m. domesticus* F1 hybrids, this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.10 and Table 5.11). Pairwise comparisons between the *M. m. castaneus* x *M. m. domesticus* F2 hybrids and the *M. m. castaneus* x *M. m. domesticus* F1 hybrids indicate that there are significant differences in mean AVB values ($p<0.05$) however this is not significant at the Bonferroni corrected p-value of 0.016. Ther are no significant differences in mean AVR and AVG values (Table 5.12). When compared to the parental populations the F2 hybrids had larger mean AVR, AVG and AVB values than both *M. m. castaneus* and *M. m. domesticus* (Table 5.10), thus, on average, having a lighter mean ventral coat colour than both parental groups. They are also significantly different from both parental groups for ventral coat colour, it is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.11). Pairwise comparisons between the *M. m. castaneus* x *M. m. domesticus* F2 hybrids and *M. m. domesticus* show that there are no significant differences in mean AVR, AVG and AVB values (Table 5.12). Pairwise comparison using t tests between *M. m. castaneus* x *M. m. domesticus* F2 hybrids and *M. m. castaneus* indicate that there are significant differences in AVG, AVR and AVB values ($p<0.05$; Table 5.12) this is not significant at the Bonferroni corrected p-value of 0.012.

PCA results for the *M. m. castaneus* x *M. m. domesticus* F2 hybrids, which were compared to the *M. m. castaneus* x *M. m. domesticus* F1 hybrids, *M. m. castaneus* and *M. m. domesticus,* are presented in Figure 5.2B and Table 5.14. PC1 accounts for 80.9% of the variation, ventral coat colour measurements have large negative loadings (AVR(-0.55), AVG(-0.49) ,AVR(-0.48)) and dorsal measurements have smaller negative loadings (ADR(-0.3),ADG(-0.28), ADB(-0.22)). PC1 is represented by the x-axis in Figure 5.2B. The *M. m. castaneus* x *M. m. domesticus* F2 hybrids overlap with *M. m. domesticus* and *M. m. castaneus* x *M. m. domesticus* F1 hybrids on the x-axis; as the PC1 scores become more negative the ventral coat becomes

lighter in colour and takes on the grey brown colour and the dorsal coat colour becomes a lighter brown. As you move from negative PC1 scores to positive PC1 scores the ventral coat colour moves from a lighter grey brown colour (on the negative end of the spectrum) to a dark brown brown ventral coat seen in the *M. m. castaneus* parent. The F2 hybrids are closer to the negative end of the spectrum because they have a lighter grey brown ventral coats when compared to *M. m. castaneus* that they separate from along PC1 (Figure 5.2B). Variation in PC1 is largely due to variation in the ventral coat colouring the F2 hybrids have a lighter ventral coat colour similar to the *M. m. domesticus* parental population. There are some F2 hybrids with extreme phenotypes having PC1 scores outside of the range of variation for the *M. m. domesticus* indicating that they have lighter dorsal and ventral coat colours. PC2, which represents 16% of the variation, the dorsal coat measurements have large negative loadings (ADR(-0.55), ADG(-0.54), ADB(-0.43)) while the ventral coat has small positive loadings (AVR(-0.3), AVG(0.273), AVB(0.22)). The hybrids mostly overlap with *M. m. castaneus* and *M. m. domesticus* along this axis with the hybrids having similar values for dorsal coat colours and the hybrids and the parental groups all having various shades of brown dorsal coats which differ slightly. However the F2 hybrids have larger ADR, ADG and ADB values than the F1 hybrids and we see a trend of F2 hybrids with a lighter dorsal coat.

Dorsal ventral patterning results F2 hybrids

M. m. castaneus x *M. m. musculus* F2 hybrids

When looking at dorsal ventral patterning the *M. m. castaneus* x *M. m. musculus* F2 hybrids have larger mean DVR, DVG and DVB values than *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 5.15), with significant differences between the F1 and F2 hybrids for dorsal ventral contrast values($p<0.01$; Table 5.16). The mean DVC of the *M. m. castaneus* x *M. m. musculus* F2 hybrids is more similar to that of *M. m. musculus*, with the DVR and DVG of the *M. m. castaneus* x *M. m. musculus* F2 hybrids being slightly smaller and the mean DVB values being slightly larger (Table 5.15). They are, however, significantly different from each other, this is significant at the Bonferroni corrected p-value of 0.012($p<0.01$; Table 5.16). The *M. m. castaneus* x *M. m. musculus* F2 mean DVR, DVG and DVB values are significantly larger than that of *M. m. castaneu*s as expected, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.15 and 5.16).

M. m. castaneus x *M. m. domesticus* F2 hybrids

The *M. m. castaneus* x *M. m. domesticus* F2 hybrids have mean DVC values larger than *M. m. castaneus* x *M. m. domesticus* F1 hybrids, and this is the case for the DVR, DVG and DVB values with a significant difference between the two for DVC ($p<0.01$; Table 5.15 and 5.16). The *M. m. castaneus* x *M. m. domesticus* F2 hybrids also have mean DVR and DVG values smaller than *M. m. domesticus* parental and slightly larger mean DVB value but this difference is not significant ($p=0.11$; Table 5.15 and 5.16). The *M. m. castaneus* x *M. m. domesticus* F2 hybrids have larger mean DVR, DVG and DVB values than *M. m. castaneus* and they are significantly different in terms of their DVC patterning, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.01$;Table 5.15 and 5.16). F2 hybrids thus have an increased DVC pattern which is introduced in the F1 hybrids and persists in the F2 sample.

Dorsal ventral contrast values	Parents									F1 Hybrids						F2 Hybrids					
	M. m. musculus			*M. m. casteneaus*			*M. m. domesticus*			*M. m. castaneus* x *M. m. domesticus*			*M. m. castaneus* x *M. m. musculus*			*M. m. castaneus*x *M. m. domesticus*			*M. m. castaneus*x *M. m. musculus*		
	n	Mean	SD	n	Mean	SD	N	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
DVR	31	108.9	11.8	23	46.92	15.2	12	70	14.9	18	60.11	19.7	11	76.5	23	25	66.55	18.05	24	101.4	17.7
DVG	31	96.47	9.53	23	52.25	14.7	12	70.5	13.9	18	63.63	17.7	11	78.1	9.06	25	68.65	15.32	24	95.94	16.9
DVB	31	80.7	12.4	23	46.61	13.6	12	62.6	13.9	18	58.25	16.2	11	63.7	8.98	25	67.28	16.04	24	85.74	15.5

Table 5.15: Mean and SD for DVR, DVB andDVG and n for F1, F2 hybrids and parents

Comparison	p value
M. m. castaneus* x *M. m. musculus v ***M. m. castaneus* x *M. m. musculus* F2**	p<0.01
M. m. musculus v ***M. m. castaneus* x *M. m. musculus* F2**	p<0.01
M. m. castaneus v ***M. m. castaneus* x *M. m. musculus* F2**	p<0.01
M. m. castaneus* x *M. m. domesticus v ***M. m. castaneus* x *M. m. domesticus* F2**	p<0.01
M. m. castaneus v ***M. m. castaneus* x *M. m. domesticus* F2**	p<0.01
M. m. domesticus v ***M. m. castaneus* x *M. m. domesticus* F2**	0.11

Table 5.16: Manova results for comparisons between F2 hybrids and their F1 hybrid parents as well as between F2 hybrids and the parental groups for DVC.

Results B1 backcrossed hybrids

Pelage results for B1 hybrids

M. m. musculus x *M. m. domesticus* B1 hybrids

When the *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 backcrossed hybrids are compared to their parents they have lower mean ADB, ADG and ADR values than both *M. m. musculus* x *M. m. domesticus* F1 hybrids and *M. m. musculus* (Table 5.17). They are not however significantly different from the *M. m. musculus* x *M. m. domesticus* F1 hybrids (p=0.25) and they are significantly different from *M. m. musculus,* this is significant at the Bonferroni corrected p-value of 0.012 (p<0.01; Table 5.18). They have mean ADR, ADG and ADB values which are smaller than the expected MPV (Table 5.17). Pairwise comparisons using t-tests indicate that there are no significant differences between the mean ADR, ADG and ADB values of *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids and *M. m. musculus* x *M. m. domesticus* F1 hybrids (Table 5.19). T-test results for comparisons between *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 and *M. m. musculus* show that there are significant differences between mean ADR (p=0.02) and ADB (p=0.03) values but not between ADG values (Table 5.19). The *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have larger mean ADR, ADG and ADB values than both *M. m. musculus* x *M. m. domesticus* F1 hybrids and *M. m. domesticus*, which indicates that on average they have a lighter dorsal coat colour (Table 5.17). They are not, however, significantly different from *M. m. musculus* x *M. m. domesticus* F1 hybrid for dorsal coat colour (p=0.15) but are significantly different from *M. m. domesticus*, this is significant at the Bonferroni corrected p-value of 0.016 (p<0.01; Table 5.18). T-test results show that there are no significant differences between mean ADR, ADG and ADB values in comparisons between *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. musculus* x *M. m. domesticus* F1 hybrids (Table 5.19). There are also no significant differences in ADR, ADG and ADB values between the *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. domesticus* (Table 5.19). The *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have mean ADR, ADG and ADB values which are larger than the expected MPV (Table 5.17).

For the ventral coat the *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids have a larger mean AVR value than the *M. m. musculus* x *M. m. domesticus* F1 hybrids, but smaller mean AVG and AVB values, which could indicate a darker ventral coat on average in the B1 hybrids (Table 5.17). However, this

difference is not significant (p=0.20; Table 5.18). When compared *M. m. musculus* the *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 has lower mean AVR and AVG values with slightly larger mean AVB values (Table 5.17), indicating that *M. m. musculus* has a lighter ventral coat on average than the B1 hybrids. This difference is significant at the Bonferroni corrected p-value of 0.012 (p<0.01; Table 5.18). Pairwise t-tests between *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids and *M. m. musculus* x *M. m. domesticus* F1 hybrids show that there are no significant differences between AVR and AVG values while there are significant differences between AVB values (p=0.02; Table 5.19). Pairwise comparisons using t-tests show that there are significant differences in mean AVR at the Bonferroni corrected p-value of 0.012 (p<0.01) values between *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids and *M. m. musculus,* but not between AVG and AVB values (Table 5.19). The *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have larger mean AVR and slightly smaller mean AVB and AVG values than the *M. m. musculus* x *M. m. domesticus* F1 parent (Table 5.17), the overall difference in ventral coat colour is not significant (0.15; Table 5.18). Pairwise t-tests show that there are no significant differences in mean AVR, AVG and AVB values between the *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. musculus* x *M. m. domesticus* F1 hybrids (Table 5.19). When compared to *M. m. domesticus* the *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have larger mean AVR, AVG and AVB values indicating a lighter ventral coat colour than *M. m. domesticus* (Table 5.17) with the difference being significant (p<0.01; Table 5.19). Pairwise comparisons using t-tests indicate that there are significant differences in AVR, AVG and AVB, but this is not significant at the Bonferroni corrected p-value of 0.012(p<0.05) values between *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. domesticus* (Table 5.19). The standard deviation for the AVR, AVG and AVB measurements of the *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids are much larger than that of either parent with it being twice as large as the SD values of the original parental populations (Table 5.17), indicating that there is a great deal of variation ventral coat colour in backcrosses into the *M. m. domesticus* parental population.

PCA results for the *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids, *M. m. musculus* x *M. m. domesticus* F1, *M. m. musculus* and *M. m. domesticus* are represented in Figure 5.3A and Table 5.20. PC1 is represented on the x-axis of Figure 5.3A. It represents 76.8% of the variation, with the ventral coat measurements having large negative loadings (AVR (-0.61), AVG(-0.49),(-0.39)) and positive scores indicating grey brown ventral coat colour, becoming darker as the score increases, and negative scores indicating a lighter ventral coat, becoming more yellow/agouti as you move to the extreme negative

scores of PC1. The dorsal coat has slightly smaller negative loadings (ADR(-0.32), ADG(-0.27),ADB(-0.39)); again, the more positive the score the darker brown the dorsal coat is, and the more negative the score the lighter the dorsal coat is (moving closer to a lighter chestnut brown colour). Most of the variation in coat colour is due to variation in ventral coat colour. PC1 represents the overall lightness or darkness of the dorsal and ventral coat. *M. m. musculus* has the lightest dorsal and ventral coat colour with the slightly larger ADR, ADG, ADB values and much larger AVR, AVG and AVB values having negative PC1 scores. *M. m. domesticus* has the darker coat and smaller ADR, ADG, ADB, AVR, AVG and AVB values having larger positive scores along this axis. Variation in ventral coat colour accounts for most of the variation along PC1. The *M. m. musculus* x *M. m. domesticus* F1 hybrids overlap with both parents along this axis, having a wider range of coat colours, as do the *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids. PC2 (y-axis) accounts for 12.8% of the variation, with the dorsal coat measurements have negative loadings along this axis (ADR(-0.48),ADG(-0.48),ADB(-0.41)) and the ventral coat measurements have positive loadings (AVG(0.26),AVB(0.53)). *M. m. domesticus* and *M. m. musculus* overlap along this axis, while the *M. m. musculus* x *M. m. domesticus* F1 hybrid, which has a larger AVG and AVB values, separates from the parental groups along the y-axis due to its lighter ventral coat colour (cream colour as opposed the yellow/agouti colour of the *M. m. musculus* parent). Thus as the scores become more positive, the ventral coat becomes lighter in colour moving from a yellow/agouti/brown colour to a cream colour. The *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids overlap with both the parental groups and the *M. m. musculus* x *M. m. domesticus* F1 parents along this axis. The *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids occupy the region where there is overlap between the original parental populations and the *M. m. musculus* x *M. m. domesticus* F1 hybrids (Figure 5.3A).

When looking at the at the PCA results for the *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids PC1 accounts for 77.9% of the variation and is represented by the x-axis in Figure 5.3B. Again the ventral measurements have large negative loadings (AVR(-0.58),AVG(-0.51),AVB(-0.5)), while the dorsal values have smaller negative loadings (ADR(-0.28),ADG(-0.29),ADB(-0.17)) for PC1. As the PC1 scores become more negative the dorsal coat colour becomes a lighter shade of brown and the ventral coat colour becomes more of a yellow/agouti colour, while the dorsal coat becomes a darker brown when the PC scores become more positive, and the ventral coat becomes a brown grey colour as the PC scores become more positive. Again, the original parental groups *M. m. musculus* and and *M. m. domesticus* separate along the x-axis, *M. m. musculus*, with the lighter dorsal and a much lighter ventral coat, has negative PC scores for PC1, and *M. m. domesticus,* with a darker dorsal and ventral coat, has

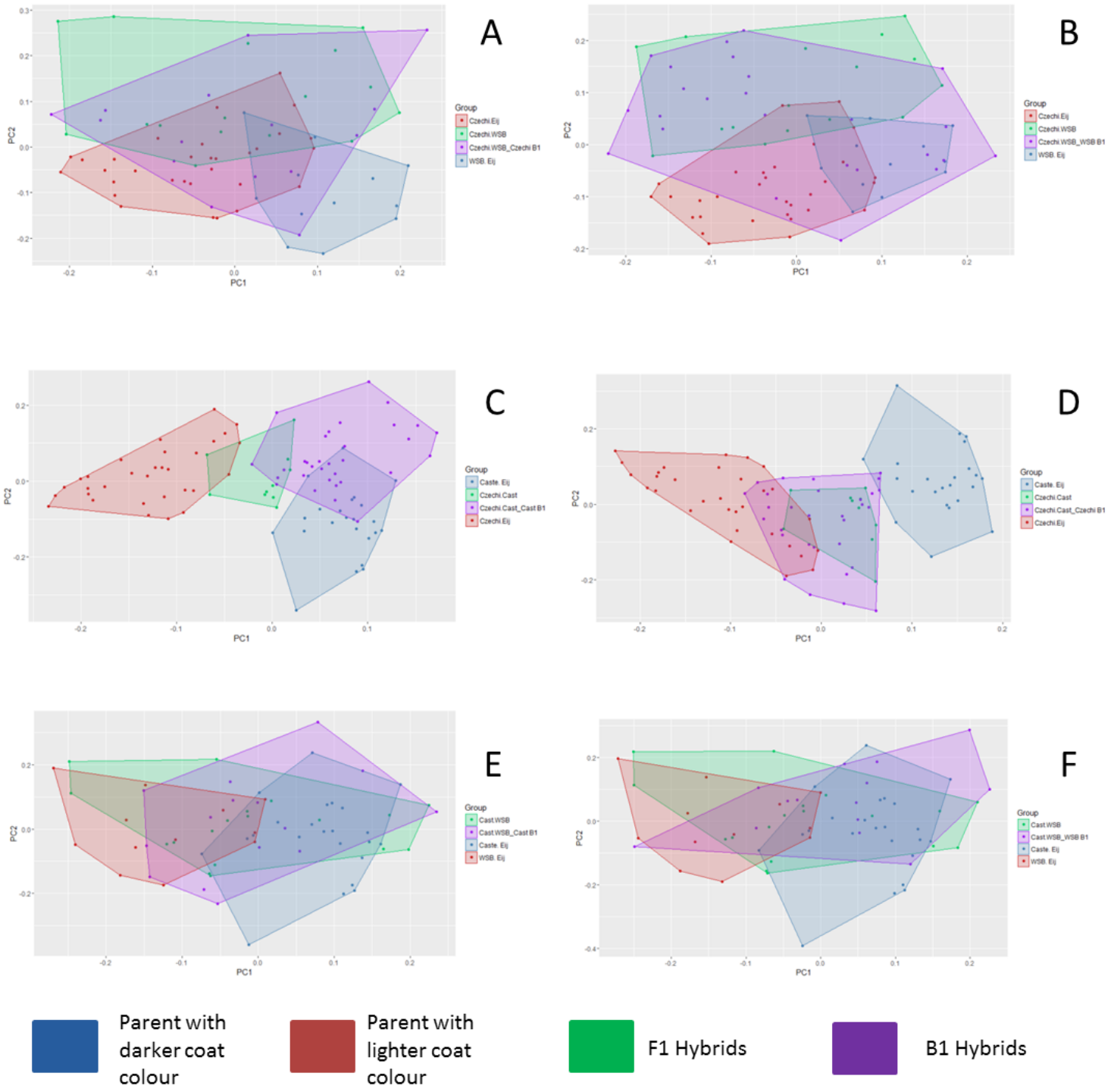

Figure 5.3: PCA based on average dorsal and ventral colour measurements of parents, F1 and B1 hybrids. (A) Distribution of Czechi.eij and WSB.Eij parents and their F1 and Czechi.WSB_Czechi B1 hybrids according to the first two principal components. (B) Distribution of Czechi.eij and WSB.Eij parents and their F1 and Czechi.WSB_WSB B1 hybrids according to the first two principal components. (C) Distribution of Czechi.eij and Cast.Eij parents and their F1 and Czechi.Cast_Cast B1 hybrids according to the first two principal components. (D) Distribution of Czechi.eij and Cast.Eij parents and their F1 and Czechi.Cast_Czechi B1 hybrids according to the first two principal components. (E) Distribution of WSB.Eij and Cast.Eij parents and their F1 and Cast.WSB_Cast B1 hybrids according to the first two principal components. (F) Distribution of WSB.Eij and Cast.Eij parents and their F1 and Cast.WSB_WSB B1 hybrids according to the first two principal components. *M. m. casteneaus* = Cast.Eij, *M. m. domesticus* = WSB.Eij, *M. m. musculus* = Czechi.Eij.

Average R,G,B values	Parents						F1 Hybrid			B1 Hybrids						Expected MPV	
	M. m. musculus			*M. m. domesticus*			*M. m. musculus* x *M. m. domesticus*			*M. m. musculus* x *M. m. domesticus_M. m. musculus* B1			*M. m. musculus* x *M. m. domesticus_M. m. domesticus* B1				
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	*M. m. musculus* x *M. m. domesticus_M. m. musculus* B1	*M. m. musculus* x *M. m. domesticus_M. M. domesticus* B1
ADR	32	105.2	14.42	21	90.44	12.66	31	93.54	15.34	19	91.68	20.6	28	96.43	19.89	99.37	91.99
ADG	32	91.78	14.39	21	84.98	12	31	83.64	15.11	19	80.76	14.1	28	87.38	17.88	87.71	84.31
ADB	32	59.47	13.15	21	63.22	10.13	31	57.05	14.82	19	52.55	18.3	28	64.01	13.94	58.26	60.13
AVR	42	212.8	16.95	32	159.5	17.08	18	184.7	28.69	34	194.7	29	32	186.3	31.89	198.75	172.1
AVG	42	186	16.64	32	155	15.48	18	181.9	24.85	34	179.6	26	32	180.5	32.22	183.95	168.45
AVB	42	137.1	18.34	32	126.4	15.57	18	157.9	22.38	34	140	27.2	32	156.7	38.28	147.5	142.15
Average R,G,B values	*M. m. musculus*			*M. m. castaneus*			*M. m. castaneus* x *M. m. musculus* F1			*M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1			*M. m. castaneus* x *M. m. musculus M. m. musculus* B1			*M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1	*M. m. castaneus* x *M. m. musculus_ M. m. musculus* B1
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD		
ADR	32	105.19	14.42	32	76.18	15.12	20	84.9	15.56	38	64.67	15.43	34	84.92	13.58	80.54	95.04
ADG	32	91.78	14.39	32	68.8	14.48	20	75.61	16.52	38	56.93	13.17	34	73.66	12.9	72.20	83.70
ADB	32	59.47	13.15	32	47.13	11.48	20	51.32	13.92	38	39.93	7.876	34	48.66	11.14	49.22	55.40
AVR	42	212.8	16.95	29	126.9	11.58	18	166	23.18	36	143.5	15.91	32	178.9	17.96	146.45	189.4
AVG	42	186	16.64	29	124.8	10.16	18	150.1	19.06	36	135.1	13.24	32	162.2	11.26	137.45	168.05
AVB	42	137.1	18.34	29	96.66	8.38	18	107.2	17.11	36	100.2	14.53	32	121.4	14.11	101.92	122.14
Average R,G,B values	*M. m. castaneus*			*M. m. domesticus*			*M. m. castaneus* x *M. m. domesticus*			*M. m. castaneus* x *M. m. domesticus_M. m. domesticus* B1			*M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1			*M. m. castaneus* x *M. m. domesticus_M. m. domesticus* B1	*M. m. castaneus* x *M. m. domesticus_ M. m. castaneus* B1
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD		
ADR	32	76.18	15.12	21	90.44	12.66	25	80.86	20.01	17	74.64	15.4	19	83.25	15.27	85.65	78.52
ADG	32	68.8	14.48	21	84.98	12	25	73.36	18.47	17	69.41	13.2	19	76.53	15.13	79.17	71.08
ADB	32	47.13	11.48	21	63.22	10.13	25	49.92	14.17	17	49.64	10.1	19	54.76	11.24	56.57	48.525
AVR	29	126.9	11.58	32	159.5	17.08	24	145.5	18.56	23	141.9	26.8	32	140.3	12.94	152.5	136.2
AVG	29	124.8	10.16	32	155	15.48	24	140.3	16.37	23	139.3	24.6	32	137.3	11.6	147.65	132.55
AVB	29	96.66	8.38	32	126.4	15.57	24	109.6	18.48	23	115	23.6	32	111.7	12.94	118	103.13

Table 5.17: Mean, and SD for measurements of ventral AVR, AVG, AVB and dorsal ADR, ADG and ADB and n for parents, F1 and B1 hybrids as well as expected MPV for crosses between F1 hybrids and parental populations.

Comparison	Dorsal Coat	Ventral Coat
	p value	p value
M. m. musculus x *M. m. domesticus* _*M. m. musculus* B1 v *M. m. musculus* x *M. m. domesticus* F1	0.25	P<0.01
M. m. musculus x *M. m. domesticus* _*M. m. musculus* B1 v *M. m. musculus*	P<0.01	P<0.01
M. m. musculus x *M. m. domesticus* _*M. m. musculus* B1 v *M. m. domesticus*	P<0.01	P<0.01
M. m. musculus x *M. m. domesticus* _*M. m.domesticus* B1 v *M. m. musculus* x *M. m. domesticus* F1	0.15	0.20
M. m. musculus x *M. m. domesticus* _*M. m. domesticus* B1 v *M. m. musculus*	P<0.01	P<0.01
M. m. musculus x *M. m. domesticus* _*M. m. domesticus* B1 v *M. m. domesticus*	P<0.01	P<0.01
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. castaneus*x *M. m. musculus* F1	p<0.01	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. musculus*	p<0.01	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. castaneus*	p<0.01	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. castaneus*x *M. m. musculus* F2	p<0.01	p<0.01
*M. m. castaneus*x *M. m. musculus* _*M. m. musculus* B1 v *M. m. castaneus*x *M. m. musculus* F1	0.3518	p<0.05
*M. m. castaneus*x *M. m. musculus* _*M. m. musculus* B1 v *M. m. castaneus*	p<0.01	p<0.01
*M. m. castaneus*x *M. m. musculus* _*M. m. musculus* B1 v *M. m. musculus*	p<0.01	p<0.01
*M. m. castaneus*x *M. m. musculus* _*M. m. musculus* B1 v *M. m. castaneus*x *M. m. musculus* F2	p<0.01	p<0.01
*M. m. castaneus*x *M. m. domesticus*_*M. m. domesticus* B1 v *M. m. castaneus*x *M. m. domesticus* F1	p<0.05	p<0.01
M. m. castaneus x *M. m. domesticus*_*M. m. domesticus* B1 v *M. m. castaneus*	p<0.05	p<0.01
M. m. castaneus x *M. m. domesticus*_*M. m. domesticus* B1 v *M. m. domesticus*	p<0.01	p<0.01
M. m. castaneus x *M. m. domesticus*_*M. m. domesticus* B1 v *M. m. castaneus*x *M. m. domesticus* F2	p<0.01	p<0.05
M. m. castaneus x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus*x *M. m. domesticus*	0.06	p<0.05
M. m. castaneus x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. domesticus*	0.10	p<0.01
M. m. castaneus x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus*	p<0.05	p<0.01
*M. m. castaneus*x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus* x *M. m. domesticus* F2	0.16	p<0.01

Table 5.18: Results for MANOVA (p values) used to determining if there is a significant difference in average dorsal coat colour and average ventral coat colour between B1 hybrids and their respective parental populations as well as F2 hybrids were applicable.

Comparison	ADR	ADG	ADB	AVR	AVG	AVB
	p value	p value	p value	p value	p value	p value
M. m. musculus x *M. m. domesticus*_*M. m. musculus* B1 v *M. m. musculus* x *M. m. domesticus* F1	0.66	0.5	0.15	0.24	0.76	0.02
M. m. musculus x *M. m. domesticus*_*M. m. musculus* B1 v *M. m. musculus*	0.02	0.03	0.09	p<0.01	0.22	0.6
M. m. musculus x *M. m. domesticus*_*M. m. musculus* B1 v *M. m. domesticus*	0.82	0.4	0.01	p<0.01	p<0.01	0.02
M. m. musculus x *M. m. domesticus*_*M. m. domesiticus* B1 v *M. m. musculus* x *M. m. domesticus* F2	0.61	0.46	0.13	0.86	0.86	0.9
M. m. musculus x *M. m. domesticus*_ *M. m. domesiticus* B1 v *M. m. domesticus*	0.21	0.58	0.82	p<0.01	p<0.01	p<0.01
M. m. musculus x *M. m. domesticus*_ *M. m. domesiticus* B1 v *M. m. musculus*	0.06	0.3	0.2	p<0.01	0.38	0.01
*M. m. castaneus*x *M. m. musculus*_*M. m. castaneus* B1 v *M. m. castaneus*x *M. m. musculus* F1	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	0.05
*M. m. castaneus*x *M. m. musculus*_*M. m. castaneus* B1 v *M. m. castaneus*	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	0.21
*M. m. castaneus*x *M. m. musculus*_*M. m. castaneus* B1v *M. m. musculus*	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
*M. m. castaneus*x *M. m. musculus*_*M. m. castaneus* B1 v *M. m. castaneus*x *M. m. musculus* F2	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
*M. m. castaneus*x *M. m. musculus*_*M. m. musculus* B1 v *M. m. castaneus*x *M. m. musculus* F1	0.43	0.25	0.21	0.11	p<0.05	p<0.01
*M. m. castaneus*x *M. m. musculus*_*M. m. musculus* B1 v *M. m. musculus*	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
*M. m. castaneus*x *M. m. musculus*_*M. m. musculus* B1 v *M. m. castaneus*	0.19	0.76	0.67	p<0.01	p<0.01	p<0.01
*M. m. castaneus*x *M. m. musculus*_*M. m. musculus* B1 v *M. m. castaneus*x *M. m. musculus* F2	p<0.05	p<0.01	p<0.01	0.12	p<0.01	p<0.01
*M. m. castaneus*x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus*x *M. m. domesticus* F1	0.43	0.27	0.06	0.4	0.66	0.41
*M. m. castaneus*x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus*	0.1	p<0.05	0.01	p<0.01	p<0.01	p<0.01
*M. m. castaneus*x *M. m. domesticus*_*M. m. castaneus* v *M. m. domesticus*	0.35	0.2	0.06	p<0.01	p<0.01	p<0.01
*M. m. castaneus*x *M. m. domesticus*_*M. m. castaneus* v *M. m. castaneus*x *M. m. domesticus* F2	0.04	0.08	0.12	p<0.01	p<0.01	p<0.01
*M. m. castaneus*x *M. m. domesticus*_ *M. m. domesiticus* B1 v *M. m. castaneus*x *M. m. domesticus* F1	0.62	0.07	0.55	0.24	0.55	0.62
*M. m. castaneus*x *M. m. domesticus*_ *M. m. domesiticus* B1 v *M. m. domesticus*	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.05
*M. m. castaneus*x *M. m. domesticus*_ *M. m. domesiticus* B1 v *M. m. castaneus*	0.21	0.47	0.99	0.06	0.03	p<0.01
*M. m. castaneus*x *M. m. domesticus*_ *M. m. domesiticus* B1 v *M. m. castaneus*x *M. m. domesticus* F2	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01

Table 5.19: Results for pairwise t tests to determine if there are significant differences in mean ADR, ADG, ADB, AVR, AVG and AVB between B1 hybrids, original parental groups, F1 hybrids and F2 hybrids.

positive PC scores along this axis. The *M. m. musculus* x *M. m. domesticus* F1 hybrids overlap with both parental groups along the x-axis as do the *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids, with some of the B1 hybrids being at the extreme ends of both parental groups. PC2 (y-axis) represents 11.8% of the variation and the dorsal coat measurements have negative loadings along this axis (ADR(-0.44),ADB(-0.38),ADG(-0.21)); while the AVR ventral measurement has a negative loading (-0.31), AVG has a small positive loading (0.15) and AVB has a large positive loading (0.71). Negative PC2 scores indicate a brown or yellow ventral coat represented by the parental group while positive scores are associated with larger AVB values which are the result of the cream coloured ventral coat seen in F1 hybrids and B1 hybrids. Negative PC2 scores are also associated with a lighter brown dorsal coat with positive PC scores associated with the darker dorsal coat of the the F1 hybrids which separate from the parents along the y-axis. The *M. m. musculus* x *M. m. domesticus* F1 hybrids again separate from the parents along this axis mainly due to the difference in ventral coat colour of the *M. m. musculus* x *M. m. domesticus* F1 hybrids which have larger AVB values in relation to the AVR and AVG. The *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids overlap with both parents along the y-axis represented by PC2, however the hybrids tend to cluster in the same space as *M. m. domesticus* or *M. m. musculus* x *M. m. domesticus* F1 hybrids with very few *M. m. musculus* x *M. m. domesticus_M. m. domesticus* B1 hybrids occupying the intermediate region in terms of morphology (Figure 5.3B).

M. m. castaneus x *M. m. musculus* B1 hybrids

The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have smaller mean ADR, ADG and ADB values than when compared to *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 5.17) the difference in dorsal colour is significant ($p<0.01$; Table 5.18). Pairwise comparisons between *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids and *M. m. castaneus* x *M. m. musculus* F1 hybrids indicate that there are significant differences in mean ADR, ADG and ADB values ($p<0.01$; Table 5.19). This indicates that the B1 hybrids had a darker dorsal coat than the F1 parents. The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids also had smaller average ADR, ADG and ADB values than *M. m. castaneus* and this difference is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.17 and 5.18). Pairwise comparisons between *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids and *M. m. castaneus* show that there is a significant differences in ADR, ADG and ADB mean values between the two ($p<0.01$; Table 5.19). This indicates that the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have darker dorsal coats on average than both parental groups, they thus have

smaller ADR, ADG and ADB values than the expected MPV(Table 5.17). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids also have smaller average ADR, ADG and ADB values than *M. m. musculus* and the difference in dorsal colour is significant the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.17 and 5.18). Pairwise comparisons between *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids and *M. m. musculus* using t-tests show that there are significant differences in ADR, ADG and ADB mean values, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 10).The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids also have smaller mean ADR, ADG and ADB values than the *M. m. castaneus* x *M. m. musculus* F1 hybrids, however they are not significantly different ($p= 0.35$; Table 5.17 and 5.18). Pairwise comparisons between *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 and *M. m. castaneus* x *M. m. musculus* F1 hybrids indicate that there are no significant differences in ADR, ADG and ADB values (Table 5.19). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have smaller mean ADR, ADG and ADB values than the expected MPV (Table 5.17). The B1 hybrids are on average darker in terms of dorsal coat colour than the parental groups for the *M. m. castaneus* x *M. m. musculus* B1 backcrossed hybrids.

When looking at the ventral coat colour the *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have smaller AVR, AVG and AVB values than the *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 5.17), which indicates that they have a darker ventral coat than the F1 hybrids; the difference is significant ($p<0.01$; Table 5.18). Pairwise comparisons using t-tests show that there are significant differences in AVR, AVG and AVB, this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$) values between the *M. m. castaneus* x *M. m. musculus* F1 hybrids and the *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids (Table 5.19). When compared to *M. m. musculus* the *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have smaller mean AVR, AVG and AVB values (Table 5.17) indicating that they have darker ventral coat colours than *M. m. musculus*; this difference is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.18). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have mean AVR, AVG values which are smaller than the expected MPV while having AVB values similar to the expected MPV (Table 5.17). When looking at the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids they have a darker ventral coat colour than the *M. m. castaneus* x *M. m. musculus* F1 hybrids with smaller AVR, AVG and AVB values (Table 5.17) and the difference in colour is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.18). Pairwise comparisons between the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids and *M. m. musculus* indicate that there are significant differences in mean AVR, AVG and AVB values ($p<0.01$;

Table 5.19). When compared to the *M. m. castaneus* parents the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have larger mean AVR, AVG and AVB values indicating that they have a significantly lighter ventral coat, this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.17 and 5.18). Pairwise comparisons between mean *M. m. castaneus* and *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids indicate that there are significant differences in mean AVR, AVG and AVB values, this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.19). The mean AVR, AVG and AVB values for the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids are similar to the expected MPV (Table 5.17).

The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids thus have a darker dorsal coat than all the parental groups (including the *M. m. castaneus* x *M. m. musculus* F1 hybrids) while having a lighter ventral coat than *M. m. castaneus*, thus looking slightly different than all the parental groups, by having a larger dorsal ventral contrast but with a darker dorsal and ventral coat. PCA results for the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids, plotted with both original 'purebred' parental groups and the *M. m. castaneus* x *M. m. musculus* F1 hybrid parent, are displayed in Figure 5.3C and Table 5.20. The x-axis in Figure 5.3C represents PC1 which accounts for 87.2% of the variation. For PC1 ventral measurements have large negative loadings (AVR(-0.64), AVB(-0.5),AVG(-0.42)), with negative PC scores associated with a yellow/agouti ventral coat of *M. m. musculus* while positive PC scores are associated with the darker brown ventral coat of *M. m. castaneus*. The dorsal coat has smaller negative loadings (ADR(-0.31),ADG(-0.27),ADB(-0.16)), with negative scores associated with the lighter brown chestnut coloured dorsal coat of *M. m. musculus* and positive PC scores associated with the darker brown dorsal coat of *M. m. castaneus*. The x-axis represents the overall lightness or darkness of the dorsal and ventral coat and colour variation in ventral coat colour accounts for most of the variation. The *M. m. musculus* parent with lighter brown overall dorsal coat and a much lighter yellow/agouti ventral coat resulting in larger ADR, ADG, ADB, AVR, AVG and AVB values has negative scores for PC1. *M. m. musculus* separates from *M. m. castaneus* on the x-axis which has the darker brown dorsal coat and an only slightly lighter ventral coat with smaller ADR, ADG, ADB, AVR, AVG and AVB values, and thus having positive scores for PC1. The *M. m. castaneus* x *M. m. musculus* F1 hybrids have an intermediate phenotype with ventral coats which are a dark mustard brown colour a dorsal coat which is brown in colour, F1 hybrids occupie the intermediate space along PC1. The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids overlap with *M. m. castaneus* and on slightly with the darker *M. m. castaneus* x *M. m. musculus* F1 hybrids along PC1 due to the fact that it has a darker dorsal coat colour and ventral coat which is a light brown and does

not have the yellow/agouti/mustard brown colour of the *M. m. castaneus* x *M. m. musculus* F1 parents. PC2, which accounts for 8.8% of the variation and is represented by the y-axis of Figure 5.3C, the dorsal coat measurements have larger negative loadings on this axis (ADR(-0.54),ADG-(0.57)),ADB(-0.47)), while the ventral coat has smaller positive loadings (AVR(0.37), AVB(0.15)). The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids overlap with *M. m. musculus* and the *M. m. castaneus* x *M. m. musculus* F1 parent on the y-axis. PC2 is representative of the DVC with smaller PC scores associated with a smaller DVC and larger scores associated with larger DVC. The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids overlap with the *M. m. castaneus* x *M. m. musculus* F1 hybrids and *M. m. musculus* along the y- axis. This is because it has a larger DVC while the parent with a smaller DVC which is *M. m. castaneus* separates from the other groups along this axis.

PCA results for the *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids compared to both parental groups and the F1 parents are displayed in Figure 5.3D and Table 5.20. The x-axis in Figure 5.3D represents PC1 which accounts for 82.2% of the variation, again this represents the overall lightness or darkness of the dorsal and ventral coat.The ventral coat accounts for most of the variation. Negative PC scores are associated with lighter brown dorsal and ventral coat colour as above and positive scores are associated with darker colours. For PC1 the ventral coat measurements have large negative loadings (AVR(-0.65),AVB(-0.52),AVG(-0.42)), and the dorsal coat measurements have small negative loadings (ADR(-0.25),ADG(-0.21),ADB(-0.14)). The two parental groups occupy opposite ends of the graph on x-axis, the lighter *M. m. musculus* has larger mean dorsal and ventral coat colour measurements thus negative PC1 scores and *M. m. castaneus* which has a darker brown dorsal and ventral coat has positive PC1 scores. The *M. m. castaneus* x *M. m. musculus* F1 hybrids occupy intermediate region with intermediate dorsal and ventral values when compared the parental groups on the x-axis. The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids overlap with *M. m. castaneus* x *M. m. musculus* F1 hybrids along the x-axis and overlap with *M. m. musculus*. PC2 accounts for 8.9% of the variation the dorsal coat measurements have large negative loadings (ADR(-0.545),ADG(-0.57),ADB(-0.47)), and the ventral coat measurements have positive loadings (AVR(0.37),AVG(0.15)). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids once again overlap with *M. m. castaneus* x *M. m. musculus* F1 hybrids along this axis as well as the *M. m. musculus* parent.

***M. m. musculus* x *M. m. domesticus* B1 backcrosses**												
M. m. musculus x *M. m. domesticus_M. m. musculus* B1							*M. m. musculus* x *M. m. domesticus_M. M. domesticus* B1					
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
SD	44.59	18.23	15.51	4.65	2.04	1.37	49.2	19.2	16.92	4.78	2.063	1.42
Proportion of Variance	0.77	0.13	0.09	0.001	0	0	0.78	0.12	0.09	0.007	0.001	0
Cumulative Proportion	0.77	0.90	0.99	0.99	1	1	0.78	0.9	0.99	0.99	0.999	1
Loadings												
Colour measurement	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
ADR	-0.32	-0.48	0	0.63	0	-0.51	-0.28	-0.4	0.29	0.61	0.16	-0.5
ADG	-0.29	-0.48	-0.24	0	0.12	0.78	-0.25	-0.4	0.41	0	0	0.78
ADB	-0.19	-0.41	-0.37	-0.73	0	-0.34	-0.17	-0.2	0.50	-0.74	0	-0.35
AVR	-0.61	0	0.65	-0.23	0.38	0	-0.57	-0.3	-0.61	-0.25	0.38	0
AVG	-0.5	0.27	0	0	-0.8	0.10	-0.51	0.15	-0.15	0	-0.82	-0.1
AVB	-0.39	0.539	-0.62	0	0.41	0	-0.5	0.71	0.308	0	0.38	0
***M. m. castaneus* x *M. m. musculus* B1 backcrosses**												
M. m. castaneus x *M. m. musculus_M. m. musculus* B1							*M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1					
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
SD	50.67	18.70	12.93	4.99	3.34	1.19	57.66	18.4	10.36	4.68	3.63	2.037
Proportion of Variance	0.82	0.11	0.05	0.01	0	0.00	0.87	0.09	0.03	0.01	0.00	0.00
Cumulative Proportion	0.82	0.93	0.99	0.99	1	1	0.87	0.96	0.99	0.99	0.99	1
Loadings												
Colour measurement	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
ADR	-0.25	-0.54	-0.25	0.57	0	0.50	-0.31	-0.5	-0.25	0.58	0	0.50
ADG	-0.21	-0.57	0	0	0	-0.79	-0.27	-0.6	0	0	0	-0.79
ADB	-0.14	-0.47	0	-0.79	0	0.36	-0.16	-0.5	0	-0.79	0	0.36
AVR	-0.65	0.378	-0.51	-0.15	0.37	0	-0.65	0.37	-0.51	-0.15	0.4	0
AVG	-0.52	0.15	0.12	0	-0.82	0	-0.5	0.15	0.12	0	-0.83	0
AVB	-0.41	0	0.81	0.15	0.41	0	-0.42	0	0.81	0.15	0.38	0
***M. m. castaneus* x *M. m. domesticus* B1 backcrosses**												
*M. m. castaneus*x *M. m. domesticus_M. m. castaneus* B1							*M. m. castaneus* x *M. m. domesticus_M. M. domesticus* B1					
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
SD	34.67	16.12	6.35	3.63	1.94	1.18	33.58	16.9	6.32	3.25	1.55	1.29
Proportion of Variance	0.79	0.17	0.026	0.01	0	0.00	0.77	0.19	0.03	0.01	0.00	0.00
Cumulative Proportion	0.79	0.96	0.99	0.99	1	1	0.77	0.96	0.99	0.99	0.99	1
Loadings												
Colour measurement	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
ADR	-0.26	-0.59	0	0.571	0	0.50	-0.25	-0.6	0	0.55	0	-0.55
ADG	-0.25	-0.56	0	0	0	-0.79	-0.24	-0.6	0	0	0.13	0.77
ADB	-0.2	-0.42	-0.11	-0.8	-0.1	0.34	-0.2	-0.4	-0.14	-0.81	0	-0.29
AVR	-0.58	0.24	0.61	-0.14	0.47	0	-0.58	0.23	0.59	0	-0.49	0.11
AVG	-0.54	0.22	0	0.11	-0.8	0	-0.53	0.23	0	0	0.80	-0.13
AVB	-0.46	0.25	-0.78	0	0.33	0	-0.47	0.24	-0.79	0.142	-0.29	0

Table 5.20: PCA results for parents, F1 hybrids and B1 hybrids.

M. m. castaneus x *M. m. domesticus* B1 hybrids

The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have smaller mean ADR, ADG and ADB values than *M. m. domesticus* and the *M. m. castaneus* x *M. m. domesticus* F1 parents thus they have darker brown dorsal coats than both parental groups, this difference is significant ($p<0.05$; Table 5.17 and 5.18). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. castaneus* x *M. m. domesticus* F1 hybrids indicate that there are no significant differences in ADR, ADG and ADB values (Table 5.19). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. domesticus* show that there are significant differences in mean ADR, ADG and ADB values, this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.19). The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have smaller ADR, ADG and ADB values than the expected MPV (Table 5.17). The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids have larger mean ADR, ADG and ADB values than both *M. m. castaneus* and the *M. m. castaneus* x *M. m. domesticus* F1 hybrids and thus on average have lighter dorsal coats than their parents (Table 5.18), they are not significantly different from the *M. m. castaneus* x *M. m. domesticus* F1 hybrids ($p=0.06$), but they are significantly different from *M. m. castaneus* ($p<0.05$; Table 5.18). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids and *M. m. castaneus* x *M. m. domesticus* F1 hybrids indicate that there are no significant differences in mean ADR, ADG and ADB values (Table 5.19). Pairwise comparisons between the *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids and *M. m. castaneus* show that there are significant differences between mean ADG, and ADB ($p<0.05$) values but not mean ADR values ($p=0.1$; Table 5.19). The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids are not significantly different from the other original parental group *M. m. domesticus* for dorsal coat colour ($p=0.10$; Table 5.18), but they have smaller mean ADR, ADG and ADB values (Table 5.17). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids and *M. m. domesticus* parents show that there are no significant differences in mean ADR, ADG and ADB values (Table 5.19). *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids have larger ADR, ADG and ADB values than the expected MPV (Table 5.17).

The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids have larger mean AVR, AVG and AVB values than *M. m. castaneus* and the difference is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.17 and 5.18). Pairwise comparisons between *M. m. castaneus* and *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids using t-tests show that they have significant differences in mean AVR, AVG and AVB values ($p<0.01$; Table 10). They have smaller mean AVR and AVG values than

M. m. castaneus x *M. m. domesticus* F1 hybrids and slightly larger mean AVB values (Table 5.17), indicating that they have on average a darker ventral coats than their F1 parents. The difference is significant ($p<0.05$; Table 5.18). Pairwise comparisons between *M. m. castaneus* x *M. m. domesticus* F1 hybrids and *M. m. castaneus x M. m. domesticus_M. m. castaneus* B1 hybrids show that there are significant differences in ADR, ADG and ADB values, these are significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.19). The *M. m. castaneus x M. m. domesticus_M. m. castaneus* B1 hybrids have larger mean AVR, AVG and AVB values than the expected MPV (Table 5.17). The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have smaller average AVR, AVG and AVB values than both *M. m. castaneus* x *M. m. domesticus* F1 hybrids and *M. m. domesticus* (Table 5.17), which indicates that they also have darker ventral coats, these differences are significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.18). Pairwise comparisons between the *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. domesticus* indicate that there are significant differences in AVR, AVB and AVG values ($p<0.05$; Table 5.19). Pairwise comparisons indicate that there are no significant differences in AVR, AVG and AVB values between the *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids and *M. m. castaneus* x *M. m. domesticus* F1 hybrids (Table 5.19). The *M. m. castaneus* x *M. m. domesticus_M. m. domesticus* B1 hybrids have AVR, AVG and AVB values smaller than the expected MPV (Table 5.17). The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have a much larger SD for the ventral coat than either parental group (Table 5.17). The SDs for the ventral coat measurements are once again large for backcrosses into *M. m. domesticus* populations.

The PCA results for the *M. m. castaneus* x *M. m. domesticus _M. m. domesticus* B1 hybrids are displayed in Figure 5.3E and Table 5.20. PC1 represented by the x-axis in Figure 5.3E accounts for 79.5% of the variation, the ventral coat colour measurements have large negative loadings (AVR(-0.57),AVB(-0.529),AVG(-0.46)). Negative PC1 scores areassociated with a brown grey ventral coat and positive scores associated with a brown ventral coat. The dorsal coat colour measurements have small negative loadings (ADR(-0.26),ADG(-0.24),ADB(-0.19)), the negative PC1 scores are associated with a lighter brown dorsal coat and the positive scores associated with darker brown coat. Along this axis the B1 hybrids overlap with *M. m. castaneus* as well as *M. m. domesticus*. PC2 accounts for 19.4% of the variation and is displayed on the y-axis again the dorsal measurements have large negative loadings (ADR(-0.56),ADG(-0.57),ADB(-0.43)) and ventral measurements have small positive loadings (AVR(0.23),AVB(0.22) AVG(0.23)). Variation in the dorsal coat colour accounts for most of the variation in PC2, DVC also pays a role i. The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids overlap with the parents along this axis, but

some B1 hybrids with very dark dorsal coats have large positive PC2 scores and are outside of the range of variation of both parental groups and are different from *M. m. domestics* being backcrossed into.

PCA results for the *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids compared to, the purebred parental groups and the F1 hybrids are displayed in Figure 5.3F and Table 5.20. PC1 accounts for 76% of the variation and is represented by the x-axis (Figure 5.3F). This accounts for overall lightness/darkness of the coat colour, the ventral coat measurements have large negative loadings (AVR(-0.58),AVB(-0.54) AVG(-0.47)), again negative scores mean a brown grey ventral coat colour while positive scores mean a brown coloured ventral coat. The dorsal coat has smaller negative loadings negative loadings (ADR(-0.25),ADG(-0.24),ADB(-0.19)). *M. m. castaneus* and *M. m. domesticus* parents separate along this axis and the *M. m. castaneus* x *M. m. domesticus* F1 hybrids overlap with both parental groups along this axis. *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids overlap with the *M. m. castaneus* parents as well as the *M. m. castaneus* x *M. m. domesticus* F1 hybrids. PC2 accounts for 17% of the variation and is represented by the y-axis, the dorsal coat measurements have large negative loadings (ADR(-0.58),ADG(-0.56),ADB(-0.42)) while the ventral coat has small positive loadings (AVR(0.24),AVG(0.22),AVG(0.25)). *M. m. domesticus* and *M. m. castaneus* largerly overlap along this axis and so do the F1 and *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids.

Dorsal ventral patterning results for B1 hybrids

M. m. musculus x *M. m. domesticus* B1 hybrids

The *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids have larger mean DVR values and smaller mean DVG and DVB values than *M. m. musculus* x *M. m. domesticus* F1 hybrids this difference is significant, at the Bonferroni corrected p-value of 0.012($p<0.01$; Table 5.21 and 5.2). When compared to *M. m. musculus* they have smaller mean DVR values and larger mean DVG and DVB values, the difference is significant, but not at the Bonferroni corrected p-value of 0.012 ($p<0.05$; Table 5.21 and 5.22). Overall the *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids have mean DVR, DVG and DVB values close to the expected MPV (Table 5.21). The *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have smaller mean DVR, DVG and DVB values than *M. m. musculus* x *M. m. domesticus* F1 hybrids this difference is not significant ($p= 0.31$; Table Table 5.21 and 5.22). When compared to *M. m. domesticus*

they have larger DVR, DVG and DVB values, the difference is significant ($p<0.05$; Table Table 5.21 and 5.22). The *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have DVR, DVG and DVB values larger than the expected MPV (Table 5.21).

M. m. castaneus x *M. m. musculus* B1 hybrids

The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have larger mean DVR and DVG values than *M. m. castaneus* x *M. m. musculus* F1 hybrids and smaller mean DVB values (Table 5.21). This difference in DVC is however not significant ($p=0.09$; Table 5.22). When compared to *M. m. castaneus* they have larger DVR, DVG and DVB values and a much larger contrast in dorsal ventral patterning (Table 5.21). This difference is significant, at the Bonferroni corrected p-value of 0.01 ($p<0.01$; Table 5.22). Overall the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have larger mean DVR, DVG and DVB values than the expected MPV (Table 5.22). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have larger mean DVR, DVG and DVB values than *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 12), this difference is not significant ($p=0.17$; Table 5.22). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have smaller mean DVR, DVG and DVB values than *M. m. musculus* indicating that they have a smaller difference in colour between the dorsal and the ventral coat (Table 5.21). This difference is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.22). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have mean DVR, DVG and DVB values which are similar to the expected MPV (Table 12).

M. m. castaneus x *M. m. domesticus* B1 hybrids

The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have a slightly smaller mean DVR and larger mean DVG and DVB values than the *M. m. castaneus* x *M. m. domesticus* F1 hybrids (Table 5.21), this difference is not significant ($p=0.19$; Table 5.22). The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have smaller mean DVR, DVG and DVB values than *M. m. domesticus*, the difference in DVC is significant, but not at the Bonferroni corrected p-value of 0.012 ($p<0.05$; Table 5.22). The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have a slightly smaller mean DVG and DVB values than the expected MPV and a DVR value which is much smaller than the expected DVR MPV (Table 5.21). The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids have slightly

smaller mean DVR and DVG values with slightly larger mean DVB values than *M. m. castaneus* x *M. m. domesticus* F1 hybrids (Table 5.21). The differences in mean DVC between the *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids and *M. m. castaneus* x *M. m. domesticus* F1 hybrids are not signficant (p=0.14; Table 5.22). The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids have larger mean DVR, DVG and DVB values than *M. m. castaneus* (Table 5.21), with the difference in DVC being significant (p<0.05; Table 5.22). Overall the *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids had larger DVR, DVG and DVB values than the expected MPV (Table 12).

Results comparing variance between parents and hybrids

F1 hybrids compared to parental groups

For ventral coat colour, the *M. m. castaneus* has the smallest variance, *M. m. domesticus* has the largest variance and *M. m. musculus* has intermediate variance for AVR, AVG and AVB measurements (Table 5.23). This difference in variance is not significant for comparisons between *M. m. castaneus* and *M. m. domesticus* and between *M. m. domesticus* and *M. m. musculus* (Table 5.24). This difference is significant for comparisons between *M. m. castaneus* and *M. m. musculus* (Table 5.24). The *M. m. castaneus* x *M. m. domesticus* F1 hybrids have the smallest variance in AVG and AVR, while *M. m. castaneus* x *M. m. musculus* F1 hybrids have the smallest AVB variance (Table 5.23). *M. m. castaneus* x *M. m. musculus* F1 hybrids have intermediate AVR and AVG variance while *M. m. musculus* x *M. m. domesticus* F1 hybrids have the largest variance in AVR, AVG and AVB (Table 5.23). Compared to the parental groups, the *M. m. musculus* x *M. m. domesticus* F1 hybrids had larger variance in AVR, AVG and AVB than both parents (Table 5.23). There is a significant difference in variance of AVR values (p<0.05), but not variance of AVG and AVB values (Table 5.24). The *M. m. castaneus* x *M. m. musculus* F1 hybrids have bigger variance in AVR, AVB and AVG than both parents (Table 5.23). This difference is significant in for variance in AVR (p<0.05) and AVB (p<0.01) while not being significant for AVG values (Table 5.24). The *M. m. castaneus* x *M. m. domesticus* F1 hybrids have much larger variance than both parental groups (Table 5.23), the difference is not significant for AVR and AVG but is significant for AVB, however it is not at the Bonferroni corrected p-value of 0.012 (p<0.05; Table 5.24). Overall there is more variation in the ventral coat colour than there is in dorsal coat colour. The F1 hybrids also have larger variances in ventral coat colour compared to the parental groups, but in most cases, this was not significant.

Dorsal ventral contrast values	Parents						F1 Hybrid			B1 Hybrids						Expected mid parental values	
	M. m. musculus			*M. m. domesticus*			*M. m. musculus* x *M. m. domesticus*			*M. m. musculus* x *M. m. domesticus_M. m. musculus* B1			*M. m. musculus* x *M. m. domesticus_M. m. domesticus* B1				
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	*M. m. musculus* x *M. m. domesticus_M. m. musculus* B1	*M. m. musculus* x *M. m. domesticus_M. m. domesticus* B1
DVR	31	108.9	11.8	23	70.04	14.89	16	96.6	18.98	15	104	17.2	25	91.05	22.78	102.77	83.32
DVG	31	96.47	12.4	23	70.48	13.87	16	104	17.94	15	99.4	19.4	25	95.01	30.87	100.135	87.14
DVB	31	80.7	9.53	23	62.58	13.93	16	106	16.77	15	88.3	16.9	25	94.27	23.06	93.35	84.29
Dorsal ventral contrast values	*M. m. musculus*			*M. m. castaneus*			*M. m. castaneus* x *M. m. musculus*			*M. m. castaneus*x *M. m. musculus_M. m. castaneus* B1			*M. m. castaneus*x *M. m. musculus_M. m. musculus* B1			*M. m. castaneus*x *M. m. musculus_M. m. castaneus* B1	*M. m. castaneus*x *M. m. musculus_M. m. musculus* B
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD		
DVR	31	108.9	11.8	23	46.92	15.2	11	76.5	16.47	36	78.9	15.08	30	93.45	18.95	61.71	92.72
DVG	31	96.47	9.53	23	52.25	14.7	11	78.1	8.98	36	78.2	10.64	30	88.25	16.87	65.175	87.28
DVB	31	80.7	12.4	23	46.61	13.56	11	63.7	9.06	36	60.5	10.81	30	72.89	14.41	55.155	72.2
Dorsal ventral contrast values	*M. m. domesticus*			*M. m. castaneus*			*M. m. castaneus*x *M. m. domesticus*			*M. m. castaneus*x *M. m. domesticus_M. m. domesticus* B1			*M. m. castaneus*x *M. m. domesticus_M. m. castaneus* B1			*M. m. castaneus*x *M. m. domesticus_M. m. domesticus* B1	*M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD		
DVR	23	70.04	14.9	23	46.92	15.2	18	60.1	20	14	59.8	13.59	15	58.6	15.4	65.08	53.52
DVG	23	70.48	13.9	23	52.25	14.7	18	63.6	18	14	65.1	11.57	15	63.1	13.9	67.06	57.94
DVB	23	62.58	13.9	23	46.61	13.5	18	58.3	16	14	62	12.77	15	60.9	16.4	60.42	52.43

Table 5.21: Mean, SD for measurements of DVR, DVG, DVB and n for parents, F1 and B1 Hybrids

Comparison	p value
M. m. musculus x *M. m. domesticus* _*M. m. musculus* B1 v *M. m. musculus* x *M. m. domesticus* F1	p<0.01
M. m. musculus x *M. m. domesticus* _*M. m. musculus* B1 v *M. m. musculus*	p<0.05
M. m. musculus x *M. m. domesticus* _*M. m. musculus* B1 v *M. m. domesticus*	P<0.01
M. m. musculus x *M. m. domesticus* _ *M. m. domesticus* B1 v *M. m. musculus* x *M. m. domesticus*	0.3185
M. m. musculus x *M. m. domesticus* _ *M. m. domesticus* B1 v *M. m. musculus*	p<0.01
M. m. musculus x *M. m. domesticus* _ *M. m. domesticus* B1 v *M. m. domesticus*	p<0.05
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. castaneus* x *M. m. musculus* F1	0.093
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. musculus*	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. castaneus*	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. castaneus* B1 v *M. m. castaneus* x *M. m. musculus* F2	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. musculus* B1 v *M. m. castaneus* x *M. m. musculus*	0.17
M. m. castaneus x *M. m. musculus* _*M. m. musculus* B1 v *M. m. castaneus*	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. musculus* B1 v *M. m. musculus*	p<0.01
M. m. castaneus x *M. m. musculus* _*M. m. musculus* B1 v *M. m. castaneus* x *M. m. musculus* F2	p<0.01
M. m. castaneus x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus* x *M. m. domesticus*	0.19
M. m. castaneus x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus*	p<0.01
M. m. castaneus x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. domesticus*	p<0.01
M. m. castaneus x *M. m. domesticus*_*M. m. castaneus* B1 v *M. m. castaneus*x *M. m. domesticus* F2	0.14
M. m. castaneus x *M. m. domesticus*_ *M. m. domesticus* B1 v *M. m. castaneus* x *M. m. domesticus* F1	0.19
M. m. castaneus x *M. m. domesticus*_ *M. m. domesticus* B1 v *M. m. castaneus*	p<0.05
M. m. castaneus x *M. m. domesticus*_ *M. m. domesticus* B1 v *M. m. domesticus*	0.18
M. m. castaneus x *M. m. domesticus*_ *M. m. domesticus* B1 v *M. m. castaneus* x *M. m. domesticus* F2	p<0.05

Table 5.22: MANOVA results for comparisons between B1 hybrids and their respective original parental populations as well as F2 hybrids were applicable.

Variation in dorsal and ventral colour of F2 hybrids

When comparing variances for the dorsal coat,the *M. m. castaneus* x *M. m. musculus* F2 hybrid have larger variation than all the parental groups, including the F1 hybrids for ADR and ADG values, while having smaller variance in ADB than the *M. m. castaneus* x *M. m. musculus* F1 hybrids and *M. m. musculus* (Table 5.23). These differences in variance are not significant (Table 5.24). The *M. m. castaneus* x *M. m. domesticus* F2 hybrids have larger variance in ADR, ADG and ADB measurements than *M. m. castaneus*, *M. m. domesticus* and *M. m. castaneus* x *M. m. domesticus* F1 hybrids (Table 5.23) These differences in variance are significant for ADR, ADG and ADB measurements, but it is not significant at the Bonferroni corrected p-value of 0.012($p<0.05$; Table 5.24).

When comparing variances for the ventral coat the *M. m. castaneus* x *M. m. musculus* F2 hybrids have smaller variance in AVR and AVG than the *M. m. castaneus* x *M. m. musculus* F1 hybrids while having larger AVB variance (Table 5.23). The *M. m. castaneus* x *M. m. musculus* F2 hybrids also has larger variances in AVR, AVG and AVB than *M. m. castaneus* (Table 5.23), and larger variances in AVR, AVB than *M. m. musculus* while having smaller variance in AVG (Table 5.23). These difference in variance are significant for AVR, but not at the Bonferroni corrected p-value of 0.012 ($p<0.05$) and AVB at the Bonferroni corrected p-value of 0.012 ($p<0.01$) but not for AVG measurements (Table 5.24). The *M. m. castaneus* x *M. m. domesticus* F2 hybrids have larger variances in AVR, AVG and AVB measurements than *M. m. castaneus, M. m. domesticus* and *M. m. castaneus* x *M. m. domesticus* F1 hybrids. These differences in variance are not significant for AVR and AVG measurements while it is significant for AVB measurements, but not at the Bonferroni corrected p-value of 0.016 ($p<0.05$; Table 5.24).

In summary, the *M. m. castaneus* x *M. m. domesticus* F2 hybrids have larger variances than previous generations for dorsal coat values, which we did not see in the F1 hybrids, as well as larger variance in AVB values. The *M. m. castaneus* x *M. m. musculus* F2 also has larger varainces in dorsal coat colour but this is not significant while have larger and signicnantly different variance in AVB measurements across the bourd when compared to the F1 hybrid and parental groups. In the F2 hybrids we see a trend towards increased AVB variance as well as increased dorsal coat colour variance.

Variation in dorsal and ventral coat colour in B1 hybrids

Results for comparisons of variances between B1 hybrids, F1 hybrids and the "pure bred" parental groups will be presented below. Variances were determined and compared for the dorsal coat colour measurements as well as the ventral coat colour measurements.

M. m. musculus x *M. m. domesticus* B1 hybrids

The *M. m. musculus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have larger variance in ADR and ADG measurements than the *M. m. musculus* x *M. m. domesticus* F1 hybrids, and smaller variance in ADB (Table 5.23). They also have larger variances than *M. m. musculus* and *M. m. domesticus* (Table 5.23). The *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids have larger variance in ADR, ADG and

ADB than the *M. m. musculus* x *M. m. domesticus* F1 hybrids, *M. m. musculus* and *M. m. domesticus* (Table 5.23). These differences in variance are not significant (Table 5.24).

For the ventral measurements, the *M. m. musculus* x *M. m. domesticus_M. m. domesticus* B1 hybrids have larger variances for AVR, AVG and AVB measurements than *M. m. musculus* x *M. m. domesticus* F1 hybrids and *M. m. domesticus* (Table 5.23), this difference in variance is significant, at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 15). The *M. m. musculus* x *M. m. domesticus_M. m. musculus* B1 hybrids have larger variances in AVR, AVG and AVB measurements than *M. m. musculus* x *M. m. domesticus* F1 hybrids and *M. m. musculus* (Table 14). The differences in variance of ventral measurements are significant for AVR ($p<0.01$), AVG ($p<0.01$) the Bonferroni corrected p-value of 0.012 and AVB measurements but not at the Bonferroni corrected p-value of 0.016 ($p<0.05$; Table 5.24).

In summary both *M. m. musculus* x *M. m. domesticus* B1 hybrids have larger significantly different variances in ventral coat colour than the F1 hybrids and the parents. They have larger variances in dorsal coat colour but these differenes are not significant.

M. m. castaneus x *M. m. musculus* B1 hybrids

The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have larger variance in ADR and smaller variance in ADG and ADB than *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 5.23). *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have smaller variance in ADR, ADG and ADB measurement than *M. m. musculus* (Table 5.23). These differences in variance are not significant for ADR and ADG but are for ADB values, but this is not significant at the Bonferroni corrected p-value of 0.012($p<0.05$; Table 5.24). *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have larger variance in ADR, and smaller variance in ADG and ADB measurements than *M. m. castaneus* x *M. m. musculus* F1 hybrids (Table 5.23). They have larger variance in ADR and ADG and smaller variance in ADB than *M. m. castaneus* (Table 5.23). The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids has larger variances than *M. m. musculus* for ADR measuements while having smaller variance in ADG and ADB values (Table 5.23). These differences are not significant for variation in ADR and ADG values but they are significant for variance in ADB, this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.23).

The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have smaller variances than *M. m. castaneus* x *M. m. musculus* F1 hybrids for AVR, AVG and AVB measurements (Table 5.23). They have larger variances than *M. m. castaneus* for AVR, AVG and AVB measurements (Table 5.23). The *M. m. castaneus* x *M. m. musculus_M. m. musculus* B1 hybrids have larger variances in AVR measurements than *M. m. musculus,* while having smaller variances for the AVG and AVB values (Table 14). These differences are not significant for difference in variance in AVR and AVG but they are significant for differences in variance in ADB, this is significant at the Bonferroni corrected p-value of 0.012 ($p<0.01$; Table 5.24). The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have smaller variances in AVR, AVG and AVB measurements than the *M. m. castaneus* x *M. m. musculus* F1 hybrids and larger variances than *M. m. castaneus* (Table 5.23). The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids also have smaller variances in AVR, AVG and AVB values than *M. m. musculus* (Table 5.23). These differences in variances are not significant for AVR and AVG values but are significant for measurements in AVB values ($p<0.01$; Table 5.24) this is significant at the Bonferroni corrected p-value of 0.012.

In summary for the dorsal coat we see a decreased variation in the *M. m. castaneus* x *M. m. musculus* B1 hybrids, this difference is significant, we see the same trend in the ventral coat coat colour with overall smaller variances, and AVB being significantly different in terms of variance when compared to the parents.

M. m. castaneus x *M. m. domesticus* B1 hybrids

The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids have larger variances than *M. m. castaneus* for ADR, ADG and ADB measurements and smaller variances than the *M. m. domesticus* (Table 5.23). When compared to *M. m. castaneus* x *M. m. domesticus* F1 hybrids it has larger variances (Table 5.23). These differences in variances are not significant (Table 5.24). The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have larger variances than both the *M. m. castaneus* and *M. m. domesticus* for ADR, ADG and ADB measurements (Table 5.23). The *M. m. castaneus*x *M. m. domesticus_ M. m. domesticus* B1 hybrids have smaller ADR, ADG and ADB variances than the *M. m. castaneus* x *M. m. domesticus* F1 hybrid parents (Table 5.23). These differences in variance are not significant (Table 5.23).

The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids have larger variance than *M. m. castaneus* for the ventral coat measurements AVR, AVG and AVB but smaller variances than *M. m.*

domesticus (Table 5.23). They also have smaller variances than *M. m. castaneus* x *M. m. domesticus* F1 hybrids for AVR, AVG and AVB (Table 5.23). The differences in variance between the *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids and their parental groups are not significant (Table 5.24). The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrids have larger variances than both *M. m. castaneus*, *M. m. domesticus* and *M. m. castaneus* x *M. m. domesticus* F1 hybrids for AVR, AVG and AVB measurements (Table 5.23). This difference in variance is significant in for AVB measurements, but this is not significant at the Bonferroni corrected p-value of 0.016 ($p<0.05$) but not for AVR and AVG measurements (Table 5.24).

In summary the *M. m. castaneus* x *M. m. domesticus* B1 hybrids have decreased variance in dorsal coat colour but this is not significant. The *M. m. castaneus* x *M. m. domesticus_M. m. castaneus* B1 hybrids has larger variances than their purebred parents but smaller variances than the F1 hybrids for ventral coat colour but these differences are not significant. The *M. m. castaneus* x *M. m. domesticus_ M. m. domesticus* B1 hybrid has larger variation in ventral coat colour when comparend to the F1 hybrids and the pure bred strains but only the difference in AVB variance is significant.

Generation	Group	Variance					
		ADR	ADG	ADB	AVR	AVG	AVB
Parents	*M. m. castaneus*	124.90	106.48	73.21	134.14	70.21	103.28
	M. m. musculus	207.88	207.06	173.00	287.36	276.86	336.43
	M. m. domesticus	160.16	143.93	102.52	291.64	239.55	242.45
F1 Hybrids	*M. m. musculus* x *M. m. domesticus*	236.89	230.08	197.66	823.29	617.75	500.77
	*M. m. castaneus*x *M. m. musculus*	178.00	209.98	156.00	452.21	332.12	237.32
	*M. m. castaneus*x *M. m. domesticus*	114.20	110.85	72.72	450.38	347.46	265.80
F2 Hybrids	*M. m. castaneus*x *M. m. musculus*	335.52	242.89	122.85	339.58	266.01	430.37
	*M. m. castaneus*x *M. m. domesticus*	400.59	341.11	200.73	344.35	267.82	341.53
B1 Hybrids	*M. m. musculus* x *M. m. domesticus_ M. m. domesticus*	395.45	319.60	194.25	1115.33	1182.65	1696.03
	M. m. musculus x *M. m. domesticus_M. m. musculus*	424.72	333.37	198.41	839.41	677.02	741.29
	M. m. castaneus x *M. m. musculus_M. m. musculus*	184.51	166.41	124.01	322.43	126.78	198.99
	M. m. castaneus x *M. m. musculus_M. m. castaneus*	238.00	173.51	62.01	253.10	175.24	211.03
	M. m. castaneus x *M. m. domesticus_M. m. castaneus*	139.98	138.55	85.94	169.06	151.15	178.38
	*M. m. castaneus*x *M. m. domesticus_ M. m. domesticus*	246.72	181.78	109.67	443.51	419.14	380.06

Table 5.23: Variances for parents, F1, F2 and B1 hybrids.

Comparisons	ADR		ADG		ADB		AVR		AVG		AVB	
	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value
M. m. castaneus v *M. m. musculus* v *M. m. domesticus*	1.03	0.36	2.42	0.09	4.31	p<0.05	4.51	p<0.05	2.91	0.06	8.65	p<0.01
M. m. castaneus v *M. m. domesticus*	0.39	0.53	0.89	0.35	1.08	0.30	3.94	0.05	3.65	0.06	3.65	0.06
M. m. domesticus v *M. m. musculus*	0.43	0.52	1.06	0.31	2.40	0.13	0.17	0.68	0.11	0.74	0.98	0.33
M. m. castaneus v *M. m. musculus*	2.20	0.16	4.71	p<0.05	8.14	p<0.05	9.77	p<0.01	5.70	p<0.05	17.55	p<0.01
M. m. castaneus x *M. m. musculus* vs *M. m. musculus* v *M. m. castaneus*	1.21	0.30	2.40	0.10	3.63	0.31	3.75	p<0.05	2.40	0.10	8.07	p<0.01
*M. m. castaneus*x *M. m. domesticus* v *M. m. castaneus* v *M. m. domesticus*	0.23	0.79	0.53	0.59	0.63	0.53	2.00	0.14	2.15	0.12	3.89	p<0.05
M. m. musculus x *M. m. domesticus* v *M. m. musculus*v *M. m. domesticus*	0.40	0.67	0.70	0.50	0.87	0.42	5.35	p<0.05	2.94	0.06	0.85	0.43
M. m. castaneus x *M. m. domesticus* F1 v *M. m. castaneus*x *M. m. domesticus* F2 v *M. m. castaneus* v *M. m. domesticus*	3.08	0.03	3.08	p<0.05	3.04	p<0.05	1.31	0.28	1.48	0.22	4.13	p<0.05
M. m. castaneus x *M. m. domesticus* F1 v *M. m. castaneus* x *M. m. domesticus* F2	4.50	0.04	3.99	0.05	5.06	p<0.05	0.11	0.75	0.08	0.78	0.94	0.34
M. m. castaneus x *M. m. domesticus* F2 v *M. m. domesticus*	2.62	0.11	2.35	0.13	1.90	0.18	0.01	0.94	0.00	0.97	0.65	0.42
M. m. castaneus x *M. m. domesticus* F2 v *M. m. castaneus*	5.87	p<0.05	6.50	p<0.05	6.24	p<0.05	2.71	0.11	3.50	0.07	14.21	p<0.01
M. m. castaneus x *M. m. musculus* F2 v *M. m. musculus*vs *M. m. castaneus* v *M. m. castaneus*x *M. m. musculus* F1	1.65	0.18	1.96	0.12	2.54	0.06	2.90	p<0.05	1.84	0.14	8.31	p<0.01
M. m. musculus x *M. m. domesticus_ M. m. domesticus* v *M. m. musculus*v *M. m. domesticus*v *M. m. musculus* x *M. m. domesticus* F1	2.00	0.12	1.087	0.358	0.69	0.56	11.48	0.00	15.71	0.00	20.21	0.00
M. m. musculus x *M. m. domesticus_M. m. musculus* B1 v *M. m. musculus* v *M. m. domesticus* v *M. m. musculus* x *M. m. domesticus* F1	1.557	0.20	0.89	0.45	0.66	0.58	5.73	0.00	4.09	p<0.01	3.62	p<0.05
*M. m. castaneus*x *M. m. domesticus_ M. m. domesticus* B1 v *M. m. castaneus* v *M. m. domesticus* v *M. m. castaneus* x *M. m. domesticus* F1	0.5185	0.67	0.47	0.70	0.4698	0.70	2.08	0.11	2.42	0.07	3.60	p<0.05
M. m. castaneus x *M. m. domesticus_M. m. castaneus* B1 v *M. m. castaneus* v *M. m. domesticus*v *M. m. castaneus*x *M. m. domesticus*	0.156	0.93	0.36	0.78	0.49	0.69	1.9733	0.12	1.83	0.15	2.59	0.06
M. m. castaneus x *M. m. musculus_M. m. castaneus* B1 v *M. m. musculus*v *M. m. castaneus* v *M. m. castaneus*x *M. m. musculus* F1	2.0613	0.11	2.05	0.11	4.52	0.00	2.46	0.07	1.92	0.13	5.09	p<0.01
M. m. castaneus x *M. m. musculus_M. m. musculus* v *M. m. musculus*v *M. m. castaneus* v *M. m. castaneus*x *M. m. musculus* F1	0.7661	0.52	1.61	0.19	3.12	0.03	2.53	0.06	2.31	0.08	5.36	p<0.01

Table 5.24: Results for Levene's test used to determine if there are significant differences in variances between parents and F1, F2 and hybrids.

Results for sthe specific cross between *M. musculus* and *M. spretus*

Below are the results for the sub-specific cross generated between a *M. musculus* (represented by *M. m. domesticus*) and *M. spretus*. Only F1 hybrids were generated for this cross. Differenes in average dorsal and ventral coat colour, DVC and variances are compared between the parents and the parents and the F1 hybrids.

Results for comparison between F1 hybrids and parents

M. m. domesticus has larger mean ADR, ADG and ADB values than *M. spretus* (Table 5.25); this difference is significant at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.26). Thus *M. m. domesticus* on average has a lighter dorsal coat than the *M. spretus*. The *M. m. domesticus* x *M. spretus* F1 hybrids have smaller mean ADR, ADG and ADB mean values than both *M. m. domesticus* and *M. spretus*. These averages are smaller than the expected MPV (Table 5.25). Thus, *M. m. domesticus* x *M. spretus* F1 hybrids have darker dorsal coats than both parental groups, this difference is significant when compared to *M. m. domesticus*, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.01$) but not in comparison to the *M. spretus* ($p=0.10$; Table 5.26).

M. m. domesticus also has larger mean AVR and AVG values than *M. spretus* but it has smaller AVB values (Table 5.25). The ventral coat of the *M. spretus* is not brown but grey in colour thus though it might have smaller AVR and AVG values than *M. m. domesticus* (brown ventral coat) it is different in colour colour. The difference in ventral coat colour between *M. m. domesticus* and *M. spretus* is significant ($p<0.01$; Table 5.26). The *M. m. domesticus* x *M. spretus* F1 hybrids have smaller AVR values than *M. m. domesticus* and larger AVG and AVB mean values (Table 5.25), this difference in ventral coat colour is significant ($p<0.01$; Table 5.26). When compared to the *M. spretus* parents they have larger mean AVR, AVG, and AVB values, this difference is significant, at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.26). The *M. m. domesticus* x *M. spretus* F1 hybrids have larger AVR, AVG and AVB values than the expected MPV (Table 5.25).

PCA results are displaced in Table 5.27 and Figure 5.4. PC1 is on the on the x-axis and the PC2 on the y-axis. PC1 accounts for 56% of the variation AVR (0.54) has the largest positive loading, dorsal coat colour measurements also have positive loadings (ADR (0.49), ADG (0.46), ADB (0.39)) and AVG has a smaller

positive loading (0.31). *M. spretus* and *M. m. domesticus* separate along PC1 because *M. spretus* has smaller ADR, ADG and ADB values and a darker dorsal coat while also having smaller mean AVR and AVG values. Negative PC1 scores indicate that the ventral coat is becoming more grey and the the dorsal coat is becoming a darker shade of brown while positive scores indicate that the dorsal coat is becoming lighter brown and the ventral coat is becoming lighter brown grey as well. The hybrids overlap with *M. spretus* group along PC1 and separate from *M. m. domesticus* parents. PC2 accounts for 33% of the variation with ventral measures having negative loadings for ventral measurements (AVB (-0.61),AVR (-0.43), AVG (-0.48)) and small positive loadings for dorsal measurements (ADR (0.25), ADG (0.28) , ADB (0.27)). The *M. m. domesticus* x *M. spretus* F1 hybrids separate from *M. spretus* along they-axis with larger AVR, AVG and

AVB values than than the *M. spretus* parent. As the PC2 scores become positive the ventral coat becomes a darker shade of grey which we see in the *M. spretus* parents. Negative PC2 scores indicate that the AVB values are becoming larger along with the other ventral measuremtns and the coat colour is becoming a ligher shade of grey which we see in the F1 hybrids.

Again, in a cross involving *M. m. domesticus* we see the hybrid offspring having a transgressive phenotype outside of the range of variation of both parental groups. This is due to the lightening of the ventral coat colour of the *M. m. domesticus* x *M. spretus* F1 hybrids. With the F1 hybrids having larger AVG and AVB values than both parental groups while having smaller AVR values than the *M. m. domesticus* parents.

Dorsal and ventral colour measurements	Parents						F1 Hybrids			
	M. m. domesticus			*M. spretus*			*M. m. domesticus* x *M. spretus*			
	n	Mean	SD	n	Mean	SD	n	Mean	SD	*M. m. domesticus* x *M. spretus*
ADR	21	90.44	12.65546	19	73.71	7.39	15	68.44	6.51	82.075
ADG	21	84.98	11.99746	19	71.47	8.15	15	65.56	7.47	78.225
ADB	21	63.22	10.1263	19	52.30	9.42	15	48.12	8.57	57.76
AVR	32	159.5	17.07858	25	134.60	11.26	25	149.30	9.83	147.05
AVG	32	155	15.4784	25	143.90	10.91	25	158.00	8.65	149.45
AVB	32	126.4	15.56963	25	137.00	12.21	25	147.90	7.96	131.7

Table 5.25: Mean, sample size (n) and SD for measurements of average dorsal ADR, ADG, ADB and average ADR, ADG and ADB values for parents, of species cross and F1 hybrids as well as expected MPVf or crosses producing F1 hybrids

Comparison	Average dorsal data	Average Ventral data
	p value	p value
M. m. domesticus x *M. spretus* F1 v *M. m. domesticus*	p<0.01	p<0.01
M. m. domesticus x *M. spretus* F1 v *M. spretus*	0.10	p<0.01
M. m. domesticus v *M. spretus*	p<0.01	p<0.01

Table 5.26: Results for MANOVA (p values) used to determining if there is a significant difference in average dorsal coat colour and average ventral coat colour between F1 species cross *M. m. domesticus* x *M.spretus* hybrids and their *M. m. domesticus*and *M. spretus* parents.

M. m. domesticus x M. spretus F1	PC 1	PC2	PC3	PC4	PC5	PC6
SD	21.32	16.21	8.44	3.82	1.27	0.75
proportion of variance	0.56	0.33	0.09	0.02	0.00	0.00
cumulative proportion	0.56	0.89	0.98	1.00	1.00	1.00
	Loadings					
	PC 1	PC2	PC3	PC4	PC5	PC6
ADR	0.49	0.25	0.00	-0.61	0.00	0.56
ADG	0.46	0.28	0.25	-0.21	0.25	-0.74
ADB	0.39	0.27	0.43	0.71	-0.14	0.25
AVR	0.54	-0.43	-0.47	0.13	-0.49	-0.19
AVG	0.31	-0.48	0.00	0.13	0.78	0.21
AVB	0.00	-0.61	0.72	-0.22	-0.25	0.00

Table 5.27: PCA results for species cross parents and F1 hybrids.

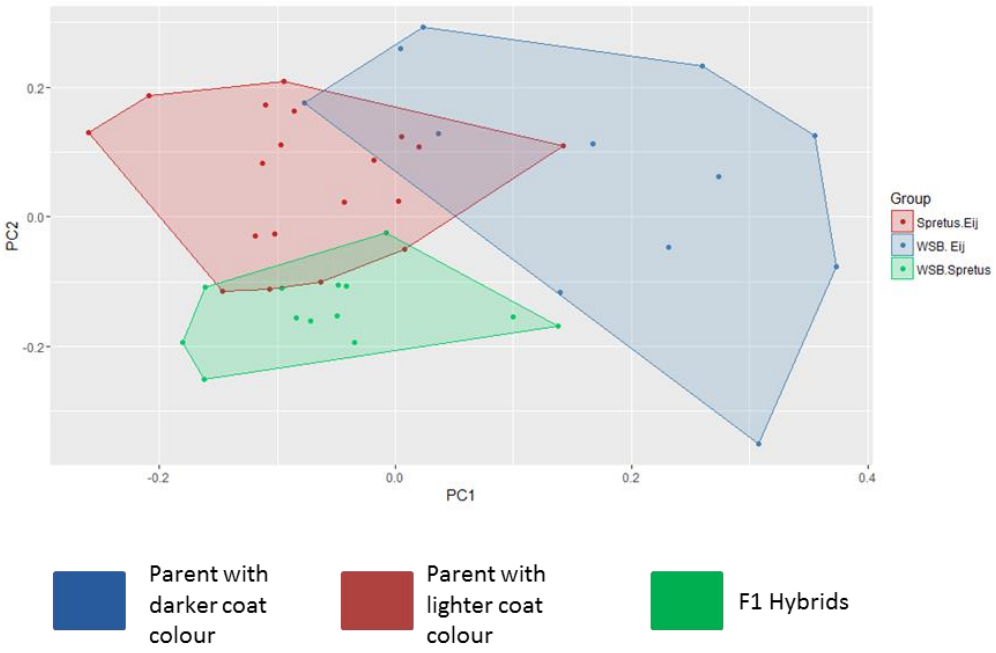

Figure 5.4: Results for specific crosses PCA based on average dorsal and ventral colour measurements of parents, F1 and B1 hybrids. The plot shows the distribution of WSB.Eij and Spretus.Eij parents and their F1 hybrids according to the first two principal components. *M. m. domesticus* = WSB.Eij, *M. Spretus* = Spretus.Eij

DVC comparisons between F1 hybrids and parents

M. spretus has smaller mean DVR values than *M. m. domesticus* and larger DVG and DVB mean values (Table 5.28). This difference in DVC is significant, the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.29). When compared to *M. spretus* the *M. m. domesticus* x *M. spretus* F1 hybrids have larger mean DVR, DVG and DVB values (Table 5.28). The *M. m. domesticus* x *M. spretus* F1 hybrids also have larger mean DVR, DVG and DVB mean values than *M. m. domesticus* (Table 5.28). In both cases the difference is significant, at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.29). The larger DVC of the the *M. m. domesticus x M. spretus* F1 hybrid mice is due to dorsal coat becoming darker while the ventral coat becomes lighter thus resulting in a heterotic phenotype in the *M. m. domesticus x M. spretus* F1 hybrids. Thus the DVC is larger than the expected MPV (Table 5.25).

Measurement of dorsal ventral contrast	Parents						F1 Hybrids			Expected mid parental values
	M. m. domesticus			*M. spretus*			*M. m. domesticus x M. spretus* F1			
	n	Mean	SD	n	Mean	SD	n	Mean	SD	*M. m. domesticus x M.Spretus* F1 hybrids
DVR	23	70.04	14.9	19	61.01	8.0616	13	79.23	5.63	65.52
DVG	23	70.48	13.9	19	72.12	9.3092	13	90.68	6.72	71.3
DVB	23	62.58	13.9	19	84.04	11.788	13	98.56	7.74	73.31

Table 5.28: Mean, standard deviation, sample sizes for differences in dorsal ventral patterning between F1 hybrids and parents

Comparisons of variance between F1 hybrids and parents

When the parents are compared *M. m. domesticus* has larger variances in dorsal and ventral coat colour than *M. spretus* (Table 5.30), this difference in variance is significant for ADR and AVR ($p<0.05$) but not for ADG, ADB, AVB, and AVG (Table 5.31). The F1 hybrids have smaller variances than both parents for the dorsal and the ventral coat colour measurements (Table 5.30). For the dorsal coat colour this difference in variance is only significant for variance in ADR, but this is not significant at the Bonferroni corrected p-value of 0.016 ($p<0.05$) and not for ADG and ADB when compared to *M. m. domesticus* (Table 5.30). When comparing the ventral coat colour the differences in variance of AVR, AVG and AVB are significant when compared to *M. m. domesticus*, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.31). There are no significant differences in variance between *M. spretus* and *M. m. domesticus x M. spretus* F1 hybrids for dorsal or ventral coat colour measurements (Table 5.23).

Comparison	p value
M. m. domesticus x M. spretus F1 v *M. m. domesticus*	p<0.01
M. m. domesticus x M. spretus F1v *M. spretus*	p<0.01
M. m. domesticus v *M. spretus*	p<0.01

Table 5.29: Manova results for comparisons between parents and F1 hybrids and their parents

Group	Variances					
	ADR	ADG	ADB	AVR	AVG	AVB
M. m. domesticus	**160.2**	**144**	**102.52**	**291.64**	**239.55**	**242.45**
M. spretus	**54.60**	**66.41**	**88.68**	**126.85**	**118.94**	**148.98**
M. m. domesticus x *M. spretus* F1	**42.37**	**55.79**	**73.39**	**96.70**	**74.74**	**63.34**

Table 5.30: Variances for parents and *M. m. domesticus* x *M. spretus* F1 hybrids

Comparison	ADR		ADG		ADB		AVR		AVG		AVB	
	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value	F value	p Value
M. spretus v *M. m. domesticus x M. spretus* F1	0.02	0.90	0.24	0.62	0.65	0.43	0.36	0.55	0.48	0.49	1.72	0.20
M. spretus v *M. m. domesticus*	5.70	0.02	2.41	0.13	0.01	0.93	5.11	0.03	3.53	0.07	2.38	0.13
M. m. domestics v *M. m. domesticus x M. spretus* F1	5.06	0.03	3.31	0.08	0.64	0.43	8.24	0.01	7.38	0.01	10.05	0.00

Table 5.31: Results for Levene's test used to determine if there are significant differences in variances between *M. m. domesticu* x *M. spretus* F1 hybrids and their parents.

Long limb bone results for sub-specific F1 hybrids and parents

Forelimb Measurements

Maximum lengths of forelimb long bones

The results for the limb measurements are represented in Figure 5.5 and Table 5.32 and 5.33 below. When comparing the humerus maximum lenghts between the parents *M. m. musculus* has the shortest, *M. m. casteneaus* is intermediate and *M. m. domesticus* has the longest (Figure 5.5A; Table 5.32). Only the difference between *M. m. musculus* and *M. m. domesticus* is significant, at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.33). *M. m. musculus* has the smallest mean maximum ulna length, *M. m. casteneaus* is intermediate and *M. m. domesticus* has the longest (Figure 5.5B; Table 5.32), these differences are not significant (Table 5.33). Hybrids were all significantly larger than the parental groups for long bones measured in the forelimbs, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 23 and 24).

When comparing the total length of the forelimbs, *M. m. domesticus* has longest, *M. m. casteneous* is intermediate and *M. m. musculus* is smallest for mean length (Figure 5.5C and Table 5.32). Only the difference between *M. m. domesticus* and *M. m. musculus* is significant, at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.33).

Relationships between the long bones of the forelimb

When comparing the forelimb ratios the ulna is longer than the humerus in all mice. When comparing the ratios, *M. m. domesticus* has the smallest difference in length between the ulna and the humerus with

a ratio closest to 1, *M. m. casteneous* has an intermediate ratio and *M. m. musculus* has the largest difference in length between the humerus and the ulna with the smallest ratio (Figure 5.4D and Table 32). This difference in ratio is significant when comparing *M. m. musculus* and *M. m. casteneous*, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.001$), it was also significant when comparing *M. m. musculus* and *M. m. domesticus*, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 5.33). When comparing the parents to the hybrids the *M. m. domesticus* x *M. m. casteneous* F1 hybrids have a smaller ratio than both its parents indicating that there was a bigger difference between the size of the ulna and the humerus (Table 5.32). This indicates that the ulna is larger than the humerus in the *M. m. domesticus* x *M. m. casteneous* F1 hybrid, these differences are significant ($p<0.001$; Table 5.33). This ratio is also much smaller than the expected MVP (Table 5.32). We see the same pattern of ulnas being on average slightly larger in relation to the humerus when compared to the parents across the F1 hybrids (Table 5.32). This is consistently significantly different, between all F1 hybrids and their respective parental groups, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 5.33), and the F1 hybrids have a smaller ratio than the expected MVP (Table 5.32).

Thus, the F1 hybrids have longer forelimb long bones, and the relationship between the long bones in is different from what we see in the parental groups. The ulna grows longer in relation to the humerus than would be expected.

Hind limb measurements

Maximum lengths of hind limb long bones

When comparing the parents, *M. m. musculus* have the shortest fibulas on average, *M. m. casteneaus* is intermediate and *M. m. domesticus* has the longest (Table 5.32). The F1 hybrids all have longer average fibulas than their their parents (Figure 5.5F; Table 5.32), these differences are significant ($p<0.001$; Table 5.33). *M. m. casteneaus* have the shortest femurs, *M. m. domesticus* are intermediate and the *M. m. musculus* have the longest on average (Figure 5.5E; Table 32). The F1 Hybrids are all longer than their parents on average, in terms of femur length (Table 5.32) and these differences are significant, at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 23).

In terms of the hindlimbs total length measurement (femur max length + fibula max length), *M. m. domesticus* have the longest hindlimbs , *M. m. musculus* are intermediate and *M. m. casteneous* hasthe

smallest on average (Figure 5.5G and Table 5.32). *M. m. domesticus* and *M. m. casteneous* were significantly different, at the Bonferroni corrected p-value of 0.016 ($p<0.001$) and *M. m. casteneous* and *M. m. musculus* are significantly different, but this is not significant at the Bonferroni corrected p-value of 0.016 ($p<0.05$; Table 5.33). The hybrids all have longer hind limbs on average than the parental groups and these differences are all significant, at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 5.32 and 5.33). The hybrids also have hindlimbs longer than the expected MPV (Table 5.32).

Relationships between the long bones of the hind limb

The ratio between the long bones of the hindlimb were determined, *M. m. casteneous* mice have shorter femurs than fibulas on average, *M. m. domesticus* and *M. m. musculus* have a ratio close to one indicating that the femur and the fibula are similar size (Figure 5.5H and Table 5.33). The femur is slightly larger than the fibula in the *M. m. musculus* mice (Table 5.33). The *M. m. domesticus* x *M. m. casteneous* F1 hybrids have a ratio slightly larger than *M. m. casteneous* but smaller than the *M. m. domesticus* and similar to the MPV (Table 5.32). The difference between *M. m. casteneous* and *M. m. domesticus* x *M. m. casteneous* F1 hybrids is not significant ($p=0.06$) while the difference between the F1 hybrids and *M. m. domesticus* parents is, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 5.33). The *M. m. casteneous* x *M. m. musculus* F1 hybrids have larger ratios than *M. m. casteneous* and smaller than the *M. m. musculus* (Table 5.32), this is slightly larger than the expected MPV (Table 5.33). The *M. m. casteneous* x *M. m. musculus* F1 hybrids have significantly different ratios when compared to both parents, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 5.33). *M. m. musculus* x *M. m. domesticus* F1 hybrids have have a slightly smaller ratio than *M. m. musculus* and *M. m. domesticus*, thus being smaller than the expected MPV (Table 5.32). This difference is significant, at the Bonferroni corrected p-value of 0.016 ($p<0.01$; Table 5.33).

In summaryin the hindlimbs we don't see the same patterns as we do in the forelimb in terms of the relationship between the long bones changing as much. The hybrids while having significantly longer hindlimbs are similar to the expected MPV when looking at the relationship between the size of the femur and the fibula (Table 5.32).

Comparisons of IM

When comparing the IM indices which is a ratio used to compare forelimb and hindlimb proportions, the mice all have shorter forelimbs than hindlimbs. The *M. m. musculus* mice have the smallest ratio, *M. m. domesticus* is intermediate and *M. m. casteneous* has the largest ratio (Figure 5.4I, Table 5.32). These

differences in IM are all significant, this is significant at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 5.33). When comparing the F1 hybrids to the parental groups, the *M. m. casteneous* x *M. m. domesticus* F1 hybrids had a smaller average IM than *M. m. casteneous,* while having a slightly larger IM than *M. m. domesticus* (Table 5.32). This difference in IM is significant when compared to *M. m. casteneous,* at the Bonferroni corrected p-value of 0.016 ($p<0.001$) but not when compared to *M. m. domesticus* ($p= 0.36$; Table 5.33). The *M. m. casteneous* x *M. m. domesticus* F1 hybrids thus has an IM smaller than the expected MPV (Table 5.33). The *M. m. casteneous* x *M. m. musculus* F1 hybrids has an IM larger than *M. m. musculus* and smaller than *M. m. casteneous* (Table 5.32). The F1 hybrid has an IM close to the expected MPV (Table 5.32). These differences in IM are significant, at the Bonferroni corrected p-value of 0.016 ($p<0.001$; Table 23). The *M. m. musculus* x *M. m. domesticus* F1 hybrids has an average IM larger than their *M. m. musculus* parent but smaller than *M. m. domesticus* (Table 5.32). The average IM for the *M. m. musculus* x *M. m. domesticus* F1 is close to the expected MPV (Table 5.32). The difference is significant when compared to the *M. m. domesticus,* but not at the Bonferroni corrected p-value of 0.016 ($p<0.05$) but not when compared to the *M. m. musculus* parent ($p=0.95$; Table 5.33).

Thus, though the hybrids have overall longer long bones in both the fore- and hindlimbs than the parents for all crosses, the relationship between the for limbs and hind limbs in two of the crosses is not significantly different from the parent with the smaller IM. Thus the hybrids long bones grow longer than expected but the relationships between the long bones are maintained.

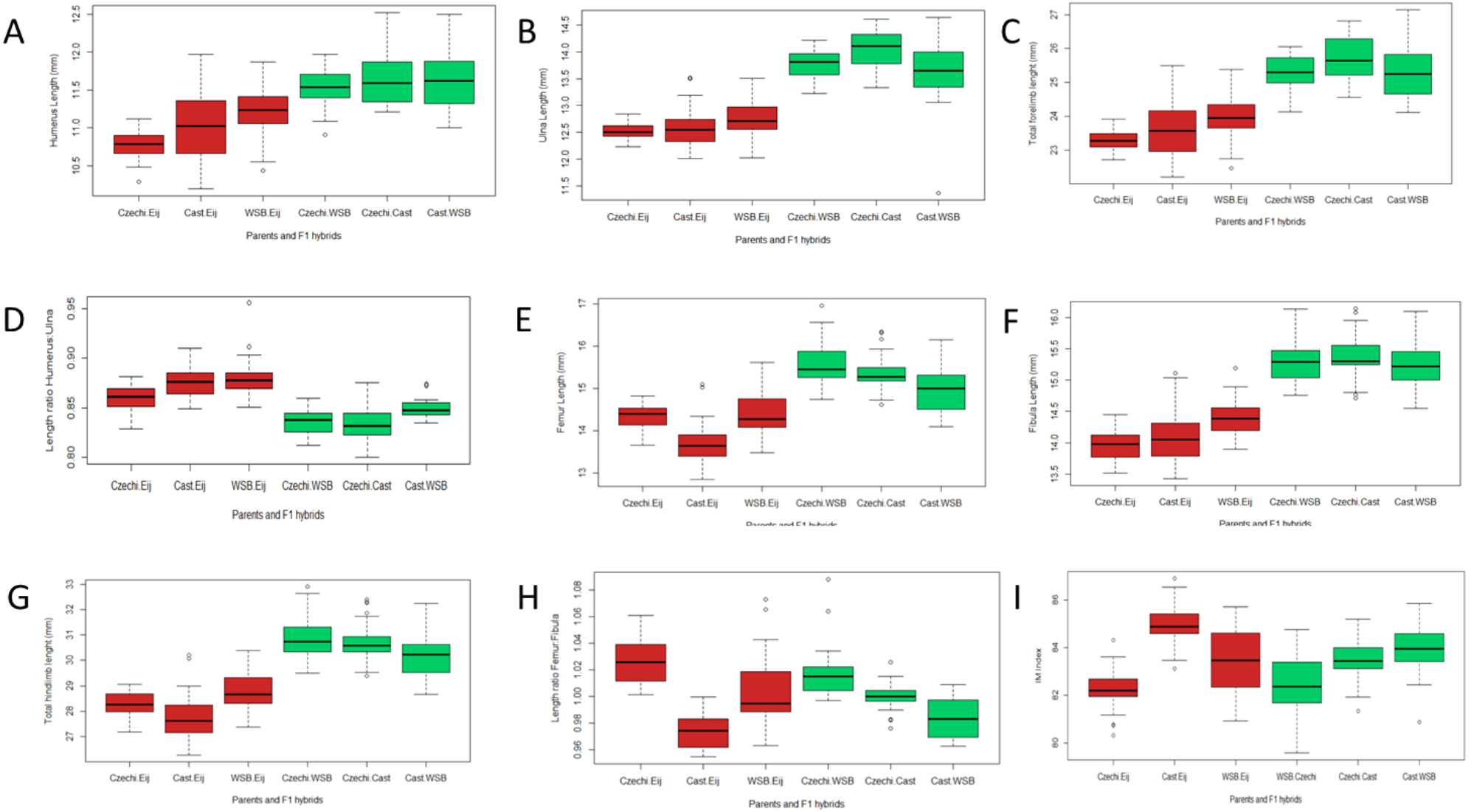

Figure 5.5: Box plot of forelimb and hindlimb measurements of parents (red) and F1 hybrids (green). (A) Humerus maximum length, (B)ulna maximum legth, (C) hindlimb total length, (D) length ratio humerus:ulna, (E)femur maximum length, (F) fibula maximum length, (G) total hindlimb length, (H) length ratio femur:fibula, (I)IM (inter-membral) index. *M. m. casteneaus* = Cast.Eij, *M. m. domesticus* = WSB.Eij, *M. m. musculus* = Czechi.Eij

Mearsurement	*M. m. casteneaus*			*M. m. musculus*			*M. m. domesticus*			*M. m. casteneaus* x *M. m. domesticus* F1			*M. m. casteneaus* x M. *m. musculus* F1			*M. m. musculus* x *M. m. domesticus* F1			mid parental ranges		
	n	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	n	Mean	sd	*M. m. casteneaus* x *M. m. domesticus* F1	*M. m. casteneaus* x *M. m. musculus* F1	*M. m. musculus* x *M. m. domesticus* F1
Total forelimb length	30	23.61	0.82	26	23.29	0.30	30	23.94	0.59	30	25.37	0.80	29	25.32	0.47	29	25.70	0.67	23.77	23.45	23.61
Humerus	30	11.02	0.45	26	10.77	0.18	30	11.21	0.33	30	11.64	0.39	29	11.68	0.36	29	11.54	0.25	11.12	10.90	10.99
Ulna	30	12.59	0.39	26	12.52	0.16	30	12.73	0.32	30	13.65	0.60	29	14.02	0.36	29	13.78	0.26	12.66	12.56	12.62
Humerus:ulna ratio	30	0.88	0.02	26	0.86	0.01	30	0.88	0.02	30	0.85	0.01	29	0.83	0.01	29	0.83	0.02	0.88	0.87	0.87
Total hindlimb length	30	27.76	0.91	29	28.33	0.46	30	28.79	0.75	30	30.23	0.95	31	30.88	0.82	36	30.72	0.78	28.27	28.04	28.56
Femur	30	13.69	0.52	29	14.35	0.26	30	14.41	0.53	30	14.99	0.56	31	15.36	0.43	36	15.57	0.50	14.05	14.02	14.38
Fibula	30	14.06	0.41	29	13.98	0.24	30	14.38	0.28	30	15.25	0.40	31	15.36	0.36	36	15.32	0.35	14.22	14.02	14.18
Femur:Fibula ratio	30	0.97	0.01	29	1.03	0.02	30	1.00	0.03	30	0.98	0.01	31	1.02	0.02	36	1.00	0.01	0.99	1.000	1.01
IM index	30	85.04	0.89	26	82.21	0.93	30	83.43	1.38	30	83.92	1.04	29	83.44	0.92	29	82.42	1.34	84.23	83.62	82.82

Table 5.32: Mean, standard deviation and sample sizes for all samples for differences in forelimb and hindlimb measurements.

Comparision	Total forelimb length	Humerus maximum length	Ulna maximum length	Humerus:Ulna ratio	Hindlimb Total Length	Femur maximum length	Fibula maximum length	Femure:Fibula ratio	IM index
	p value	p value	p value	p value	p value	p value	p value	p value	p value
M. m. casteneaus* v *M. m. domesticus	0.19	0.128	0.46	0.29	p<0,001	p<0.001	p<0.01	p<0.001	p<0.001
M. m. casteneaus* v *M. m. musculus	0.19	0.034	0.53	p<0.001	0.03	p<0.001	0.98	p<0.001	p<0.001
M. m. musculus* v *M. m. domesticus	p<0.01	p<0.001	0.18	p<0.001	0.05	0.65	p<0.001	p<0.001	p<0.001
M. m. casteneaus* x *M. m. domesticus* v *M. m. casteneaus	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	0,065	p<0.001
M. m. casteneaus* x *M. m. domesticus* v *M. m. domesticus	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	0.36
M. m. casteneaus* x *M. m. musculus* v *M. m. musculus	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
M. m. casteneaus* x *M. m. musculus* v *M. m. casteneaus	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
M. m. musculus*x *M. m. domesticus* v *M. m. musculus	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	P<0.05	0.95
M. m. musculus*x *M. m. domesticus* v *M. m. domesticus	p<0.001	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	P<0.05	P<0.05

Table 5.33: Results from pairwise comparisons of forelimb and hindlimb measurements compared between parents and parents and F1 hybrids to determine if there were significant differences in means.

Chapter 6: Discussion

Phenotypic outcomes of hybridization in F1 hybrids

Based on previous research we expect hybrids to have intermediate morphologies under an additive genetic model (Grant and Grant, 1994). They can also be transgressive or overlap with one of the parental groups (Rieseberg *et al*., 1999). F1 hybrids tend to be significantly different from their parents for average trait measurements (Grant and Grant, 1994; Fuzessy et al., 2014). One of the factors thought to affect phenotypic outcomes is genetic distance (Stelkens and Seehausen, 2009; Stelkens et al., 2009). It is predicted that the more distantly related two organisms are the more likely it is that their hybrid offspring will display transgressive traits (Stelkens and Seehausen, 2009). This is due more genetic differences being fixed between the parental groups over time (Stelkens and Seehausen, 2009). It was found that there was a positive correlations between the frequency of transgressive traits and genetic distance for both cichlids (58% and 78%) (Stelkens *et al*., 2009), and in a meta-analysis of the frequency of transgressive phenotypes and genetic distance for a variety of organisms (genetic distance accounted for 43% of the variance in transgression frequency) (Stelkens and Seehausen, 2009). However, it is also thought that there is a u-shaped relationship between genetic distance and the production of transgressive traits (Chen, 2013). More distantly and closely related crosses are thought to produce more transgressive phenotypes, while those of intermediate genetic distance are thought to result in less transgressive phenotypes (Chen, 2013). The more phenotypically similar two parental species are the more likely it is that they will have trangressive traits, this is thought to be the result of DSD in the parental groups for the genes underlying the development of the trait. In this case genetic differences accumulate even though the parents look similar phenotypically.

Effect of hybridization on pelage in F1 hybrids

A lot of the research available for mammalian hybrid coat colour variation is from mixed hybrid populations consisting of subsequent generations of hybrids such as F2 and backcrossed hybrids (Bynum, 2002; Fuzessy *et al*., 2014). There are data available for F1 gibbons/siamang hybrids; the hybrids had a mosaic morphology, having a colour and texture similar to the siamang and a white ring around its face like a gibbon (Wolkin and Myers, 1980). Howler monkey hybrids have transgressive phenotypes as a result of new colour combinations (Aguiar *et al*., 2008). Other groups have

intermediate but variable phenotypes, spanning the range of variation seen in the parental groups (Fuzessy *et al*., 2014).

In the F1 mice we see different outcomes in terms of average morphology for each of the sub-specific and specific crosses. *M. m. castaneus* and *M. m. domesticus* produced F1 hybrids which overlap with their parents. These parental taxa are morphologically similar in overall coat colour, and while their F1 hybrids overlap with both parents Figure 5.1A. They have an overall larger DVC, which is similar to *M. m. domesticus*.

The *M. m. castaneus* and *M. m. musculus* cross produces hybrids that have an intermediate phenotype. All measurements for the dorsal coat, ventral coat and DVC were close the MPV. Here we see the F1 hybrids being what you would expect under and additive genetic model Figure 5.1B.

The *M. m. musculus* x *M. m. domesticus* F1 hybrids differ from the other two crosses in having a new pattern that displays new combinations of dorsal and ventral coat colour not present in either of the parents. The *M. m. musculus* and *M. m. domesticus* F1 hybrids are transgressive in this regard. Transgressive phenotypes can take the form of absolutely larger or smaller size or very different colours, they can also take the form of new patterns and combinations of traits, such as the new patterns in howler monkey hybrids (Aguiar *et al*., 2008). Previous work on mouse hybrid mandibles showed that there weren't only changes in size but also changes in the relationship between different regions of the mandible resulting in a transgressive phenotype (Renaud *et al*., 2012). The dorsal and the ventral coat can be seen as different regions of the body with different developmental cues determining the colour produced (Candille *et al*., 2004; Hoekstra, 2006). These patterns are set up during development (Hoekstra, 2006), and the range of colours in the parental groups indicate that there are differences in the developmental underpinnings of these patterns (Millar *et al*., 1995; Candille *et al*., 2004). There are also differences in gene expression patterns to maintain these colours as hair grows and is replaced through-out the life span of the mouse (Millar *et al*., 1995). This produces the variation in dorsal ventral patterning we see in the parents.

The specific cross (*M. m. domesticus* x *M. spretus*) also results in F1 hybrids that have transgressive coat colour patterning, as displayed in Figure 5.4. These F1 hybrids separate from the *M. spretus* parent along both PC1 and PC2. The hybrid has a darker dorsal coat, and also has a grey ventral coat which is a different shade from the *M. spretus* parent ventral coat. The ventral coat values are intermediate between the two parental groups but still larger than the expected MPV. The F1 hybrids also have a transgressive DVC when compared to *M. spretus* and *M. m. domesticus,* with a new combination of dorsal and ventral coat colours.

Thus, overall there are a diverse range of outcomes for coat colour in F1 hybrid mice. *M. spretus* and *M. m. domesticus* are the most distantly related taxa, followed by the *M. m. domesticus* and *M. m. musculus* sub-species cross. *M. m. domesticus* and *M. m. musculus* form a hybrid zone (Jones *et al*., 2010), however hybridization results in male hybrid sterility (Jones *et al*., 2010). Thus genetic distance could play a role in the transgressive phenotype of the F1 hybrids for these two crosses. The cross between the two most closely related groups, *M. m. musculus* and *M. m. castaneus,* produces an intermediate phenotype. The *M. m. castaneus* and *M. m. domesticus* cross produces F1 hybrids which overlap with the parental groups morphologically. Thus with increasing genetic distance we see the hybrids producing transgressive coat colour traits. However, more work needs to be done to understand the role of genetic distance in producing transgressive phenotypes (Stelkens and Seehausen, 2009; Stelkens et al., 2009; Chen, 2013). It is also hypothesized that genes under directional selective pressure are less likely to produce transgressive phenotypes because genetic changes will be in the opposite direction thus hybrids are likely to be intermediate (Chen, 2013). Natural selection acts on coat colour and causes changes in coat colour to occur for substrate matching in mice so that they are not visible to predators (Hoekstra, Drumm and Nachman, 2004). Dorsal ventral patterning serves to make mice less visible from a horizontal plain, thus there is a great deal of selective pressure on coat colour (Hoekstra *et al*., 2004). Not enough data are available for the coat colour variation of the strains used in this experiment, but these selective pressures might explain the patterns we are seeing and why we some hybrids have transgressive phenotypes while others are very similar to their parents.

The transgressive phenotypes produced by the *M*. *m. musculus* x *M. m. domesticus* and the *M. m. domesticus* x *M. spretus* F1 hybrids could also be due to other factors. We don't see the same pattern in the sub-specific cross between *M. m. castaneus* and *M. m. domesticus* which produces F1 hybrids that are phenotypically similar to their parents, even though they have the same level of divergence as the *M*. *m. musculus* x *M. m. domesticus* cross. As mentioned above genetic distance accounts for a lot of the variation in the frequency of transgressive traits, but there are other factors that can produce transgressive traits in hybrids (Reisenberg 1999). In particular, *M*. *m. domesticus* is a parent in both crosses that produce hybrids with transgressive phenotypes. Thus, the transgressive phenotypes might not be the result of genetic distance but due to the genetic background of the *M*. *m. domesticus* parent, which modifies the gene expression patterns during development when it is crossed with a strain where dorsal ventral patterning is established during development such as *M*. *m. musculus* and *M. spretus. M. m. domesticus* has a mutation which results in the production of white spots on their foreheads for all *M*. *m. domesticus* mice used for the project, and some mice also have white spots on their belly. This is a result of a mutation in the *Kit* gene which is important for the

distribution and survival of melanoblasts during development (https://www.jax.org/strain/001145, (Jackson, 1994). The variant found within the WSB.Eij strain is thought to result in the death of melanosomes during development in the regions where the white spots develop (Jackson, 1994). Thus a cross between *M. spretus* and *M. m. castaneus* is required to determine if these transgressive traits are due to the *M. m. domesticus* genetic background, is a result of genetic distance or some other factors.

Are there broad patterns in terms of the outcome of hybridization in coat colour in the F1 hybrids?

One broad pattern identified was that in hybrids where one parent had a perceivable difference in colour between the dorsal and ventral coat and a large DVC the hybrids are also likely to have a large DVC, and there will be a discernable difference in colour between the dorsal and the ventral coat. The resultant morphology might be intermediate or extreme in the hybrid. The hybrids which are the result of the crosses where *M. m. domesticus* is a parent have larger average DVC than is expected producing transgressive dorsal ventral patterning on two occasions. Dorsal ventral patterning is one of the most common patterns among mammals and vertebrates and is established early on in development (Hoekstra, 2006). Dorsal-ventral patterning is common among mammals, because it reduces shadow in well-lit environments (Caro, 2005; Kamilar, 2009; Kamilar and Bradley, 2011). Dorsal-ventral patterning is also common in small primates, which are more vulnerable to predation, however this only holds true if these primates mostly adopt a horizontal position (Kamilar, 2009; Kamilar and Bradley, 2011). It was shown that primates such as marmosets and tamarins which are small and adopt vertical positions, don't usually evolve counter shading (Kamilar, 2009; Kamilar and Bradley, 2011).

Chinese and Indian rhesus macaques have a bipartite pattern, as do their hybrids when crossed with long tailed macaques). The hybrids are thus more similar to their rhesus macaque parents in terms of coat colour than their long tailed macaque parents which have a mono-colour pelage (Clarke and O'Neil, 1999; Hamada et al., 2006). Hybrids howler monkeys have new coat colour patterns not seen in either of the parental groups, with different regions of the body displaying colours corresponding to the overall coat colour of either parent (Aguiar *et al*., 2008). Thus it seems like hybrids are more likely to have coat colour patterning if one of the parents has coat colour patterning, even if the other is mono-colour. Disruption in development of the coat might result in hybrids being

more likely to develop coat patterns than to have a mono-colour pelage, even in the event when both parents have monocolour pelage as is the case with the howler monkeys.

Another factor might be cryptic coat patterning not detectable to the eye. For the *M. m. casteneaus* and *M. m. domesticus* mice the subtle differences in dorsal and ventral coat colour cannot be detected with the naked eye. However measurement of colour variation using the digital photographs clearly shows that the ventral coat in mice is always lighter than the dorsal coat. Other mammals, including other primates, might also have subtle differences in colour between different regions of the body which are magnified when hybridization occurs. Thus standardizing methods for measuring colour and understanding coat pattern morphology will be necessary to really see broad patterns in morphology and to understand the relationship between pelage variation and cranial/skeletal variation. Patterning is established early on in development, and it is likely that the same genes that are responsible for skeletal patterning are responsible for patterning of the coat colour. This will be discussed further later on.

Do F1 hybrids have increased levels of variation?

Hybrids are predicted to be more variable than their parents because new genetic combinations are produced when hybridization occurs (Grant and Grant, 1994). It is also predicted that offspring will be variable in response to hybridization with some hybrids being similar to one of the parents, others being intermediate and some being transgressive (Grant and Grant, 1994; Ackermann et al., 2006). Increased variation has been recorded in populations which are the result of admixture, and is thought to assist hybrids with adapting to novel environments, even helping them invade new regions not occupied by the parents (Grant and Grant, 1994; Ackermann et al., 2006; Lucek et al., 2010). Though we expect increased variation in the F1 hybrids, the F2 and B1 hybrids are expected to be more variable because of new genetic combinations which form in these generations.

In this study, most of the observed variation in coat colour is due to variation in ventral coat colour. When comparing dorsal coat colour variation between the F1 hybrids and their parents, there were no significant differences. In contrast, when the ventral coat colour variation of F1 hybrids is compared to their parents, the F1 hybrids all have variances in AVR, AVG and AVB which are larger than what is seen in the parental groups. These differences in variance are significant for some measurements but not for others. Because the combination of the three values in total gives an indication of the colour variance in one measurements means that there is greater variation in the colour overall. This is in line with the expected outcome of hybridization because new genetic combinations are present in the parental groups.

The *M. m. musculus* x *M. m. domesticus* F1 hybrids have twice the amount of variation in ventral coat colour measurements when compared to the parental groups. It is possible that the F1 hybrids have larger levels of variation when compared to the parental groups because *M. m. domesticus* has variation in the presence of a white spot on the ventral coat. Thus, there was variation within the parental population already, possibly resulting in higher levels of variation in the hybrids. For the species cross (*M. m. domesticus* x *M. spretus*) there was less variation in both the dorsal and ventral coat for the F1 hybrids compared to both parents. This difference was is only significant for variation in ADB and AVB values.

Although previous data have shown that hybrids have higher levels of variation when compared to parental groups, most data available for pelage is not on pedigreed hybrids so it is difficult to determine how hybridization might affect pelage variation in F1 hybrids. Our data shows that different regions might be affected differently by hybridization in terms of variation and that it might vary between crosses.

Limb measurements of F1 hybrids compared to parents

Transgressive long bone measurements in sub-specific F1 hybrids

The long bones of the forelimb and hind limb were measured and compared for the sub-specific crosses; this included the parents and F1 hybrids. Primates, including modern humans and Neanderthals, have differences in limb proportions as well as average lengths of long bones (Jungers, 1985; Ruff and Runestad, 1992; Walker et al., 2011). Thus, it was important to determine how hybridisation affects long bones. We also looked at whether the relationship between long bones changed as a result of hybridization.

It is important to establish how post crania are affected by hybridization; a lot more information is available for limb measurements for hybrids from mixed hybrid groups. F1 hybrids finches had intermediate overall morphology for morphometric traits including tarsier and wing length, while another cross produced hybrids smaller than the expected size (i.e. MPV) (Grant and Grant, 1994). Other mammalian hybrids from multi-generational hybrid zones are not transgressive for any of the individual morphometric measurements (e.g. femur length) and fall between the ranges of variation of the parents (Sears *et al*., 2003; Hamada *et al*., 2006; Fuzessy *et al*., 2014). These hybrids are also significantly different from both parents for forelimb and hind limb measurements, tend to be closer to one of the parents for different measurements, and do not fall close to the MPV exactly. Transgressive body size has been noted for gelada and hamadryas baboon F1 hybrids and sheep-goat

hybrids; these are crosses at the genus level with divergence between the different crosses occuring at ~4.5 Ma and between 5.9 – 7.08 Ma respectively (; Jolly *et al*., 1997; Mine *et al*., 2000; Zinner *et al*., 2013). Lion-tiger hybrids are also transgressive for body size but this is a cross at the species level (Randi *et al*., 1991). A considerable amount of research on F1 hybrids has also been for agricultural purposes to produce livestock with traits which will make them more profitable (such as larger yields or different flavours and textures) (Koch *et al*., 1995).

As expected the F1 mouse hybrids studied here are all significantly different from their parents for average long bone lengths. The F1 hybrids are all transgressive, having longer long bones on average than both parental groups for all long bones measured. It is common for mammalian hybrids to be transgressive in body size, but this is usually the case for hybrids which are more distantly related as discussed above. For most of the cases where hybrids between more closely related species hybridize we do not see transgressive body size changes. These results might only reflect what happens in the F1 hybrids, with subsequent backcrossed and mixed populations possibly not being as transgressive in terms of size, and/or fitting within the range of variation of the parental groups, which is what is observed in mixed hybrid groups (Bynum, 2002; Hamada *et al*., 2006; Kelaita and Cortés-Ortiz, 2013; Fuzessy *et al*., 2014).

The transgressive phenotype could be the result of the inbred mice being outcrossed, however as previously discussed this only explains a limited amount of transgression (Chen, 2013), and it is most likely due to genetic divergence that transgression is occurring across the board. Transgression is a result of two co-adapted genomes coming together, resulting in the production of hybrids that are transgressive in terms of absolute limb lengths (Lippman and Zamir, 2007; Birchler *et al*., 2010). There are other crosses such as those between tamarin monkeys (*Saguinus fuscicollis illigeri* × *S. f. lagonotus* and *S. f. illigeri* × *S. f. leucogenys)* which displayed heterosis in cranial size (Cheverud *et al*., 1993). Thus more F1 mammalian hybrids need to be studied to understand the role of genetic distance in the production of transgressive traits and whether they only appear in the F1 hybrids.

Effects of hybridization on the relationship between long bones of the fore- and hind-limbs

Though there has been a lot of research on hybrid body size there has been less on body shape and especially on skeletal material; more work needs to be done in this regard. Limited research has shown that hybrids from multi-generation hybrids groups are not always transgressive for individual trait measurements (Fuzessy *et al*., 2014, Hamada *et al*.). However, they might be transgressive for overall body shape (Fuzessy *et al*., 2014, Hamada *et al*.). They might also be similar to their parents or

intermediate as is the case with hybrid baboons (Jolly *et al*., 1997). The relationships between the fore and hind-limbs in hybrids baboons were shown to be similar to one parent using IM as a measurement, with the other hybrid having an intermediate phenotype; this was however a small sample (Jolly *et al*., 1997). Thus we explored the relationships between the long bones of the forelimbs and hind limbs. We also looked at the IM which is a measure of the relationship between the forelimbs and the hind-limbs and tells us about how this relationship varies between different groups.

The hybrid mice are different from their parents for the relationship between the humerus and the ulna, with the ratio being smaller than that of the parents and MPV. Thus in the hybrid we see a new relationship form between the long bones measured in the fore limb. The F1 hybrids were generally intermediate and very close to the expected MPV for ratio between the femur and the fibula of the hind limb. Even though the limbs grow longer than expected and the hybrids are transgressive for total lengnt the relationship between the long bones are similar to the MPV. Thus the long bones grow as expected in relation to each other in the hind limb, but not in the forelimb. In one instance, the *M*. *m. musculus* x *M. m. castaneus* F1 hybrid is not significantly different the *M. m. castaneus* parent when comparing the average Femur: Fibular ratio.

The IM tells us about the relationship between the forelimb and the hind-limb the hybrids are not significantly different from the parent which has the smallest ratio. For the *M. m. musculus* x *M. m. domesticus* F1 hybrids compared to *M. m. musculus* and *M. m. casteneaus* x *M. m. domesticus* F1 hybrids compared to *M. m. domesticus*, there was no significant difference. Thus, for the mouse hybrids we see a pattern of transgressive growth in terms of length, and we also see a difference in the relationship between the forelimb long bones. However they have a similar relationship between the fore and hind limb length (IM) to their parents at least for two of the crosses.

Sub- specific F2 Hybrids

Do F2 hybrids tend to exhibit more transgressive traits?

F2 hybrids are the result of F1 hybrids reproducing. Only two sub-specific crosses produced F2 hybrids. These are the crosses between *M. m. castaneus* x *M. m. musculus* and between *M. m. castaneus* x *M. m. domesticus*. It is expected that F2 hybrids will be, on average, significantly different from their parents (Parsons *et al*., 2011; Renaud *et al*., 2012). We also expect F2 hybrids to have a larger range of variation when compared to F1 hybrids and their parents, and expect to find more transgressive hybrids in F2 generation (Parsons *et al*., 2011; Renaud *et al*., 2012). This is due to new

genetic combinations forming in the F2 hybrids. The effects of hybrid breakdown due to new genomic combinations are also expected to occur in F2 hybrids (Parsons *et al*., 2011; Renaud *et al*., 2012).

As previously discussed new trait combinations not seen in parental groups can produce transgressive traits (Parsons *et al*., 2011; Fuzessy *et al*., 2014). The F2 hybrids resulting from the cross between *M. m. domesticus* and *M. m. casteneaus* from this study are transgressive because they have lighter average dorsal and ventral coats than both of the parents and the F1 hybrids. They have a DVC larger than their F1 parents but smaller than *M. m. domesticus*. This can be visualised in a PCA shown in Figure 5.2B. In this Figure, F1 hybrids are not transgressive, having average dorsal and ventral coat colours in between the averages of the two parents (which are very similar in coat colour with one being slightly lighter than the other). The F1 hybrids overlap with both parental groups along PC1 and PC2 with the parents separating along PC1 (Figure 5.2B), with only a few F1 hybrids being transgressive with darker dorsal and ventral coats. However, overall the F1 hybrids overlap with the parental groups. In contrast, the F2 hybrids are transgressive.Many of the F2 hybrids separate from the parental group along PC1 and PC2 with negative PC scores (Figure 5.2B). Many of the F2 hybrids also overlap with *M. m. domesticus,* the parent with the lighter overall coat colour. Thus, we see new phenotypes being produced in the F2 hybrids that we don't see in the parents or F1 hybrids, possibly due to new genetic combinations which arise in the F2 hybrids. Thus, in one cross we see transgressive traits in the F2 generation even though their F1 parents had coat colour morphology similar to that of the parents. It is predicted that parents which are phenotypically similar are more likely to produce hybrids with transgressive traits because of DSD (Rieseberg *et al*., 1999). This might be the case with the *M. m.* castaneus x *M. m. domesticus* cross with the transgressive phenotype only showing up in the F2 hybrids.

The other cross produces *M. m. casteneaus* x *M. m. musculu*s F2 hybrids that overlap with the *M. m. musculu*s parent and are not intermediate like their F1 hybrids parents. Overall the F2 hybrids are on average lighter than their F1 parents in terms of overall coat colour. A clear trend in the F2 hybrids is that they overlap with the original parental groups with the lighter coat colour and the larger contrast in dorsal ventral patterning as seen with the PCA results (Figure 5.2). They also have phenotypes outside of the range of variation of the parental groups or the F1 hybrids. F2 hybrids also have a large range of variation which overlaps that of the parent with the lighter coat and the larger contrast as well as with the F1 hybrids.

As previously explained the variation in coat colour is due to variation in in the amount of eumelanin and pheomelanin deposited in the coat. The overall lighter coat colour of the F2 hybrids indicates that more pheomelanin is being deposited and there might be differences in gene expression

of the *Agouti* gene, or it's interaction with the *MCIR* gene. The F2 hybrid mice could have new combinations of *Agouti* and *MCIR* genes which result in this overall lighter phenotype.

Are the F2 hybrids more variable than their F1 parents?

It is expected that F2 hybrids will be more variable than their parents because new genetic combinations arise in F2 hybrids (Parsons *et al*., 2011; Renaud *et al*., 2012). Indeed, the *M. m. castaneus* x *M. m. domesticus* F2 hybrids are more variable in dorsal coat colour than the F1 hybrids, *M. m. castaneus* and *M. m. domesticus*. This difference in variation was significant when compared to the F1 hybrids and *M. m. castaneus*. However, they were not significantly different when compared to *M. m. domesticus*. The *M. m. castaneus* x *M. m. musculus* F2 hybrids generally had larger variance than the F1 hybrid and their parents for dorsal measurements, with the exception of ADB which has larger variance in *M. m. musculus* and *M. m. castaneus* x *M. m. musculus* F1 hybrids, however none of these differences were significant. Thus, the F2 hybrids are generally more variable than the F1 hybrids and parents, for dorsal coat colour.

For ventral coat colour both the *M. m. castaneus* x *M. m. domesticus* F2 and the *M. m. castaneus* x *M. m. musculus* F2 hybrids have larger variance in AVB values when compared to the F1 hybrids and parents; these differences are significant. In the case of the *M. m. castaneus* x *M. m. musculus* F2 hybrids this is most likely the result of having the a range of ventral coat colour which spans from the yellow mustard ventral coat colour with relatively low AVB values to the mustard brown ventral coat which most likely has higher AVB values. To sum, in the F2 hybrids there is increased variation in traits which may have not been as variable in the F1 hybrids.

Sub-specific backcrossed hybrids: B1 hybrids

Phenotypic outcomes of hybridization in B1 hybrids

The expectation is that B1 hybrids will be on average intermediate between the F1 hybrid and the parent being backcrossed into (Grant and Grant, 1992). We also expect to see some transgressive traits which arise in the F1 hybrid parental also present in the B1 hybrids, but not at the same high frequency when compared to F1 and F2 hybrids (Grant and Grant, 1992; Ackermann et al., 2014). Previous studies have shown that heritabilities were high for hybrid traits and we would like to determine whether traits produced in the parents are also produced in the F1 hybrids (Grant and Grant, 1992; Ackermann et al., 2014). We will be looking at the B1 hybrids from the three sub-specific crosses below.

Do traits introduced in F1 hybrids persist in B1 hybrids?

The *M. m. musculus* and *M. m. domesticus* cross produced F1 hybrids which were transgressive but did not produce F2 hybrids due to male hybrid sterility. The F1 hybrids were able to backcross into both parental groups producing B1 hybrids. The backcross into *M. m. domesticus* which has a small DVC produced B1 hybrids that cluster with the F1 parents that has a transgressive DVC phenotype, the other B1 hybrids cluster with *M. m. domesticus* as can be seen in Figure 5.3B. Thus, we see transgressive traits introduced in the parental groups in subsequent generations for this cross. There is also an overlap in phenotype between the *M. m. castaneus* x *M. m. musculus* F1 hybrids and *M. m. castaneus* x *M. m. musculus_ M. m. musculus* B1 hybrids as seen in Figure 5.3C. The *M. m. castaneus* x *M. m. musculus_ M. m. musculus* B1 hybrids have an intermediate morphology as t overlapping with *M. m. musculus*.

Thus, in some crosses we see phenotypes which are produced in the F1 hybrid parental population in subsequent generations, whether the F1 hybrids have extreme or intermediate traits. There are however B1 hybrids which look like the parental group being backcrossed into.

Are there increased levels of variation within the backcrossed B1 hybrids?

The *M. m. musculus* x *M. m. domesticus_M. m. domesticus* B1 hybrids have elevated levels of ventral coat colour variation and this is significant. Once again we see an explosion in variation in the hybrids in relation to the parents. They also had higher levels of dorsal coat colour variation but these differences were not significant. Once again a cross involving *M. m. domesticus* results in increased variation; this could be due to the fact that it has variation in its ventral coat, as previously discussed. The F1 hybrids used for this backcross also had high levels of ventral coat variation which could also be a factor in the high levels of variation seen in the B1 hybrids.

The back crossed hybrids resulting from the crosses between *M. m. castaneus* and *M. m. musculus* had lower levels of variation when compared both the F1 and F2 hybrids. The *M. m. castaneus* x *M. m. musculus_M. m. musculus* cross had lower levels of variation when compared to *M. m. musculus* dorsal coat colour. The B1 hybrids however had larger levels of variation for the AVR measurement of the ventral coat, increased AVR measurements are associated with more yellow/agouti ventral coat colour while increased in AVB is associated with the more brown, grey and cream ventral coat. This increased variation in AVR could be due to having B1 hybrids which are similar to the *M. m. musculus* parental group.

The appearance of new transgressive phenotypes in backcrossed B1 hybrids.

Though it is expected that F1 hybrids will have transgressive traits, many studies have reported that recombinants in subsequent generations are likely to result in more variability as well as the production of novel phenotypes not see in the parents or F1 hybrids (Parsons *et al*., 2011; Renaud *et al*., 2012). Some of the traits associated with the transgressive phenotype of the hybrid sunflower species *H. paradoxus* arose in backcrossed artificial hybrids of *H. annuus* × *H. petiolaris* (Lexer 2003)**.** The *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids have a dark brown dorsal and ventral coat producing a phenotype outside of the range of variation seen in either the F1 parental group or the *M. m. castaneus* parental group, and are on average significantly different from their parents. Some of the *M. m. castaneus* x *M. m. musculus_M. m. castaneus* B1 hybrids overlap with *M. m. castaneus* and *M. m. castaneus* x *M. m. musculus* F1 hybrids, while others have intermediate phenotype between the two parents. However there is another group that has trait values outside of the range of variation seen in either parental group (Figure 5.3C). In these transgressive B1 hybrids we see the coat colour becoming darker while at the same time maintaining a larger DVC. Thus there is a colour change while maintaining the relationship between the dorsal and the ventral coat, resulting in the new phenotype. These could be new recombinants in which the *Agouti* gene is not functioning correctly, or in which the *MCIR* and *Agouti* gene are not compatible, among other possible genetic causes.

It is not unusual to see transgressive traits displayed in B1 hybrids; many multigenerational hybrid populations have hybrids which are transgressive traits and these are likely backcrossed hybrids (Fuzessy *et al*., 2014). Many transgressive traits which are of adaptive significance are found in backcrossed hybrid groups (Rieseberg *et al*., 1999). The presence of transgressive phenotypes in backcrossed hybrids and descendants of the F1 hybrids is important for transgressive segregation to occur. We see the same pattern in Louisiana irises with transgressive traits being detected in backcrossed populations (Johnston, Donovan and Arnold, 2004)

Can we infer developmental changes from final pelage morphologies?

Coat colour morphology is set up in two phases. The first phase is the development of the coat colour pattern i.e. determining which colour different regions of the body will be (Jackson, 1994). This is set up early in development when the melanoblasts are derived from the neural crest and developmental genes are important for determinization, specialisation, movement and activation of

these cells (Jackson, 1994; Hoekstra, 2006). The second phase is the maintenance of those patterns, an example of this would be differential expression of genes the *Agouti* gene during the growth of the hair in mice (Jackson, 1994; Vrieling *et al*., 1994; H. Hoekstra, 2006).

Some mice have yellow/agouti ventral coats, while having dark brown dorsal coat. This dorsal ventral pattern is set up early in development with the *Agouti* gene activated at 10.5 days during embryonic development in the ventral coat (Vrieling *et al*., 1994). The gene is activated in the dorsal coat at a later stage of development (Vrieling *et al*., 1994). The pattern set up in development is maintained through the differential expression of the *Agouti* gene in the dorsal and in the ventral coat (Vrieling *et al*., 1994). The yellow/agouti ventral coat produced in mice with this dorsal ventral pattern is the result of pheomelanin being deposited in the ventral coat throughout the hair growth process (Hoekstra, 2006). The dorsal coat has a banded hair pattern with alternating black and yellow bands producing the brown colour (Jackson, 1994; Vrieling *et al*., 1994). In the dorsal region there is a pulsating *Agouti* gene expression pattern resulting in the alternating deposition of eumelanin (black/brown pigment) and pheomelanin (yellow-red pigment) producing banded hairs which form a brown dorsal coat (Jackson, 1994; Vrieling *et al*., 1994; H. Hoekstra, 2006). The ratio of eumelanin and pheomelanin determines coat colour. More pheomelanin produces lighter coats and more eumelanin produces darker coats. The differential expression of the *Agouti* gene in the dorsal and ventral coat is due to the fact that it has two different promoters (Vrieling *et al*., 1994).

The development of this dorsal ventral patterning and differential expression of the *Agouti* gene forms part of the development of general body patterning in mice and in other animals (Candille *et al*., 2004; Hoekstra, 2006). In mice with a loss of function mutation of the *Tbx15,* a reduced skeleton and cranium developed, resulting in the droopy eared phenotype (Singh *et al*., 2005). A similar loss of function mutation also results in a shift of the dorsal ventral boundary (Candille *et al*., 2004). As previously discussed these are gross malformations and if there were differences in gene expression in this gene we would expect subtle shifts in the hybrids.

M. m. musculus has a ventral coat which is agouti/yellow and a brown dorsal coat. This indicates that there is differential expression of the *Agouti* gene in the dorsal coat and in the ventral coat. The Czechi.Eij (*M. m. musculus*) strain used for this cross is known to have heightened agouti/ yellow pigment due to a coat colour genetic mutation (https://www.jax.org/strain/002799). This phenotype is most likely due to differential expression of the *Agouti* gene with constitutive expression in the ventral coat and pulsating expression in the dorsal coat, with he dorsal ventral pattern set up early in development in this strain (Vrieling *et al*., 1994; H. Hoekstra, 2006).

In this study, strains were crossed with different DVC phenotypes, and although we don't know the developmental underpinnings of these specific strains, we know that that coat colour patterning is set up early in development. The *M. m. musculus* has the agouti ventral coat phenotype and only has banded hair in the dorsal coat. The other parents have banded hairs in the dorsal and the ventral coat, and though the ventral coat is slightly lighter than the dorsal coat, there is not constitutive expression of the *Agouti* gene in the ventral coat of the *M. m. castaneus* and the *M. m. domesticus* parents, which indicates different developmental underpinning of the coat colour morphology. The *M. m. domesticus* x *M. m. musculus* and the *M. m. domesticus* x *M. spretus* hybrids are transgressive for dorsal ventral patterning. Both have ventral pelage colours not seen in either of the parents and this indicates that a different developmental dynamic was set up in the hybrids during the regional development of the ventral pelage. We see that new pattern passed onto some of the B1 hybrids. This also means that there are differences in expression of the genes which are important for determining the ratio of eumelanin and pheomelanin that occurs in the hybrids. Thus it is possible to make some interpretation on developmental disruption in hybrids if we have an understanding of the developmental pathways and how the final phenotype is produced. This might also translate into differences in skeletal patterning in the hybrids.

When the hybrids have phenotypes outside the range of variation seen in the parental group this is most likely the result of developmental establishment of dorsal ventral body patterning being disrupted during the developmental process. If the genes which control dorsal ventral patterning are disrupted and result in the transgressive coat colour patterning in some hybrids we might expect the same thing in skeletal elements also under the developmental control of genes such as *Tbx15*. Thus based on the transgressive coat colour morphology we might expect to see more transgressive features in the skeleton of these hybrids. Determining whether non-metric skeletal traits occur in conjunction with, or co-vary with, coat colour patterns in the hybrids could help us to make inferences about soft tissue traits using the fossil record.

In primates very little is known about the development of coat colour pattern. However across all mammals it is likely that this pattern is set up early in development, when melanosome fate and distribution are determined (Hoekstra, 2006). As previously discussed, the pelage and skin is an important barrier to the environment and is under a great deal of selective pressure; sexual selectuion also plays a key role in determining coat colour variation in primates (Bradley and Mundy 2008).

Chapter 7: Conclusion

Pelage and skin are understudied traits, which have undergone dramatic changes during human evolution (Jablonski 2004). We know more about the changes in skin colour throughout human evolution but we know a lot less about the changes in morphology of hair and the epidermis.. There is also a great deal of variation between mammals and primates for pelage pattern, thickness, distribution and density (Bradley and Mundy 2008; Montagna 1972; Peters and Slen 1964; Taru and Backwell 2014). Despite this the pathways important for colouration and development of these traits are often highly conserved and linked to the development of other traits such as elements of the skeleton and the crania (Bradley and Mundy 2008; Hoekstra 2006). We know that admixture did not only occur between AMH, Neanderthals and Denisovans but throughout human evolution when hair and skin traits were undergoing major morphological transitions (Hammer et al., 2011; Eriksson and Manica, 2012).

The results presented here show that crosses between more distantly related mice taxa produced transgressive traits; this might also be due to the genetic background of one of the strains used for this project. If the former is the explanation then we might expect hybrids that result from more distant crosses (i.e. separated in deeper time) to have more dramatic differences in skin and hair morphology, resulting in transgressive phenotypes. If the latter is responsible for producing transgressive phenotypes, it shows how a mutation in one gene could have a dramatic effect on the phenotypic outcomes of hybridization.

Applied to the human context generally, we might expect that a lineage with reduced hair and dark skin color leading up to AMH hybridizing with a lineage with different skin/hair phenotypes might produce hybrids with very transgressive phenotypes. Furthermore, we might expect hybrids which are a consequence of hybridization between Neanderthals and Denisovans, which are more closely related, to be intermediate or similar to parental groups for skin colour or other soft tissue trait variation (including hair morphology, hair colour, possibly fat distribution –see section discussing *TBX15* gene), while hybrids between AMH and Neanderthals/Denisovans would be transgressive for soft tissue traits. If they were intermediate or transgressive this new trait variation might have assisted with adaptation to new environments. Even in the closely related lineages we see transgressive soft tissue traits being produced in F2 and B1 hybrids, thus we still expect hybridization to have a big impact on soft tissue morphology of hybrids. These tissues are in direct interaction with the environment and

important for adaptation thus hybridization can be essential in such cases for providing the novel variation on which selection can act.

Other soft tissue traits also important for adaptation to new environments include fat catabolism and fat distribution traits (Cunnane and Crawford, 2003; Vernot and Akey, 2014; Racimo et al., 2017). These traits have also undergone important changes throughout human evolution. Modern humans are born with substantial fat deposits in comparison to chimps; these are established prenatally and developmental fat deposition genes are important for producing this phenotype (Cunnane and Crawford, 2003). This is thought to be important for the appropriate brain development of babies (Cunnane and Crawford, 2003). The *TBX15* gene is important for body planning during development in mammals, including fat distribution and the appropriate skeletal development (Candille *et al*., 2004; Singh *et al*., 2005; Lausch *et al*., 2008). An allele of the *TBX15* gene has been introgressd in modern humans from a close relative to the Denisovans (Racimo *et al*., 2017). In mice *Tbx15* affects dorsal ventral patterning (Candille *et al*., 2004). The results presented here show dramatic changes in the dorsal ventral patterning in the mouse hybrids, with transgressive phenotypes presenting new dorsal and ventral colour combinations. Thus, hybridization between modern humans and Denisovans might have had dramatic effects on development of body (possibly skeletal) patterning and fat distribution in hybrids given that the *TBX15* gene was different and had different expression patterns (Racimo *et al*., 2017); there were possibly other differences in the developmental pathway *TBX15* is in. These genetic/development differences might have affected development of Denisovan X AMH hybrids and in terms of skeletal elements the scapula and pelvis might be affected as both of these require *TBX15* for appropriate development (Candille *et al*., 2004).

The soft tissue traits (hair, skin and colour patterning) discussed here arise from the same germ layers as the skeleton or their development is controlled by the same developmental genes (Pispa and Thesleff, 2003; Candille et al., 2004; Singh et al., 2005; Lausch et al., 2008). Because they are under selective pressure during human evolution we might see major changes in the developmental pathways of hominin groups to accommodate soft tissue variation while traits such as teeth and skeletal elements will be broadly similar (Bradley and Mundy 2008; Hoekstra 2006; Jablonski 2004; Rantala 2007; Rees 2000; Walsberg 1983). DSD acting in developing systems to maintain skeletal features which are broadly similar between humans and Neanderthals, while resulting in very different soft tissue morphology, might result in skeletal traits being heterotic when the two genomes come together. This project is the first step to understanding whether selection for differences in soft tissue traits could explain divergence in skeletal and cranial traits, and therefore be responsible for some of the skeletal and cranial traits we see in hybrids upon remerger of these divergent genomes.

There are variable outcomes in the hybrids in terms of pelage, but we repeatedly see the formation of transgressive traits. These indicate that there is a disruption in the development of the dorsal and ventral coat patterning in some of the hybrids..

In terms of the result from the postcranial, material there are certain patterns which could be used to identify hybrids in the fossil record. For example, you might expect that the long bones of hybrids will have average measurements significantly different from either parental group; the F1 hybrids might be transgressive as is the case with the long bones of the F1 hybrid mice. However, more work needs to be done on F1 post cranial remains to determine if this is a common pattern resulting from hybridization. You might also expect the relationship between the long bones in the legs and arms to differ. If you were looking at AMH x Neanderthal, AMH x Denisovan or Denisovan x Neanderthal hybrids, there might be differences in the relationship between the bones of the arms but not the legs. You might also expect the IM to be similar to that of the parent with the smaller IM. Modern humans and Neanderthals are known to have substantial differences in post crania with Neanderthals being shorter, stockier, having shorter distal limbs and a different overall body shape when compared to AMH (Pearson, 2000). From studies of primate hybrids we also see that in some cases mean measurements might not be transgressive and be intermediate between parental groups while body shape might change (Fuzessy *et al*., 2014). Thus it will be important to understand patterns prevailing in mammalian hybrids and compare it to the available hybrids material from Skhul, Mladěck, Qafzeh and Amud among others. Some hypothesized hybrids such as Skhul V have associated post cranial remains with Neanderthal derived traits (Pearson, 2000).

The results shows that with soft tissue traits and traits such as coat colour we could expect transgressive phenotypes to show up after the F2 and B1 generations of hybrids, and these would provide new variation upon which selection can act. This could also have aided the adaptation of modern humans to new environments. Many of the genes which have been introgressed into modern humans are shown to have differential gene expression patterns, when compared to their non-introgressed counterparts (Swanson-Wagner *et al*., 2006). Thus the combining of the new genomes would have resulted in the production of new phenotypes. It will be important for future work to look at how developmental genes were different between modern humans and Neanderthals and how this might have affected the development of hybrid offspring. Understanding the relationship between these soft tissue traits and skeletal traits could aid with inferring soft tissue traits in hominin hybrids using fossil material. This in combination with a good understanding of developmental biology and data mining form aDNA could help paint a fuller picture of the consequence of hybridization in terms of both morphology and adaptations.

Limitations of this study

Mice are great mammalian models because they are easy to breed, and we have considerable knowledge regarding the genetic underpinnings of developmental processes and the influence of the genotype on certain phenotypes. However primates and mice are very different in terms of certain morphologies, including pelage morphologies, and humans have different pathways which determine skin colour variation. Thus when considering the relationship between traits we need to bear these differences in mind. The other limitation was that we could not use some of the digital photographs because they lacked consistency, thus we have smaller samples sizes than is ideal, making it difficult to determine the relationships between pelage colour and the skeletal traits. We also know relatively little about the genetic underpinnings of pelage colour, patterning and texture in primates, thus any patterns that we recognize will require that we make big intellectual leaps from the mouse model. However as more work is done and more studies try to better understand differences in soft tissue traits between extant primates and humans, and aDNA gives us insight into soft tissue traits of Neanderthals, we might be able to make more informed inferences about whether evolution of soft tissue traits and their developmental pathways might impact skeletal morphology of hybrids.

Future work

This is part of a larger study which aims to understand the role of hybridization on morphological variation. Future work will include comparing the pelage data to cranial and skeletal data to determine if there is any co-variation between these traits. It will also be important to think about which measurements might be informative for determining the relationship between traits. In the case of dental traits, variation in *EDAR* expression is associated with thickness of hair and tooth morphology. Thus it might be informative to determine if there are differences in hair thickness of baboon and mouse hybrids which could be an indicator of variation in expression of this gene causing the dental non-metric traits we see in the hybrid baboons, and add to determining if variation in ectodermal development genes are the cause of the non-metric trait variation in the hybrids.

It will also be interesting to determine if there is a relationship between dorsal ventral patterning and other skeletal traits. The scapula and pelvis both require proper expression of the *Tbx15* gene to develop fully. It might be interesting to investigate whether there is a relationship between dorsal ventral patterning and these skeletal elements.

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